Efficacy of a Novel Contact Pathway Inhibitor, Ir-CPI, on in vitro Clotting Induced by PCI Catheter Segment

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Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

WILEY rpth research and practice in thrombosis and haemostasis | ISTH

ABSTRACT SYMPOSIUM ATHEROTHROMBOSIS & STROKE

ASY 13.1 | Impact of Venous Thromboembolism on Formation and Progression of Carotid Atherosclerosis - The Tromsø Study

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Background: Venous thromboembolism (VTE) is associated with increased risk of arterial cardiovascular diseases (CVD). The mechanism behind this relationship remains unsettled, but atherosclerosis may play a key role, as atherosclerosis is a major risk factor for arterial CVD. No previous study has examined the association between incident VTE and risk of future formation and progression of carotid atherosclerosis. We hypothesized that inflammatory processes secondary to VTE could promote formation and progression of carotid atherosclerosis.

Aims: To investigate whether incident VTE was associated with subsequent carotid atherosclerosis formation and progression in a population-based observational study.

Methods: Subjects attending ≥2 ultrasound examinations of the right carotid artery, including measurement of total plaque area (TPA), in the Tromsø Study in 1994-95, 2001-02 and/or 2007-08 were eligible for the study. We identified 150 subjects diagnosed with first-lifetime VTE between the initial and follow-up visit, and randomly selected 600 age- and sex-matched subjects without VTE between the visits.

Results: Subjects with VTE and presence of carotid plaque(s) at the first visit had 4.1 mm^2 (β 4.13, 95% CI -1.72 to 9.98) larger change in TPA between the first and second visit compared to subjects without VTE after adjustment for change in high-sensitivity C-reactive protein (hs-CRP) and traditional atherosclerotic risk factors. This association remained after restricting the analyses to VTE events diagnosed in the initial time-interval between the carotid ultrasounds, but was not statistically significant due to low study power. No association was found between incident VTE and novel carotid plaque formation.

Conclusions: Incident VTE was associated with atherosclerosis progression in those with already established plaques, but not with novel plaque formation. The association between VTE and carotid plaque progression was not mediated by low-grade inflammation assessed by hs-CRP.

ASY 13.2 | Carotid Atherosclerotic Plaque Matrix Metalloproteinase-2 Content Predicts Adverse Outcome in Patients Undergoing Endarterectomy

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Background: Matrix metalloproteinases (MMPs), participate in the vascular remodeling associated with atherosclerotic plaque development and rupture, responsible for ischemic cardiovascular events.

Aims: To investigate the association between plaque MMP-2 levels with the occurrence of clinical adverse events in a large cohort of patients undergoing endoarterectomy (CEA).

Methods: A biobank was created (Athero-Matrix Biobank) by collecting carotid plaque specimens from all patients undergoing CEA between 2009 and 2015. Carotid plaque tissue was removed, cleaned in sterile saline, extracted and analysed for MMP-2 content by SDS-PAGE zymography (total, pro and active forms) and TIMP-2 by ELISA. Relevant previous clinical history, risk factors and treatments were systematically collected. Cardio-cerebrovascular events occurring during follow-up were registered. Patients were followed up for a median 3 years (1-11 years).

Results: 690 atherosclerotic plaque specimens were collected. A total of 150 cardio-cerebrovascular events occurred during follow-up (1.6% myocardial infarction, 0.3% TIA, 2.4% stroke, 6.2% vascular death, 5.8% restenosis, 8.1% new carotid revascularization, 4.9% stenosis of the contralateral carotid). Significantly higher carotid plaque active MMP-2 levels, active MMP-2/pro-MMP-2 and active MMP-2/TIMP-2 ratios were found in patients who developed an event at follow-up (23.4 ± 4.5 vs 8.7 ± 1.0 ng/μg protein, 0.9 ± 0.2 vs 0.3 ± 0.05, 0.6 ± 0.1 vs 0.3 ± 0.04, respectively). Higher carotid plaque active MMP-2 was also predictive of vascular death, new

carotid revascularization, and stenosis of the contralateral carotid (30 ± 9.3 vs 11 ± 1.3 ng/μg protein, 16.3 ± 4.0 vs 9.0 ± 1.1, 34 ± 17.4 vs 9.2 ± 1.0, respectively). MMP-2 levels in plaques were significantly lower in statin-treated subjects.

Conclusions: Active MMP-2 in plaques predicts adverse cardiovascular outcome in patients undergoing CEA. MMP-2 may represent a biomarker for atherosclerosis progression and drugs reducing active MMP-2 in plaques may be of special interest.

ASY 13.3 | Vascular Matrix Metalloproteinase-9 Enhances Blood Brain Barrier Breakdown and Ischemic Brain Injury in Experimental Thromboembolic Stroke

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Background: Thromboembolic ischemic stroke causes blood brain barrier (BBB) breakdown, fibrin deposition, brain swelling, hemorrhage and ischemic cell death. Elevated levels of matrix metalloproteinase-9 (MMP-9) correlate positively with ischemic brain injury in human stroke and animal models. Still, the effects of intravascular vs brain MMP-9 on stroke outcomes are poorly understood.

Aims: We examined the effects of MMP-9 deficiency and MMP-9 supplementation on outcomes following thromboembolic stroke in mice.

Methods: Thromboembolic stroke was induced by injecting autologous clots into the proximal middle cerebral artery in mice. After 24 h, brain swelling, hemorrhage and cerebral infarction were quantitated. MMP-9 expression, BBB breakdown, fibrin deposition (immunostaining) and cell death (TUNEL staining) were quantitatively determined. The data were analyzed by Student's t-test or one way ANOVA.

Results: After 24 h stroke, MMP-9 expression was specifically upregulated in the ischemic hemisphere and co-localized with neutrophils and the endothelium. In comparison to MMP-$9^{+/+}$ mice, MMP-$9^{-/-}$ mice showed a significant decrease in cerebral infarction ($p < 0.05$), swelling ($p < 0.01$) and a nonsignificant decrease in brain hemorrhage. BBB breakdown ($p < 0.05$), fibrin deposition ($p < 0.01$) and cell death ($p < 0.01$) were also significantly decreased in MMP-$9^{-/-}$ mice. Intravenous MMP-9 supplementation (45 min before stroke) to MMP-$9^{-/-}$ mice reversed the beneficial effects of MMP-9 deficiency. Cerebral infarct and swelling were restored in MMP-9 supplemented MMP-$9^{-/-}$ mice to levels equivalent to MMP-$9^{+/+}$ mice demonstrating the role of intravascular MMP-9 in ischemic stroke injury.

Conclusions: Intravascular MMP-9 has profound deleterious effects on BBB breakdown, brain swelling, hemorrhage and cell death in ischemic stroke. Targeted inhibition of vascular MMP-9 may prove beneficial for reducing ischemic brain injury.

Financial support: NIH (NINDS NS089707 & NHLBI HL092750) to G. Reed, AHA Postdoctoral fellowship to S Singh.

ASY 13.4 | High Risk of Ischemic Stroke in Patients with Acute Pulmonary Embolism and Patent Foramen Ovale: Paradoxical Embolism Confirmed as an Important Mechanism. The EPIC FOP Prospective Multicentre Study (Clinical Trials.gov Number NCT01216423)

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Background: Cryptogenic stroke is more frequent among patients with patent foramen ovale (PFO) but the underlying mechanism of stroke in these patients remains unclear. We hypothesized that paradoxical embolism should be the main mechanism.

Aims: We aimed to compare the prevalence of recent ischemic stroke in patients with symptomatic pulmonary embolism (PE) according to whether PFO is detected or not.

Methods: In a prospective study of 374 patients with symptomatic documented PE, a cerebral magnetic resonance imaging (MRI) was systematically performed within 15 days of onset of PE symptoms. Recent ischemic stroke was diagnosed by hypersignal on diffusion-weighted imaging and restricted apparent diffusion coefficient on cerebral MRI. PFO was assessed by contrast trans-thoracic echocardiography. The prevalence of ischemic stroke was compared between PE patients with PFO and those without PFO.

Results: Contrast trans-thoracic echocardiography was conclusive in 324 patients and showed PFO in 43 patients and no PFO in 281. Median age was 66 years (IQR: 54-77), deep vein thrombosis was associated in 45% of PE and 55% of patients had a modified severity score (s-PESI) of 0 (no difference between PFO and non-PFO group). One patient in the PFO group and 8 in the non-PFO group were excluded because of absence of MRI or > 15 days delay in MRI. The prevalence of ischemic stroke was significantly higher in the PFO group than in the non-PFO group (9/42 (21.4%) and 15/273 (5.5%), respectively, Fisher test $p = 0.0016$).

Conclusions: We found that among patients with acute PE, those with a PFO have a higher risk of ischemic stroke than PE patients without a PFO. This finding supports the hypothesis of paradoxical embolism being an important mechanism of ischemic stroke. The implications of this finding on duration of anticoagulation remain to be determined.

ASY 16.1 | bApolipoprotein C-III is a Predictor of Activated Factor VII-antithrombin Complex Levels: A New Link between Plasma Lipids and Coagulation Pathway

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Background: Activated factor VII-antithrombin (FVIIa-AT) complex is a potential biomarker of prothrombotic diathesis, reflecting tissue factor (TF) exposure, and has been associated with mortality in patients with coronary artery disease (CAD). Previous works indicated plasma lipids as predictors of FVIIa-AT variability.

Aims: To evaluate the relationships between FVIIa-AT plasma concentration and lipid/apolipoprotein profile.

Methods: Within the framework of the Verona Heart Study we selected 460 subjects (120 CAD-free and 340 CAD, 68.9% males, mean age 59.9 ± 10.3 years) not taking anticoagulant drugs and for whom plasma samples were available for FVIIa-AT assay and for a complete lipid profile, including apolipoprotein (Apo) A-I, B, C-III, and E. FVIIa-AT plasma levels were measured by ELISA.

Results: There were significant direct correlations of FVIIa-AT levels with total and HDL cholesterol, triglyceride, Apo A-I and Apo C-III. Apo A-I ($R = 0.164$, $P = 4 \times 10^{-4}$) and Apo C-III ($R = 0.236$, $P = 3 \times 10^{-7}$) showed the strongest correlations. Including all the lipid parameters in an adjusted regression model only Apo A-I and Apo C-III remained significant predictors of FVIIa-AT levels, with Apo C-III explaining 5.6% of FVIIa-AT variability. Such results were confirmed after adjustment for sex, age, CAD diagnosis, and renal function.

The rs964184 polymorphism (tagging also APOC3 gene locus), which has been linked with cardiovascular risk and plasma lipids by genome-wide association studies, was associated not only with Apo C-III levels but also with FVIIa-AT plasma concentration, being higher in the carriers of the risk allele G (CC 81.6 [77.9-85.5], CG 90.0 [83.2-97.4], and GG 98.5 [77.8-124.6] pM, P = 0.015).

Conclusions: Our results indicate a strong association between Apo C-III and FVIIa-AT levels, thereby supporting the hypothesis that Apo C-III, one of the most important actor in lipid metabolism, may influence TF-FVIIa pathway with prothrombotic effects.

ASY 16.2 | Markers of Vascular Function Are Associated with Procoagulation Factors in the General Population

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Background: The strength and direction of the associations between vascular function and procoagulant factors remain unclear, especially in the general population.

Aims: We investigated whether three measures of vascular function (i.e. estimated glomerular filtration rate (eGFR), urinary albumin creatinine ratio (UACR), and arterial stiffness measured by pulse wave velocity (PWV)) are associated with an increased procoagulant state.

Methods: In this cross-sectional analysis of baseline measurements of the NEO Study eGFR, UACR, serum fibrinogen, and coagulation factors (F)VIII, FIX, and FXI were determined in all participants (n = 6,536). PWV was assessed by MRI in a random subset (n = 2433). eGFR, UACR and PWV were analysed on a continuous scale and per percentile based categories. eGFR and UACR were grouped into 6 categories (>50th [reference] to < 1st percentile for eGFR and < 50th [reference], to >99th percentile for UACR), and PWV was grouped in 4 categories (< 50th [reference] to >95th percentile). We performed linear regression analysis and adjusted for age, sex, total body fat, smoking, education, ethnicity, total cholesterol, CRP, and use of vitamin K antagonists (FIX).

Results: Mean age was 56 years, mean eGFR 86.2 (12SD) ml/1.73 m² and UACR 0.45 mg/mmol (IQR 0.30; 0.71). All coagulation factors showed a procoagulant shift with vascular function, for example FVIII concentration was 22 IU/dL (95% CI: 13-32) higher in the < 1st percentile. Compared with the UACR< 50th percentile, FVIII was 12 IU/dL (3-22) higher in the >99th percentile. PWV was only associated with procoagulant factors FIX an FXI in continuous analysis: to illustrate, for every m/s difference in PWV, FIX concentrations were 1.96 IU/dL (95% CI 0.70-3.2) higher.

Conclusions: All tested measures of vascular function were associated with increased levels of procoagulant factors, supporting the hypothesis that vascular function plays a role in the etiology of venous thrombosis.

TABLE 1 Association between PWV, eGFR, UACR and coagulant factors

Procoagulant Factor	eGFR per 10 ml/min/1.73 m² lower difference (n = 6,536)	UACR (mg/mmol) per 2-fold higher difference (n = 6,536)	PWV per m/s higher difference (n = 2,433)
Fibrinogen (mg/dL), crude	1.06 (0.6 to 2.7)	5.44 (3.7 to 7.2)	2.25 (0.5 to 4.0)
Fibrinogen (mg/dL), adjusted	0.46 (1.0 to 1.9)	1.96 (0.6 to 3.3)	0.60 (−1.9 to 3.1)
Factor VIII (IU/dL), crude	3.15 (2.2 to 4.1)	0.36 (−0.6 to 1.3)	1.39 (0.3 to 2.4)
Factor VIII (IU/dL), adjusted	2.34 (1.3 to 3.3)	−0.93 (−1.9 to 0.1)	0.38 (−1.2 to 1.9)
Factor IX (IU/dL), crude	1.09 (0.5 to 1.7)	0.79 (0.2 to 1.4)	1.89 (1.3 to 2,5)
Factor IX (IU/dL), adjusted	1.01 (0.4, to 1.6)	0.32 (−0.2 to 0.8)	1.96 (0.7 to 3.2)
Factor XI (IU/dL), crude	0.77 (0.13 to 1.4)	1.02 (0.5 to 1.6)	0.68 (0.06 to 1.3)
Factor XI (IU/dL), adjusted	0.49 (0.1 to 1.1)	−0.05 (−0.6 to 0.5)	1.05 (0.1 to 2.0)

ASY 16.3 | TFPI in Human Atherosclerotic Plaques

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Background: The coagulation inhibitor tissue factor pathway inhibitor (TFPI) is present in human atherosclerotic plaques and the levels are elevated compared to in healthy arteries. TFPI induces a protective effect in atherosclerotic mice models, but it is not known which isoform of TFPI is important and what regulates TFPI levels in the atherosclerotic plaque.

Aims: To uncover the expression pattern and regulation of TFPI and its isoforms (α and β) in relation to atherosclerotic disease in humans.

Methods: Human carotid endarterectomies were collected and processed, while human monocyte-derived macrophages were isolated from buffy coats of blood donors, differentiated into M1 and M2 subtypes with GM-/M-CSF and LPS + INFg/IL-4, respectively, and subsequently stimulated with cholesterol crystals (CCs). mRNA and protein levels of TFPI isoforms were quantified using qRT-PCR and ELISA, respectively, while protein detection in the plaques were performed with IHC.

Results: TFPI mRNA levels were significantly increased in human atherosclerotic plaques compared to healthy vessels, and both isoforms were elevated. However, Ct values indicated that TFPIα was the prominent isoform in the plaques. TFPI mRNA levels correlated significantly with the macrophage markers CD68, CCR7, CD163, and ADRP, but not with the B cell markers CD45 or CD3 g. TFPI protein co-localized with CD68, CD80 and CD163 in plaque sections. Interestingly, TFPI mRNA levels were elevated in M2 macrophages compared to the M1 phenotype and further increased when the M2 macrophages were incubated with the atherogenic agent CCs. This increase in TFPI mRNA levels was dependent on ER stress induction in the M2 macrophages.

Conclusions: Both isoforms of TFPI were increased in human plaques with TFPIα as the major isoform. Alternatively activated M2 macrophages may contribute to the elevated levels of TFPI observed in atherosclerotic plaques, indicating a novel protective mechanism of these cells in atherosclerosis.

ASY 16.4 | Protease-activated Receptor 2 Deficiency Attenuates the Formation of Atherosclerosis

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Background: Protease-activated receptor 2 (PAR-2)-dependent signaling results in augmented inflammation and has been implicated in the pathogenesis of several autoimmune conditions. While PAR-2 protein is present in coronary atherosclerotic lesions, the relevance of this finding has not been investigated in experimental models.

Aims: The objective of this study was to determine the effects of PAR-2 on the development of atherosclerosis.

Methods: Male low density lipoprotein receptor deficient (*Ldlr*$^{-/-}$) mice (8-12 weeks old) that were on a *Par-2*$^{+/+}$ or *Par-2*$^{-/-}$ background were fed a fat and cholesterol-enriched diet for 12 (n = 10 each group) or 24 weeks (n = 5 each group). Bone marrow transplantations were utilized to examine non-marrow or marrow-derived effects (n = 15 for each of 4 chimeric groups). For mechanistic considerations, PAR-2 agonist peptide or scrambled peptide were utilized in oxidized LDL (oxLDL) loaded peritoneal macrophages or water-soluble cholesterol treated vascular smooth muscle cells (VSMCs).

Results: Expression of PAR-2 is increased in human coronary artery (21 fold) and mouse aortic arch (16 fold) atheroma versus control coronary and aortic arch arteries, respectively (P = 0.001). PAR-2 deficiency attenuated atherosclerosis in the aortic sinus and aortic root with no effects on total plasma cholesterol concentrations or lipoprotein distributions after 12 (P < 0.05) or 24 (P < 0.05) weeks.

These reductions were attributable to both hematopoietic and non-hematopoietic-derived PAR-2. Activation of PAR-2 augmented oxLDL-induced foam cell formation and apoptosis in conjunction with decreased expression of cholesterol transporters. Further, PAR-2 activation of VSMCs augments the transition to a macrophage-like state. **Conclusions**: Our results that indicate PAR-2 deficiency significantly attenuates the initiation (12 weeks) and reduces the progression (24 weeks) of atherosclerosis potentially via regulation of both lipid efflux from macrophages and the phenotypic modulation of VSMCs.

ASY 31.1 | Targeted Termination of Plasminogen Activation after Tissue Plasminogen Activator Therapy Reduces Ischemic Brain Injury and Hemorrhage in Experimental Ischemic Stroke

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Background: In ischemic stroke, early treatment with recombinant tissue plasminogen activator (r-tPA) is beneficial. However, r-tPA blood levels remain high for hours and studies indicate that after prolonged ischemia, r-tPA increases brain hemorrhage, early mortality and neurotoxicity.
Aims: To determine whether termination of prolonged r-tPA action is beneficial in experimental ischemic stroke.
Methods: Experimental ischemic stroke was induced by a middle cerebral artery embolus in anesthetized mice by a blinded investigator. After 2.5 hours of ischemia, mice were treated by r-tPA followed, in a randomized manner, by placebo or a monoclonal antibody antidote of r-tPA. Ischemic brain injury was assessed in a blinded fashion after 6 h.
Results: The r-tPA antidote bound specifically with sub-nanomolar affinity to r-tPA. The r-tPA antidote inhibited plasminogen activation and human clot dissolution in a dose-related fashion with greater specificity and potency than plasminogen activator inhibitor-1. In experimental ischemic stroke, when given immediately after the r-tPA bolus, during the r-tPA infusion, the r-tPA antidote caused a 4-fold reduction in hemorrhage ($p < 0.05$) and a 2.9-fold reduction in brain infarction ($p < 0.01$) compared with placebo. Treatment with the r-tPA antidote 30 min. after the r-tPA bolus also significantly reduced brain bleeding >4-fold ($p < 0.05$) and brain infarction 2.2-fold ($p < 0.01$) by comparison to placebo.
Conclusions: A specific monoclonal antibody antidote specifically blocks r-tPA plasminogen activation and clot dissolution in vitro. When administered at various times after r-tPA bolus therapy, the antidote markedly reduces ischemic brain infarction and hemorrhage. This suggests that early termination of r-tPA activity may reduce ischemic brain injury and hemorrhage in patients given r-tPA after prolonged brain ischemia.

ASY 31.2 | A Novel and Effective Fibrinolytic Approach in a Murine Model of Diabetes-associated Ischemic Stroke

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Background: Stroke is clinically characterized by a rapid loss of brain function due to the disruption of the blood flow to the brain. Diabetes influences the prognosis of stroke patients. The thrombolytic agent tissue-type plasminogen activator (tPA) is the only approved pharmacological treatment for ischemic stroke (IS). Nevertheless, tPA has been associated with adverse effects, such as cerebral haemorrhage and neurotoxicity.
Aims: We previously showed the fibrinolytic effects of matrix metalloproteinase-10 (MMP10) in an IS animal model (Orbe et al., Circulation 2011). Considering comorbidity factors, we wanted to study whether MMP10 treatment would reduce brain damage after IS in a murine diabetes model, thus avoiding the adverse effects of tPA.
Methods: Type I diabetes mellitus was induced in mice by a single dose of streptozotocin (180 mg/Kg). Two weeks later, IS was performed by injection of thrombin into the middle cerebral artery and the effect of recombinant MMP10 (6.5 μg/Kg), tPA (10 mg/Kg) or tPA/MMP10 on brain damage and functional outcome were analysed. Motor activity was assessed before and after stroke using the open field test.
Results: Our results showed that MMP10 treatment alone or combined with tPA significantly reduced the lesion volume 24 h after stroke ($p < 0.05$). At day 3 all treatments reduced infarct volume when compared to saline ($p < 0.05$), although maximal reduction was obtained with t-PA/MMP10 combination ($p < 0.05$, when compared to tPA). Moreover, the combination therapy demonstrated a decrease of neuronal degeneration 24 h after stroke ($p < 0.05$). Analysis of motor activity showed a deteriorated function associated with blood glucose levels at 24 h. None of the treatments significantly improved the impaired motor activity induced by diabetes-IS.
Conclusions: Our data suggest that MMP10 or MMP10/tPA treatment reduces brain damage more effectively than tPA after diabetes-associated IS, identifying MMP10 as a novel and effective thrombolytic agent for IS.

ASY 31.3 | Late Administration of a new Antifibrinolytic Strategy (CM352) Reduces Lesion Size and Improves Functional Outcome in a Collagenase-Induced Rat Model of Intracerebral Hemorrhage

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Background: Intracerebral hemorrhage (ICH) is an acute neurological disorder, comprising approximately 10-20% of all stroke cases, with high mortality and no proven treatment. We have previously demonstrated the efficiency of CM352, a short half-life (1.4 h) antifibrinolytic agent and matrix metalloproteinase (MMP) inhibitor, to reduce early (1 h) hematoma expansion and improve functional outcome in a rat model of collagenase-induced ICH (ISTH 2015, OR116).

Aims: To study in a collagenase-induced ICH model whether late (3 h) CM352 administration still has a beneficial effect on lesion size and functional outcome.

Methods: ICH was induced by striatal injection of collagenase. 3 h later, rats received an intravenous injection of saline (n = 6) or CM352 (1 mg/kg, n = 6). Hematoma (1 h, 3 h, 5 h and 24 h) and lesion (14d) volumes were quantified on T2-weighted magnetic resonance images (MRI). Neurologic deficit was analyzed by Bederson and Wahl scales (24 h and 14d). Motor impairment was assessed using the cylinder test and quantified by laterality index. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured to assess the effect of CM352 on hemostasis.

Results: Intracerebral hematoma was similar in all animals at 1 h (mm^3: 15.3 ± 5.2 vs 16.0 ± 1.9) and 3 h (mm^3: 38.8 ± 9.9 vs 39.8 ± 6.7) post-ICH. Late (3 h) CM352 administration reduced hematoma at 5 h (mm^3: 59.3 ± 14.2 vs 34.4 ± 4.5; $p < 0.01$) and 24 h (mm^3: 54.9 ± 14.2 vs 34.5 ± 9.5; $p < 0.05$), and lesion size up to day 14 (mm^3: 9.4 ± 2.3 vs 5.1 ± 2.4; $p < 0.05$). CM352 drastically reduced sensorimotor impairment after ICH in rats at 24 h ($p < 0.01$) and 14d ($p < 0.01$). Interestingly, it also attenuated neurological deficit at 24 h ($p < 0.01$) and 14d ($p < 0.05$). No differences were found in PT or aPTT at any time point.

Conclusions: Late CM-352 administration reduces lesion size, associated to better functional and neurological recovery, in a rat model of collagenase-induced ICH, without interfering normal hemostatic function.

ASY 31.4 | Restoring Cardiac Function after Myocardial Infarction by Targeting CD39 to Activated Platelets

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Background: CD39 is a cell membrane NTPase with anti-inflammatory and anti-platelet effects. However, its clinical use is limited by its bleeding side effect.

Aims: We aimed to harness CD39's therapeutic potential while avoiding hemostatic problems. We designed a fusion protein (Targ-CD39) consisting of the extracellular domain of CD39 and a single-chain antibody that specifically binds to the activated platelet receptor GPIIb/IIIa. Through this enrichment at activated platelets, the required systemic dose is below the dose impairing hemostasis.

Methods: Using an ischemia/reperfusion (IR) mouse model (LAD ligation for 1 hour) we achieved remarkable protection of the reperfused tissue with Targ-CD39 (0.4 µg/g, i.v. post reperfusion) compared to Non-targ-CD39 (mutated, non-binding version of Targ-CD39) and PBS. This was measured using ultrasound, histological analysis and cytokine assays.

Results: Targ-CD39 restored ejection fraction as well as fractional shortening at week 4 to a non-significant difference to pre IR injury (62.6 ± 3.8 baseline vs. 54.5 ± 5.3% EF for week 4 Targ-CD39) but significant differences to PBS and Non-Targ CD39 at week 4 (54.5 ± 5.3 Targ-CD39 vs. 38.5 ± 11.5 PBS and 40.9 ± 10.5% EF Non-Targ CD39, $p < 0.01$).

Employing advanced, clinically relevant methods of ultrasound analysis, we observed that strain showed infarct-typical changes of myocardial deformation in controls, but not in Targ-CD39 treated mice (Targ-CD39 of 22.1 ± 4.1 vs. PBS of 3.4 ± 2.5 and Non-Targ-CD39 of 7.9 ± 6.8% $p < 0.05$). Histological assessment confirmed strong reduction of infarct size ($p < 0.01$) and increase in neovascularization ($p < 0.001$). Furthermore, attenuation of post-ischemic inflammation was seen in cytokine profiling. 32 inflammatory cytokines and receptors were down-regulated more than twofold in the Targ-CD39 group, as compared to the Non-targ-CD39 control group. No marker was up-regulated.

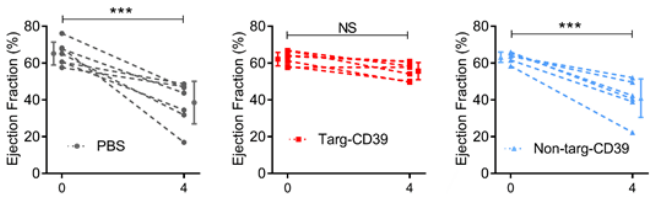

FIGURE 1 Treatment with Targ-CD39 restores ejection fraction 4 weeks post myocardial infarction

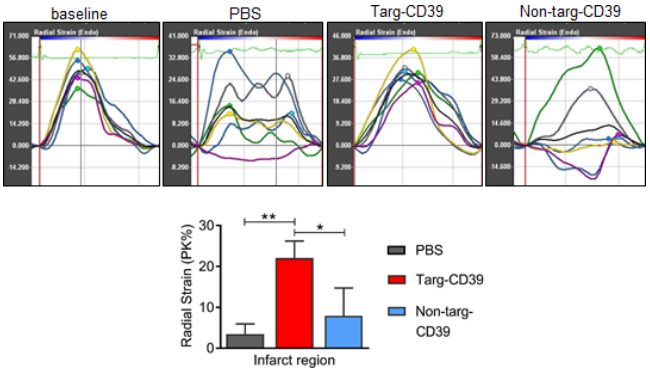

FIGURE 2 Radial strain curves showed infarct-typical changes of myocardial deformation in controls, but not in Targ-CD39 treated mice

Conclusions: In conclusion, we demonstrate that Targ-CD39 holds promise for the prevention of IR injury in reperfused myocardial infarction.

COAGULANT & ANTICOAGULANT MECHANISMS

ASY 02.1 | Human-snake Chimeras of Cofactor V Reveal Allostery between the A- and C-domains

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Background: The venom of the snake Pseudonaja textilis contains a highly stable homolog of cofactor V, ptFV. The ptFV C-domains share ~60% sequence identity with those of human FV, but have lost the ability to bind anionic membranes despite retaining a number of conserved lipid-binding motives.

Aims: Investigate whether loss of lipid association by the ptFV C-domains relates to enhanced ptFV cofactor stability.

Methods: The C-domains of constitutively active B-domainless human FV (hFV) and ptFV were exchanged and the chimeric variants hFV-ptC and ptFV-hC were expressed, purified and characterized.

Results: Thermal analysis of the FV variants employing differential scanning calorimetry (DSC) revealed a single denaturation event for hFV at 54°C. In contrast, ptFV displayed distinct denaturation events at 60°C and 70°C. Swapping of the C-domains resulted in a more stable human cofactor as hFV-ptC exhibited a denaturation peak at 58°C. No DSC profile could be obtained for ptFV-hC. Thermal FV cofactor activity decay rate constants assessed at 52°C confirmed the high stability of ptFV ($k < 0.001$) relative to hFV (k 0.39 ± 0.04). The C-domain exchange adversely affected the ptFV stability (ptFV-hC, k 0.73 ± 0.25), yet improved that of hFV (hFV-ptC, k 0.13 ± 0.04). Examination of phospholipid binding on a Biacore L1-chip coated with vesicles containing 25-5% PS anionic content showed no binding of ptFV (>1M) compared to hFV (0.5 μM). Weak binding constants were observed for hFV-ptC (≥11 μM) and ptFV-hC (≥3 μM). A purified prothrombinase assay confirmed the weak lipid-binding by the C-domains of ptFV as full cofactor activity of hFV-ptC required 10-100-fold higher concentrations of 25-5% PS vesicles compared to hFV.

Conclusions: Collectively, our results implicate allosteric crosstalk between the C- and A-domains of FV, since when linked to the hFV A-domains, the ptFV C-domains were able to bind PS-containing phospholipids and, in part, improved cofactor stability.

ASY 02.2 | Uncovering the Unique Functional APC-resistant Modifications of Snake Venom FV

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Background: Coagulation factor Va (FVa) is proteolytically inactivated by activated protein C (APC), which is key to downregulate the procoagulant response. APC cleaves FVa at several positions throughout the A2-domain, with Arg306 and Arg506 as major cleavage sites. We previously reported the functional resistance of snake venom *P.textilis* factor V (ptFV) to human APC, despite proteolysis within the A2-domain. Sequence analysis revealed the absence of the 306 site and surrounding residues in ptFV.

Aims: Assess the role of the ptFV sequence that replaces the region surrounding cleavage site Arg306.

Methods: The non-conserved ptFV region (GNPDTLT) was exchanged for the human Arg306 region (PKKTRNL), thereby generating ptFV-h306 and hFV-pt306.

Results: While human APC did not proteolyze ptFV at the 306 position, introduction of the human 306 region resulted in 306 cleavage of ptFV-h306. Conversely, this cleavage was absent in hFV-pt306. Full proteolysis of human FV (500 nM) was achieved following treatment with 10 nM APC, while a 75-fold higher APC concentration (750 nM) was required to obtain fully proteolyzed ptFV-h306, similar to ptFV. Functional analysis in a purified prothrombinase system revealed no significant differences in APC-induced inactivation, with both human FV molecules being fully inactivated following a 5 min incubation with APC. Surprisingly, both ptFV and ptFV-h306 maintained full activity for at least 15 min, despite extensive APC-mediated proteolysis.

Conclusions: These findings indicate that, conversely to human FV, APC-dependent cleavage of ptFV at Arg306 does not abrogate FVa cofactor function. This may suggest that even following APC cleavage at the positions homologous to human 306 and 506, the functional integrity of the ptFV A2-domain is stabilized such that it is able to form productive interactions. As such, ptFV provides a biological model to further study structure-function requirements that may ultimately contribute to a FV molecule with enhanced procoagulant cofactor activity.

ASY 02.3 | X-ray Crystallographic Studies of the Factor VIII C2 Domain in Complex with O-phospho-L-serine Indicate that Arg 2320 Contributes to the Phospholipid Membrane Binding Site

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Background: Factor VIII is a glycoprotein cofactor that is essential for proper regulation of blood coagulation. Genetic deficiencies in factor VIII cause hemophilia A, which affects 1 in 5,000 males worldwide. The C-terminal "C2" domain of factor VIII is essential for proper interactions with the membrane surface of activated platelets and is a major epitope that is recognized by inhibitory antibodies. Previous X-ray crystallographic studies of the factor VIII C2 domain in complex with classical anti-C2 antibodies suggested that the face of the C2 domain harboring Arg 2320 contributes to phospholipid membrane binding.
Aims: In this study, we aim to test our working model of factor VIII membrane association that involves Arg 2320. Understanding the structural mechanism by which factor VIII associates with activated platelet surfaces to form the intrinsic tenase complex with blood coagulation factor IXa will further illustrate their roles in hemostasis.
Methods: Using a combination of X-ray crystallography, site-directed mutagenesis and phospholipid binding assays, we have soaked crystals of the factor VIII C2 domain with the soluble headgroup of phosphatidylserine, o-phospho-L-serine (OPS). Hemophilia A-associated point mutants at Arg 2320 were also generated for membrane binding analysis.
Results: A 1.4 Å X-ray crystal structure of the porcine factor VIII C2 domain soaked with OPS was determined. Continuous positive density features were observed that were not attributed to the model within a binding cleft centered on Arg 2320. Subsequent to these structural findings, mutations to Arg 2320 (R2320S/T) indicate a dramatic loss in activity while the R2320M mutant is insoluble upon overexpression.
Conclusions: Taken together, these results strongly suggest that blood coagulation factor VIII binds to activated platelet surfaces through phospholipid membrane interactions with the binding site containing Arg 2320, which is in contrast to previously suggested hypotheses.

ASY 02.4 | Full Length Activated Plasma Prekallikrein Crystal Structure reveals Autoactivation Model Involving the Apple Domains

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Background: Plasma Prekallikrein (PPK) is a serine protease zymogen that circulates in plasma bound to high molecular weight kininogen (HK). It is an important component both in kallikrein-kinin system composed of PPK, plasma kallikrein (PK), HK; and in the contact system consisting of PPK, Factor XII (FXII) and HK. PPK is activated by Factor XIIa to become PK that cleaves HK to liberate bradykinin (BK), an inflammatory peptide. PK reciprocally activates the FXII zymogen to amplify generation of FXIIa which then leads to activation of FXI, which initiates the intrinsic pathway of coagulation. Animal models have shown that deficiencies of PPK had a defect in thrombus formation while maintaining hemostasis which raises the possibility that targeting PK may serve as a strategy to treat pathological thrombosis with less risk of side effects than currently used anti-coagulants.
Aims: To solve plasma kallikrein full length crystal structure and compare these with previously determined FXI.
Methods: SPR was used to obtain affinity binding constants for protein interactions; and protein crystallography was used to determine PK structures.
Results: We here report the first crystal structure of full-length plasma kallikrein revealing the 3D arrangement of the four apple domains at the N terminus and the catalytic light chain. A comparison with the previously determined FXI zymogen structure reveals the activated PK protease shifts significantly and we speculate this exposes a substrate exosite. We also solved a series of crystal structures of the heavy chain of PK in complexes with peptides derived from the cofactor HK revealing the binding on the apple domains. The binding constants for PPK bound to HK peptides were in the low nM range, our data showed that HK is a dimer bound to a PPK monomer.
Conclusions: The plasma kallikrein structure reveals the first model for autoactivation of this protease and provides a scaffold on developing new inhibitors for kallikren-kinin system and Contact activation.

ASY 21.1 | Functional Characterization of Three Novel Activated Protein C (APC) Binding Aptamers

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Background: Activated protein C (APC) is a multifunctional serine protease that controls blood coagulation and exhibits anti-inflammatory and cytoprotective functions involving different exosite structures. We have selected three aptamers (NB1, NB2 and NB3) against APC using systematic evolution of ligands by exponential enrichment (SELEX).
Aims: To characterize the binding site of NB1-3 within the APC molecule and to assess the impact of the NB1-3 aptamers on APC-substrate interactions.
Methods: The functional properties of the APC aptamers were investigated through analysis of their impact on (I) the amidolytic activity of APC using different peptide substrates, (II) APC-catalyzed FVa/FVIIIa inactivation in purified enzyme systems, (III) the anticoagulant effects of APC in the plasma matrix using calibrated automated thrombogram

(CAT), and (IV) plasma protein C-inhibitor (PCI)-mediated APC inactivation using an enzyme-capture assay.

Results: All 3 aptamers inhibit the anticoagulant functions of APC with IC50-values of 5.9 and 8.3 nM for FVa and FVIIIa inactivation, respectively, while hydrolysis rates of APC peptide substrates were only marginally influenced. Within the plasma matrix, NB3 proved to be the most effective inhibitor of anticoagulant functions of APC (IC50 = 46 nM). Most interestingly, binding of NB1 and NB2 enhanced the inactivation of APC by PCI while NB3 protected APC from PCI-induced inactivation.

Conclusions: The newly selected APC-binding aptamers share a common binding site within the basic exosite of APC but differentially influence the functional properties of APC. While all three aptamers selectively block the anticoagulant functions of APC, NB-3 only protects APC from early inactivation by PCI. This fetures qualifies NB-3 to an interesting drug candidate in clinical situations such as septicemia where selective inhibition of the anticoagulant functions without inhibiting the cytoprotective functions of APC is of particular interest.

ASY 21.2 | Inhibition of Activated Protein C Aspartyl Beta-hydroxylation Restricts Anticoagulant Function but Enhances Cytoprotective Signaling Activity

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Background: The anticoagulant enzyme activated protein C (APC) also triggers cytoprotective cell signaling via protease-activated receptor 1 (PAR1) proteolysis. These properties have prompted the development of non-anticoagulant APC mutants for clinical use, and new strategies to selectively boost APC cytoprotective signaling activity would be therapeutically advantageous.

Aims: APC is subject to post-translational aspartyl β-hydroxylation, but the functional role of this modification is poorly understood. To evaluate this, an APC mutant resistant to β-hydroxylation (D71E) was generated and characterised.

Methods: APC-D71E anticoagulant activity was measured using calibrated automated thrombography and activated factor V (FVa) degradation assays. APC-D71E cell signaling activity was assessed using reporter assays of PAR1 proteolysis and endothelial cell (EC) barrier integrity.

Results: APC-D71E was unable to restrict plasma thrombin generation or mediate protein S-independent FVa degradation, implying a new role for aspartyl β-hydroxylation in enabling APC anticoagulant substrate recognition and degradation. In contrast, APC-D71E exhibited significantly enhanced PAR1 proteolysis, and triggered downstream cytoprotective signaling on ECs 5-fold more effectively than wild type APC. Combining defective aspartyl β-hydroxylation with an amino acid substitution (N329Q) previously shown to enhance PAR1 proteolysis yielded an APC mutant (APC-D71E/N329Q) with even greater signaling potency. Remarkably, APC-D71E/N329Q exhibited no anticoagulant activity, but mediated rapid PAR1 proteolysis and reduced thrombin-induced EC barrier disruption ~100-fold more effectively than wild type APC.

Conclusions: Collectively, these data describe novel gain-of-function APC mutants with selectively amplified cytoprotective properties, and identify an intriguing new role for aspartyl β-hydroxylation ablation in preventing APC anticoagulant function, whilst simultaneously boosting cytoprotective signaling activity.

ASY 21.3 | TFPIα Protects FV-short from Rapid Inactivation by Activated Protein C

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Background: Factor V (FV)-short, an alternatively spliced B-domainless variant (missing residues 756-1458) of the coagulation *FV gene* is the molecular defect in the FV East Texas bleeding disorder. In this case, reduced thrombin generation potential is partly explained by increased levels of TFPIα and its tight association via its C-terminal basic region (BR) to the available acidic region 2 (AR2) on FV-short.

Aims: The present study investigates the mechanism by which the TFPIα-FV-short complex regulates the susceptibility of this cofactor-like molecule to proteolytic inactivation by the anticoagulant, activated protein C (APC).

Methods: Using a 56-amino acid C-terminal TFPIα BR fragment or full length TFPIα, we monitored the discontinuous proteolysis and inactivation of FV-short in the presence of APC and membranes. Cleavage products were visualized using monoclonal antibodies against the heavy chain of FV.

Results: APC cleaves both FVa and FV-short with similar profiles. However, densitometric analyses showed that both TFPIα and the BR fragment significantly inhibited proteolysis of FV-short with >80% of the starting material remaining after 15 min of incubation with APC compared to the controls. Using FV-short and other B-domainless variants (FV-DT-R306Q, and R506Q) we found that TFPIα and the BR fragment blocked cleavage at R506 suggesting the BR somehow obscures the ability of APC to engage FV-short at this site. TFPIα and the BR fragment had no effect at R306 or R679. Consistent with data showing a high affinity interaction between TFPIα-BR and AR2 of FV-short, TFPIα or the BR has no impact on FVa (lacks AR2) inactivation by APC. Together these data show the FV-short-TFPIα complex is resistant to APC proteolysis.

Conclusions: The findings are consistent with data from our laboratory showing that the internal BR of FV has a major impact on APC inactivation of the procoafactor. Thus, not only does TFPIα block the procoagulant function of FV-short it also protects it from the protein C pathway.

ASY 21.4 | Factor V-Short and Protein S as Synergistic TFPIα-cofactors

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Background: In the East Texas bleeding disorder a *F5* gene mutation activates a splice donor site resulting in the in frame deletion of 702 residues. The truncated B domain of FV-Short exposes a high affinity TFPIα binding site - FV-Short and TFPIα forming a circulating complex. Due to the increase in FV-Short in affected individuals, the TFPIα concentration increases 10-fold, which results in a bleeding phenotype. FV-Short is also present in normal plasma at low levels and circulates in complex with TFPIα.

Aims: To elucidate whether the complex formation between FV-Short and TFPIα affects the function of TFPIα as FXa inhibitor and to test whether the TFPIα-cofactor activity of protein S is affected by the presence of FV-Short.

Methods: Using purified components (FXa, TFPIα, FV-Short, protein S and negatively charged phospholipid vesicles), the TFPIα mediated inhibition of FXa was monitored with S2765.

Results: FV-Short has intrinsically weak TFPIα-cofactor activity but the presence of a low protein S concentration, which in itself is insufficient to stimulate TFPIα, results in rapid inactivation of the FXa. In the absence of FV-Short, relatively high concentrations of protein S (>25 nM) are required to achieve full TFPIα-mediated inactivation of FXa. In contrast, in the presence of FV-Short full inhibition of FXa is observed at 10-fold lower protein S. Activation of FV-Short by thrombin results in the loss of the TFPIα-cofactor activity.

Conclusions: We demonstrate that FV-Short and protein S are synergistic cofactors to TFPIα in the regulation of FXa activity. Our results suggest the formation of an efficient FXa-inhibitory complex between FV-Short, TFPIα and protein S on the surface of negatively charged phospholipids, which presumably is important for the inhibition of initially generated FXa. This is the second reported anticoagulant activity associated with FV, which emphasizes the central importance of FV as a regulator of blood coagulation.

COAGULATION SIGNALING & IMMUNITY

ASY 03.1 | Platelet Necrosis Induces Neutrophil Macro-aggregation and Pulmonary Thrombosis Following Gut Ischemia

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Background: Gut ischemia is common in critically ill patients, promoting thrombosis and inflammation in distant organs, especially the lung, leading to the development of acute respiratory distress syndrome (ARDS). The mechanisms linking gut ischemia to pulmonary thrombosis remain ill defined.

Aims: Investigate how gut ischemia leads to pulmonary thrombosis.

Methods: A murine model of gut ischemia reperfusion (IR) injury and intravital microscopies were used to monitor the interactions between platelets and neutrophils in the gut and lung vasculature.

Results: We have demonstrated that gut ischemia in the mouse induces a distinct pulmonary thrombotic mechanism triggered by dying platelets and neutrophil macro-aggregates. These neutrophil macro-aggregates lead to occlusion of pulmonary arteries and veins. Similar pulmonary neutrophil macro-aggregates were also identified in lung specimens from ARDS patients, with 20-66% of the vessels containing aggregates, but not in the lungs from patients with acute pulmonary oedema or emphysema. Intravital microscopy during gut IR injury and in vitro neutrophil perfusion studies revealed fragmentation of dying platelet membranes by rolling neutrophils, with the extracted platelet fragments bridging adjacent neutrophils to facilitate neutrophil macro-aggregation. This process is not inhibited by conventional antiplatelet agents, however, platelet specific-deletion of cyclophilin D, a mitochondrial mediator of platelet necrosis, maintained platelet membrane stability, prevented neutrophil macro-aggregation and pulmonary thrombosis, and improved respiratory function and survival post gut IR injury.

Conclusions: We have identified a distinct neutrophil-rich thrombosis mechanism following gut IR injury. This unique thrombotic response is triggered by membrane fragmentation of dying platelets by rolling neutrophils, and links gut ischemia to pulmonary thrombosis. Targeting platelet necrosis may represent a new approach to reduce pulmonary thrombosis in critically ill patients.

ASY 03.2 | Going against the Flow: How Staphylococcus Aureus and Staphylococcus Lugdunensis Adhere to the Heart Valves and Initiate Endocarditis by Binding to von Willebrand Factor

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Background: Both *S. aureus* and *S. lugdunensis* are feared and frequent causes of endocarditis, but the real reason why remains elusive. Before infecting the hearth valves, bacteria must first adhere to the valve endothelium. However, their adhesion is hampered by the high shear stress of blood flowing though the valves.

Aims: Investigate how these bacteria adhere under shear stress and if similarly to platelets they achieve this by binding to von Willebrand factor (VWF).

Methods: We studied the adhesion of *S. aureus* and *S. lugdunensis* to VWF and endothelial cells in a flow chamber. With a mesenteric microvascular perfusion mouse model we examined the interaction of fluorescent bacteria with the vessel wall in real time in vivo. To measure bacterial adhesion to the heart valves we developed a new unique endocarditis mouse model. For this we intravenously injected fluorescent bacteria and activated the valve endothelium by locally infusing histamine through a catheter placed in the carotid artery. With confocal microscopy we measured the adhesion of bacteria to the valves. We used VWF knockout mice to evaluate the role of VWF.

Results: Both *S. aureus* and *S. lugdunensis* bind to VWF under shear stress, in contrast to other staphylococci that seldom cause endocarditis. This enabled the bacteria to adhere to activated endothelial cells in vitro and to the vessel wall in vivo. In our new endocarditis mouse model we could show that *S. aureus* and *S. lugdunensis* hardly adhered to resting valve endothelium. However, by locally infusing histamine and inducing endothelial activation we could make the valves more prone to bacterial adhesion and induce endocarditis. In addition, binding of both *S. aureus* and *S. lugdunensis* to heart valves of VWF knockout mice was greatly reduced compared to wild type mice.

Conclusions: Binding to VWF allows *S. aureus* and *S. lugdunensis* to overcome shear stress and infect the hearth valves. This explains why they are so effective in causing endocarditis compared to other staphylococci.

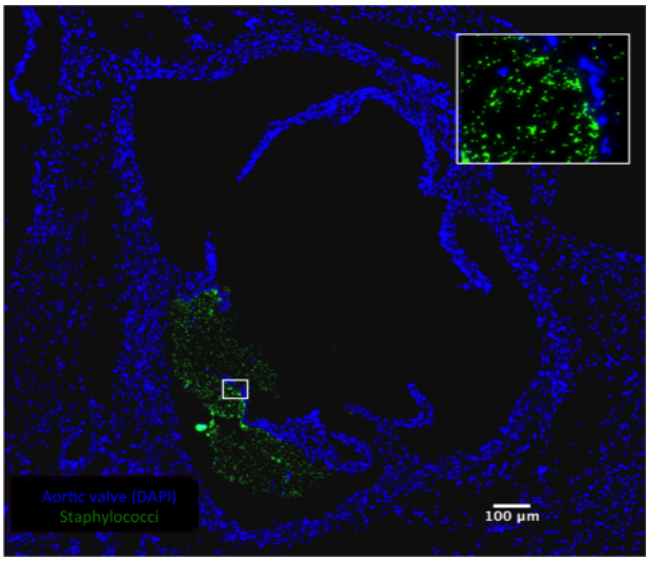

FIGURE 1 Fluorescent bacteria adhering to an aortic valve in a new endocarditis mouse model

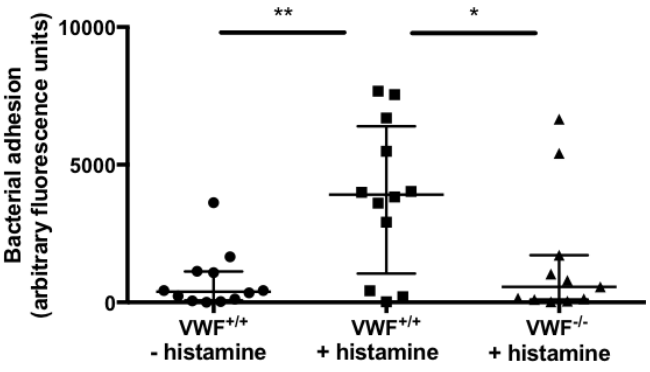

FIGURE 2 Adhesion of S. aureus to the aortic valve after activation with histamine in WT and VWF KO mice

ASY 03.3 | Soluble Short Fractions of β-1,3 Glucans Derived from Candida albicans Act as a Shield for Pathogenic Yeasts and Modulate the Activation of Platelets

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Background: Platelets play a crucial role in hemostasis, thrombosis, and pathogen clearance. Many pathogenic fungi can interact with platelets in circulating blood. The pathogenic fungus *Candida albicans* is the predominant cause of invasive forms of candidiasis. Its cell wall contains β-1,3 glucans that are known to trigger a wide range of host cell activities.

Aims: Clinically, β-glucans are released in the circulation during infection and their detection allows the early diagnosis of an invasive fungal infection, but the role of β-glucans in the modulation of platelet activities and platelet-neutrophil interactions is unknown.

Methods: Platelets pretreated with β-glucan fractions were analyzed in terms of activation, receptor expression, aggregation and adhesion to neutrophils and to *C. albicans*.

Results: Our study shows that β-glucan fractions decrease platelet aggregation, and modulate the coagulation process. The biological activities of these oligoglucosides depend on their degree of polymerization and their concentration. They also affect the regulation of platelet receptors (P-selectin and $\alpha_{IIb}\beta_3$). Interestingly, these oligoglucosides at a low concentration reduce platelet activation, and both platelet-*C. albicans* and platelet-neutrophil interactions, which allow *C. albicans* to be protected from leukocyte activation. Mechanistically, these oligoglucosides block protein kinase-C activation in platelets that affect the regulation of platelet receptors. Bedsides, the pentaglucosides reduced platelet activities through TLR4 mediated TGF-β1 production

and ATP release, and blocking this receptor by an anti-TLR4 antibody abolished the effect of the pentaglucosides on platelets suggesting that TLR4 is involved in the immuno-modulatory effects induced by β-glucans.

Conclusions: Our study offers new insights, showing that these fungal-derived oligoglucosides are not involved exclusively in the protection of *C. albicans* during infection but also in the modulation of platelet activation mediated via TLR4 stimulation.

ASY 03.4 | VWF and ADAMTS-13 Modulate the Outcome of Staphylococcus aureus Sepsis

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Background: The size and functionality of the multimeric von Willebrand factor (VWF) is regulated by its cleaving protease ADAMTS-13. Despite the correlation of VWF and ADAMTS-13 levels with disease severity and outcome in the heterogenous population of sepsis patients, animal models on this topic have yielded conflicting results and have focused mainly on gram-negative or polymicrobial abdominal sepsis.

Aims: To study the role of VWF and ADAMTS-13 in gram-positive monomicrobial *Staphylococcus aureus* (*S. aureus*) sepsis.

Methods: Analysis of blood samples of *S. aureus* bacteremia patients. Animal model for *S. aureus* sepsis, in wildtype, *Adamts13* -/- or *Vwf* -/- mice.

Results: In patients with *S. aureus* bloodstream infection, high VWF levels correlated with inflammatory parameters and inversely with kidney function. Low ADAMTS-13 levels associated with parameters of disease severity and DIC.

In an animal model for severe sepsis, mice deficient in VWF had improved survival. In contrast, *Adamts13* -/- mice showed increased mortality. Immediate clearance of bacteria was enhanced in VWF-deficient mice. The differences in mortality for the studied genotypes were associated with differential loads of organ microthrombi in liver and kidneys.

Conclusions: This is the first study that consistently shows the relation of VWF, ADAMTS-13 and their ratio to disease severity in patients and mice with *S. aureus* sepsis. VWF and its cleaving protease are not only involved in the primary adhesion of *S. aureus* to the vasculature, but also in the development of organ microthrombi containing platelets, neutrophils and bacteria, and thus potentially in end organ failure. Further research investigates whether substitution of the relative ADAMTS-13 deficiency that occurs in sepsis will reduce organ microthrombi and improve outcome in mice with *S. aureus* sepsis.

ASY 25.2 | Inflammatory Activities of rRNA-containing Microparticles from Mast Cells

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Background: Mast cells (MC) are resident tissue cells and well known effectors of allergic or anti-parasitic responses but also first line players in inflammation and innate immunity. Following activation of MC with the rapid release of pre-stored mediators through regulated exocytosis (degranulation), rapid anaphylactic reactions, allergic responses and inflammatory activities are induced, including the recruitment of leukocytes. Likewise, extracellular RNA was found to promote inflammatory and procoagulant activities *in vitro* and *in vivo*.

Aims: Studies were performed to investigate whether MC may release RNA as an additional early inflammatory agonist and self-DAMP.

Methods: Bone marrow-derived MC or the MC-line HMC-1 were stimulated to induce degranulation, which was followed by the release of histamine and b-hexosaminidase activity. Microparticles were isolated by the centrifugation method and assessed by Annexin staining and FACS analysis. Membrane components of microparticles and RNA were stained by fluorescent dyes.

Results: Stimulation of MC by various agonists like ionomycin, compound 48/80, complement anaphylatoxins, or Toll-like receptor agonists induced the release of RNA (predominantly ribosomal RNA), which was largely associated with the microparticle fraction. The liberation of RNA from degranulating MC correlated well with the release of histamine and b-hexosaminidase activity and was abolished by inhibiting the degranulation process. Staining of microparticles with the antibody against rRNA and analysis by immuncytochemistry and electronmicroscopy demonstrated that rRNA was localized inside the microparticles. Following uptake of MC-released microparticles by human umbilical vein endothelial cells, the expression of cytokines like monocyte chemoattractant protein or interleukin-6 increased in a concentration-dependent manner.

Conclusions: Results indicate that MC-derived RNA-containing microparticles play a role in amplifying the initial cytokine storm during the inflammatory response.

ASY 32.1 | Platelets Modulate the Proinflammatory Phenotype of Macrophages via the Interaction of CLEC-2 and Podoplanin

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Background: Platelets interact with macrophages to regulate thrombo-inflammatory and infectious disease. Depletion of platelets

or macrophages reduces survival during mouse models of sepsis. Furthermore, podoplanin-expressing, inflammatory macrophages induce platelet activation through CLEC-2. However the effect of CLEC-2 binding to podoplanin on macrophages is not known.

Aims: In this study, we investigate the role of the platelet ITAM receptor CLEC-2 in modulating macrophage function during sepsis.

Methods: Platelet-specific CLEC-2 deficient mice (*Clec2*$^{fl/fl}$PF4cre), hematopoietic-specific podoplanin-deficient mice (PDPN$^{fl/fl}$VAV1cre) or wild type (WT) mice were subjected to caecal ligation and puncture (CLP). A systemic clinical score, kidney and liver function, cytokine levels and bacterial load were assessed 24h post CLP. Spreading, migration and cytokine secretion were also assessed in bone marrow-derived macrophages (BMDM) using recombinant CLEC-2 or an anti-podoplanin antibody *in vitro*.

Results: We show that CLEC-2 deletion from platelets increases the clinical score and bacterial load in the peritoneum and the kidney, and exacerbates the inflammatory response. This was associated with an increase in the proinflammatory cytokines TNF-α and IL-6 and the anti-inflammatory cytokine IL-10 with increased sepsis-mediated acute kidney injury. A similar increase in bacterial load and sepsis-mediated acute kidney injury was observed in PDPN$^{fl/fl}$VAV1cre mice. *In vitro*, anti-podoplanin antibody induces a switch from an M1 proinflammatory phenotype to an M2 tissue-repair phenotype as measured by the expression of iNOS, CD206 and Egr2 on macrophages.

Conclusions: These results demonstrate that platelet CLEC-2 protects from septic shock possibly via interaction with podoplanin on macrophages and modulating their function. This interaction reprograms the macrophages toward a tissue repair phenotype M2 which is required to limit the inflammatory response.

ASY 32.2 | Activation of Circulating Platelets Leads to Innate-like Delivery of Potent Antiviral Antibodies

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Background: Platelet activation and subsequent thrombus formation is a well-defined process to maintain vascular integrity upon tissue damage. However, platelets are also activated by an array of inflammatory stimuli, including microbial infection. Further, circulating platelets contain intracellular IgG that are released upon activation.

Aims: We aimed to elucidate the physiologic function of platelet-derived IgGs and their effect on viral infections *in vitro* and *in vivo*.

Methods: IgG levels, subclass and light chain distributions in human plasma and shear-induced platelet releasate were quantified by ELISA. For *in vitro* neutralization assays, CMV-infected HUVECs were incubated or perfused with platelets or plasma of anti-CMV IgG seropositive or -negative donors before quantification of CMV titer by IF or qPCR. IgG content of neonatal Fc-receptor (FcRn)-deficient or wildtype murine megakaryocytes (MK) was measured by flow cytometry.

Results: Human platelets can store and release anti-Influenza type A and anti-CMV IgG. Under both static conditions and microvascular shear stress, platelets from anti-CMV IgG seropositive but not seronegative donors potently neutralized *in vitro* CMV-infection. In spite of containing approximately 100-fold less IgG, platelets were equally efficient at neutralization as plasma from the same donor. While platelets are not enriched for a specific subclass of IgG, nor have a specific kappa or lambda light chain preference, anti-CMV antibodies were enriched as compared with the total amount of IgG in platelets. As MKs contain FcRn, sequestration of IgG might occur in the shared microenvironment of MKs and Ig-producing plasma cells. Indeed, MK FcRn is partially responsible for IgG uptake and may thus rescue IgG from degradation after endocytosis.

Conclusions: Our data show that platelets mediate potent IgG-mediated antiviral effects directly at foci of infection, indicating that platelet activation may represent a novel mechanism for focused serological immunity.

ASY 32.3 | Platelets Orchestrate Bleomycin-induced Lung Fibrosis in Circuitry with NETs

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Background: Platelets have been demonstrated to interact with immune cells in the context of neutrophil extracellular trap (NETs) formation. NETs are released during inflammation as an innate immune defense mechanism. NETs not only exert bactericidal activities but are toxic for host cells and thus promote tissue injury. In recent years, several reports have uncovered the important roles of platelet-neutrophil interactions for NET formation not only during thrombosis but also during acute inflammatory diseases. On the other hand, our current knowledge on the contribution of NETs during chronic inflammation, namely fibrotic disorders, remains incomplete.

Aims: The aim of this study was to characterize the immunologic networks involving platelets, neutrophils and NETs during lung fibrosis.

Methods: Transgenic mice (C57BL/6 background) were studied in a model of intra-tracheal bleomycin administration for the induction of lung fibrosis.

Results: Transient depletion of Ly6G+ neutrophils (Ly6G-Cre/iDTR mice) was sufficient to decrease the build-up of extracellular matrix (collagen I,V) and cytokines such as TGFβ1 in lungs. We hypothesized

that platelets may be the major source of TGFβ1 in lung fibrosis. We used platelet-specific genetic deletion (PF4-Cre/TGFb1flox/flox mice) in our subsequent studies: These mice displayed a ≥70% reduction of TGFβ1 in plasma. TGFβ1 was reduced in broncho-alveolar lavage fluids during the acute phase of lung injury. The accumulation of collagen and the severity of lung fibrosis was significantly ameliorated in PF4-Cre/TGFb1flox/flox mice. Furthermore, the release of TGFβ1 by platelets was directly triggered by extracellular histones, which are a major component of NETs. The blockade of extracellular histones by antibodies reduced the release of TGFβ1 and other endpoints during lung fibrosis.
Conclusions: Our data suggest that platelet-neutrophil interactions including NET formation are an important pathologic mechanism during lung fibrosis.

ASY 32.4 | Platelets Interact with Neutrophils and Promote Intravascular Neutrophil Activation in Acute Glomerulonephritis

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Background: Leukocyte recruitment is central to the pathology of several forms of glomerulonephritis. We previously observed that in acute glomerulonephritis, platelets accumulate in glomerular capillaries and facilitate neutrophil recruitment and neutrophil-dependent injury. However, the nature of the platelet/neutrophil interaction and whether platelets contribute to neutrophil activation in glomeruli is unclear.
Aims: Here, we aimed to characterise platelet-neutrophil interactions in the glomerulus and investigate their role in neutrophil recruitment and activation in acute glomerular inflammation.
Methods: Spinning disc confocal intravital microscopy was used to analyse neutrophil and platelet behaviour in the mouse kidney under resting conditions and in an in situ immune complex model of glomerulonephritis.
Results: Under steady-state conditions, circulating platelets constitutively interacted with glomerular endothelial cells and neutrophils in glomerular capillaries. During inflammation, platelets that interacted with neutrophils, but not other intraglomerular cells, were retained in the glomerulus for longer durations, a response that required P-selectin. Removal of platelets or inhibition of P-selectin diminished neutrophil retention in the glomerulus and reduced neutrophil oxidant generation. To address the role of platelet activation in promoting these neutrophil responses, we inhibited the ADP/P2Y12 and thromboxane/TP receptor pathways demonstrating that neutrophil ROS production, but not recruitment, was dependent on signalling via these pathways. In contrast, inhibition of platelet activating factor only inhibited neutrophil recruitment.
Conclusions: These findings demonstrate that circulating platelets constitutively interact with neutrophils in glomerular capillaries. During inflammation, these interactions are associated with neutrophil retention and activation. Together, these data show a previously undescribed role for platelets in neutrophil activation in the acutely-inflamed glomerulus.

ASY 34.1 | Tissue Factor-PAR2 Signaling Promotes Cutaneous Inflammation in Mice

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Background: Tissue factor plays a crucial role in hemostasis and thrombosis, but its distinct function in supporting protease-activated receptor-2 (PAR2) signaling in the innate and adaptive immune system is poorly understood. PAR2 is activated by a broad array of serine proteases including the TF ligands activated coagulation factors VII (FVIIa) and X (FXa).
Aims: We here focus on TF-PAR2 signaling in cutaneous inflammation of relevance for allergic contact dermatitis in humans.
Methods: We studied contact hypersensitivity (CHS) in a murine model by inhibiting TF with an anti-TF antibody in C57BL/6 wildtype mice, preventing proteolytic activation through PAR2 mutation to abolish all (R38E) or specifically FXa (G37I) signaling, and conditionally deleting PAR2 in $CD11c^{+}$ dendritic cells (DC) and myeloid cells ($CD11c\text{-}cre^{+/-}/PAR2^{flox/flox}$ and $LysMcre^{+/-}/PAR2^{flox/flox}$, respectively). The cutaneous inflammatory reaction was assessed by the ear swelling and analysis of the cutaneous immune cell infiltrate with histology and flow cytometry. The hapten-specific Tc1-mediated T cell response was measured after hapten-specific restimulation *in vitro*, as shown by T cell proliferation and Tc1 cytokine production.
Results: TF inhibition and PAR2 mutation significantly reduce the 2,4,6-trinitrochlorobenzene (TNCB)-induced CHS reaction. In particular, complete insensitivity to proteolytic PAR2 activation (R38E) results in a stronger CHS reduction than the exclusive resistance to the proteolytic effect of FXa (G37I). Interestingly, $CD11c^{+}$ DC derived PAR2 does not influence the allergic inflammatory reaction, whereas PAR2 signaling in myeloid cells enhances the early effector phase of CHS.
Conclusions: TF-PAR2 signaling is a key mediator of inflammation in CHS and may provide novel targets in the topical and systemic treatment of cutaneous inflammatory diseases.

ASY 34.2 | Protease Activated Receptor 2 Cleavage by Coagulation Factor VIIa Contributes to Lung Inflammation

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Background: Coagulation activation is frequently observed during respiratory viral infections generating double-stranded RNA intermediates that act as toll-like receptor (TLR) 3 ligands. In addition to their roles in coagulation, proteases induce intracellular signaling by

cleavage of cell surface protease-activated receptors (PAR). PAR2 can be activated by both tissue factor (TF)-FVIIa and the ternary TF-FVIIa-FXa complex. To better understand roles of coagulation proteases in infection, we have generated PAR2 mutant mice with selective resistance to activation by coagulation proteases.

Aims: To define the role of PAR2 activating coagulation factors in the recruitment of inflammatory cells to the lung after challenge with the TLR3 agonist poly(I:C).

Methods: Adult mice received three doses of nasal administration of poly(I:C) with 24 h rest period between each administration. The inflammatory response after nasal administration of poly(I:C) was determined by measuring broncho-alveolar lavage (BAL) counts of leukocytes in wild-type and (PAR2 R38E) or FXa (PAR2 G37I) cleavage-resistant PAR2 mutant mice, as well as mice with macrophage-specific coagulation factor deficiency.

Results: Poly(I:C) stimulation of WT and FXa cleavage-insensitive PAR2 G37I mice caused a marked increase in BAL total leukocyte counts and specifically a significant increase in myeloid cell counts (neutrophils, monocytes and macrophages). In contrast, the completely cleavage insensitive PAR2 R38E strain was protected from the increase in inflammatory cell recruitment to the alveolar space. Since we had previously shown that FVII and FX can be expressed by macrophages, we studied mice with myeloid cell deficiency of coagulation factors in this model. Loss of FVIIa expression by macrophages similarly protected mice from lung inflammatory cell recruitment.

Conclusions: These new transgenic animals provide evidence that lung recruitment of inflammatory cells in response to viral challenge depends on TF-FVIIa-PAR2 signaling in myeloid cells.

ASY 34.3 | Effector-memory T cells Attenuate Thrombus Resolution

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Background: Innate immune cells are key effectors in venous thrombus formation and resolution but the participation and role of adaptive immune cells remains largely unexplored.

Aims: Investigating the functional role of T cells in venous thrombosis we observed that DVT recruits effector-memory T cell (T_{EM}) cells into the vein wall and thrombus.

Methods: Deep vein thrombosis (DVT) was induced by 80% flow reduction in the inferior vena cava (IVC) of mice. T cell recruitment and inflammatory activity was followed by flow cytometry, histology and intrathrombotic gene expression in reporter strains and upon depletion of T cells over various time points after DVT.

Results: Using reporter mice we show that DVT-recruited intravenous T_{EM} receive an immediate antigen-independent activation and produce IFN-g *in situ*. We further identify a set of DVT up-regulated cytokines and chemokines that synergize to induce antigen-independent IFN-g-production in $CD4^+$ and $CD8^+$ T_{EM} cells and demonstrate that intravenous T_{EM} activation and IFN-g-production determines neutrophil and monocyte recruitment into the vein wall and attenuates thrombus revascularization and resolution.

Conclusions: Our findings identify innate T cell activation in the vein wall as a key event in post-thrombotic inflammation and suggest T cells as a valid target for therapeutic intervention with the post-thrombotic inflammatory process.

ASY 34.4 | Caveolin-1 is a Novel Key Player in the Crosstalk between Tissue Factor and the IGF-1R

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Background: The IGF-1 receptor promotes survival and has also been implicated in the pathogenesis of e.g. cardiovascular disorders and cancer. We recently showed that binding of coagulation factor VIIa (FVIIa) to its receptor tissue factor (TF) induces a transactivation of the IGF-1R in several different cell types. This leads to increased resistance to apoptosis and nuclear translocation of the intact IGF-1R.

Aims: Lipid rafts/caveolae are known modulators of TF/VIIa- and IGF-1R signaling. To clarify the TF/FVIIa transactivation mechanism of the IGF-1R further, we investigated the interplay between TF/FVIIa, IGF-1R, and the caveolae protein caveolin-1 (Cav1).

Methods: The levels, interactions and phosphorylations of proteins were assessed by the Duolink *in situ* proximity ligation assay in intact cells and by and western blot on whole- and fractionated cell lysates. PC3 prostate or MDA-MB-231 breast cancer cells were used as model systems.

Results: Treatment with 10 nM FVIIa, but not PAR1 or PAR2 agonists, resulted in a Src-family dependent phosphorylation of Tyr14 of Cav1 in both PC3 and MDA-MB-231 cells. Cav1 downregulation singlehandedly increased the IGF-1R tyrosine phosphorylation, whereas treatment with a peptide corresponding to amino acids 82-101 of Cav1, i.e. the Cav1 scaffolding domain, blocked the phosphorylation of the IGF-1R by TF/FVIIa. The presence of the Cav1 scaffolding domain peptide consequently broke the TF/FVIIa/IGF-1R signaling pathway, which intact leads to increased resistance to death receptor-induced apoptosis, and blocked the nuclear translocation of the IGF-1R after FVIIa treatment.

Conclusions: Our data shows that Cav1 prevents IGF-1R activation in resting cells via its scaffolding domain, and that this inhibition is terminated by TF/FVIIa-induced phosphorylations of Src and Cav1. We thereby propose Cav1 to be a novel key player in TF/FVIIa signaling by its crucial role in the intra-cellular crosstalk between TF/FVIIa and the IGF-1R.

ASY 38.3 | Septic Conditions Modulate the Level of miRNAs in Platelets and Megakaryocytes that May Contribute to Abnormal Platelet Reactivity

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Background: Bone marrow may be affected in sepsis causing platelet activation, which can lead to thrombotic complications. The molecular mechanisms regulating elevated platelet activation are still not fully known.

Aims: In this study, we examined the expression of platelet miRNAs in patients with sepsis, and miRNAs in megakaryocytes (MKs) were also investigated among *in vitro* septic circumstances.

Methods: MiRNAs were investigated in leukocyte-depleted platelet samples obtained from 20 individuals suffering from sepsis and 24 age- and sex-matched healthy controls. We randomly selected 3 RNA samples from each group, and we first analyzed miRNAs using TaqMan Open Array (ABI). We further quantified miR-223 and miR-26b in all samples by UPL-probe based RT-qPCR (Roche), and target P2RY12 and SELP (P-selectin) mRNAs, respectively, were also analyzed. Platelet activation was studied through measuring surface P-selectin by flow cytometry. To prove the effect of altered miRNA profile of MKs in sepsis, miRNA analysis in MEG-01 and K562 cells (Sigma-Aldrich) was performed after the treatment with recombinant TNF-α (100 ng/mL) and/or LPS (O55:B5, 100 ng/mL) for 4-24 hours at 37°C.

Results: There was augmented platelet activation as P-selectin expression was increased in septic patients (P< 0.001). Sixty-six platelet miRNAs indicated more than two-fold decrease (e.g. miR-223, miR-26b), while 37 (e.g. miR-155) were increased at the same degree in sepsis. Consequently, P2RY12 and SELP mRNA levels were significantly upregulated (P< 0.01) in platelets. Mature MKs from K562 cells by PMA and MEG-01 cells showed enhanced miR-146a and miR-155 levels by TNF-α and/or LPS with significantly lower miR-223 and miR-26b expression after 4 and 24 hours, respectively. In addition, we could also detect their elevated target MK mRNA levels by 24 hours in response to these inflammatory triggers.

Conclusions: Decreased level of platelet miRNAs with elevated target mRNAs may result in enhanced activation of platelets in sepsis.

DIAGNOSTICS AND OMICS

ASY 04.1 | A Microfluidic Model of Hemostasis Sensitive to Platelet Function and Coagulation

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Background: Most in vitro flow chambers simulate intravascular thrombus formation, which is relevant to thrombotic events. However, relatively few simulate extravascular thrombus formation, which is more pertinent to bleeding diatheses.

Aims: To develop an in vitro model of extravascular thrombus formation whereby a hemostatic thrombus forms under physiological pressure gradients that is sensitive to platelet function and coagulation.

Methods: A microfluidic device was fabricated with an 'H' geometry that includes vascular, injury, and extravascular channels (Fig. 1A). Type I collagen, tissue factor (TF) or collagen-TF were adsorbed on the walls of the injury channel. Citrated whole blood was recalcified in a continuous mixer upstream of the vascular channel. A pressure-based flow controller enabled user-defined control of the pressure gradient across the injury channel. The velocity field and shear stresses were calculated by computational fluid dynamics (Fig. 1B). Thrombus formation was visualized with DiOC6 labeled platelets and Alexa-555 labeled fibrinogen (Fig. 1C). Blood was treated with anti-FVIII or anti-TFPI antibodies, the P2Y12 antagonist 2-MeSAMP, or vehicle prior to the assay.

Results: Collagen and TF acted synergistically to yield a stable thrombus that stops blood loss into the extravascular compartment in 7.5 ± 1.6 min. Platelets first formed a plug that stop blood flow, followed by fibrin polymerization that stabilized the thrombus. Anti-FVIII treatment resulted in an unstable thrombus that did not close the injury. Treatment with a P2Y12 antagonist prolonged the closure time two-fold. Anti-TFPI treatment resulted in a reduced closure time of 3.5 ± 0.5 min.

Conclusions: These data demonstrate a hemostatic model that is sensitive to both coagulation and platelet function that recreates the dynamics of primary and secondary hemostasis. This model can be used to study the biophysical phenomena that regulate coagulopathies and platelet dysfunction that cause bleeding.

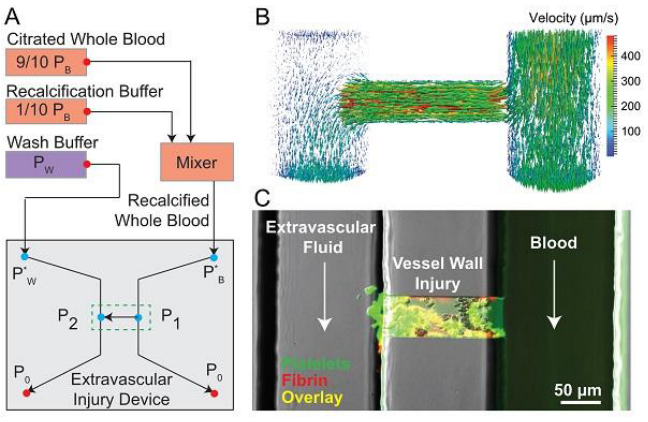

FIGURE 1 A. Microfluidic device with vascular, injury, and extravascular channels. B. Velocity field in device. C. Hemostatic thrombus formed in injury channel

ASY 04.2 | Introduction of a National Protocol for Light Transmission Aggregometry in The Netherlands: Results of More than 80 Healthy Volunteers Before and after Standardisation

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Background: Light Transmission Aggregometry (LTA) is considered the gold standard method for evaluation of platelet function. The SSC Platelet Physiology published a guideline in order to standardize LTA. The Society of Hematological Laboratories in The Netherlands (VHL) has set a goal for 2017, namely to reduce variability in LTA results by stimulating all laboratories to uniform their methods.

Aims: To establish a national LTA procedure, based on the SCC 2013 guideline, and to compare LTA results of more than 80 healthy volunteers from 8 hospital laboratories, before (2013) and after (2016) standardisation.

Methods: The SSC guideline was adapted to a national procedure. Almost all advices of the SSC were followed up e.g.: no adjustment of PRP, citrate concentration 109 mM, 21 needle gauge, fasting, resting time for whole blood and PRP, centrifugation time and speed and agonist concentrations. Results of healthy volunteers were collected in 2013 (before standardisation) and in 2016 (after standardisation) in respectively 9 and 8 hospital laboratories including more than 80 results of healthy volunteers per year (maximal aggregation %) on 4 different analysers (Chronolog, PAP-8, APACT and AggRam) using their own choice reagent brand.

Results: Before standardisation a large diversity in pre-analytical procedures and agonist concentrations were used. After standardisation all participants used the same agonist concentrations (Table 1) and (pre)analytical procedure. Median (IQR) for maximal aggregation per agonist is shown in Table 1.

Strikingly, variation increased after standardisation for ADP but decreased for collagen low and ristocetin low. Analyser specific differences were detected e.g. for ADP low Figure 1.

Conclusions: A standardised Dutch procedure for LTA, based on the SSC guideline, does not result in a smaller variability in healthy volunteers for all agonists.

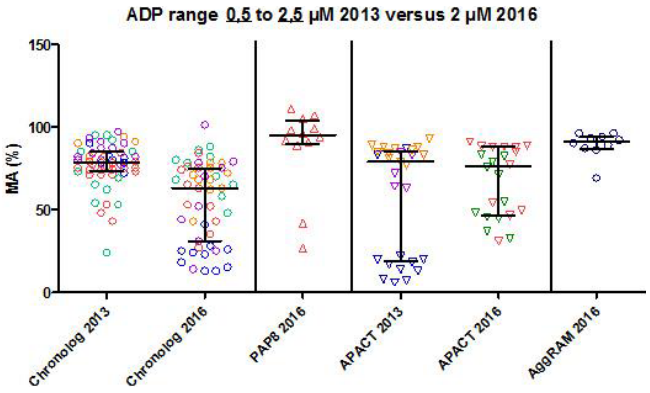

FIGURE 1 Comparison of ADP maximal aggregation of healthy volunteers between 2013 and 2016

ASY 04.4 | Novel Integrin αIIbβ3 and GPIbα Coatings that Feature Anti-fouling Properties for Platelet Research and Clinical Diagnostics

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Background: Platelets play key roles in thrombosis and hemostasis. Fibrinogen (Fg) binding to integrin αIIbβ3 (GPIIbIIIa) has been considered to be required for platelet aggregation. However, we found platelet aggregation persists even in the absence of Fg and VWF, suggesting unidentified αIIbβ3 ligands are involved in thrombosis. Additionally, ligands for GPIbα have not been fully explored.

TABLE 1 Median and IQR of maximal aggregation in 2013 and 2016 in healthy volunteers

Agonist	2013 concentrations	2013 n	2013 median (IQR)	2016 concentration	2016 n	2016 median (IQR)
ADP low (µM)	0.5;1;2;2.5	85	79 (70-85)	2	96	72 (45-88)
ADP intermediate (µM)	4;5	43	86 (82-90)	5	98	84 (72-87)
Ristocetin low (mg/mL)	0.25;0,5;0.6	86	9 (4-14)	0.5-0.7	55	7 (3-12)
Ristocetin high (mg/mL)	1;1.2; 1.25	138	92 (88-94)	1.2	86	92 (88-98)
Collagen low (µg/mL)	1;1.25; 2	102	85 (79-89)	2	86	89 (83-92)
Collagen high (µg/mL)	4, 5, 10	95	87 (84-91)	5	66	88 (80-93)
Epinephrine µM	5	47	80 (75-85)	5	53	87 (73-91)
Arachidonic acid (mM)	0.5; 1; 1.5; 1.6	70	84 (79-86)	1	64	89 (80-92)

Aims: Development of αIIbβ3- and GPIbα-bound monolayer coatings that can be applied to any hydroxyl (-OH) terminated surface (e.g. glass, metals and plastics).

Methods: Surface- and bio-chemistry synthesis.

Results: Both coatings are based on a trimethoxysilyl diethylenetriamine (DETA) monolayer which forms covalent bonds to the underlying -OH surface, facilitates covalent linkage of either αIIbβ3 or GPIbα, and is anti-fouling (resistance to non-specific protein adsorption). These two coatings were synthesized on planer (glass slide) and spherical (3 μm silica bead) surfaces. GPIbα surfaces bound both conformational and linear epitope specific anti-GPIbα mAbs. αIIbβ3 coated surfaces bound Fg as well as conformational and linear epitope specific mAbs. Furthermore, αIIbβ3 coated beads were incorporated into murine wild type platelet aggregates and VWF/Fg $^{-/-}$ platelet aggregates, demonstrating the interaction with the yet unidentified "x-ligand" responsible for VWF/Fg independent aggregation.

Conclusions: These data indicate αIIbβ3 and GPIbα adopt ligand binding conformations when immobilized on DETA. Moreover, DETA remarkably resists non-specific interactions and maintains a very low background signal regardless of detection method, even without employing a blocking agent (e.g. BSA). This work presents the first use of anti-fouling organic-monolayer attached platelet surface receptors and demonstrates the enormous potential that these synthetic coatings possess in platelet research. Identification of novel αIIbβ3 and GPIbα ligands and evaluation of these coatings for auto- and allo-antiplatelet antibody detection remains ongoing.

ASY 25.1 | Microparticles as a Biomarker to Detect Thrombosis in Patients with Implanted Ventricular Assist Devices

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Background: Left ventricular assist devices (LVADs), increasingly common for heart failure management, have associated thrombosis as a major cause of mortality. LVAD thrombosis is detected by elevated plasma lactate dehydrogenase levels (LDH); however, this method has limited sensitivity. Sub-micron cellular microparticles (MPs) are derived by outward blebbing of the plasma membrane in response to hemostatic activation, inflammation, altered rheology or infection.

Aims: To determine whether MPs are more sensitive than clinical parameters to predict thrombotic events in LVAD implanted patients.

Methods: Citrated blood samples were collected periodically from 26 consented patients implanted with a Thoratec HeartMate II LVAD and once from 12 healthy controls. Plasmas were processed by ultracentrifugation to pellet MPs which were quantitated by flow cytometry using polystyrene beads < 1μm, PKH67 (biological membrane dye), and monoclonal antibodies CD41-PE (platelets), CD45-PE [leukocytes (WBCs]), CD146-PE [endothelium (ECs)], CD235-PE [erythrocytes (RBCs)]. Documented thrombosis, LDH, platelet count, aPTT, D-dimer, fibrinogen, INR were recorded.

Results: MP levels in patients without thrombosis remained below the 500 MP normal count threshold. Patients with thrombotic events had elevated levels (2000-9000 MPs) a median 50 days (35-391 days) prior to LDH elevation. MP levels reverted to normal with resolution of thrombosis. Platelet and RBC MPs were elevated earlier and greater than WBC and EC MPs, and occasionally only RBC MPs were elevated when thrombosis was present. There was no obvious association with thrombosis for platelet count, D-dimer, INR time out of therapeutic range or any other clinical parameter.

Conclusions: Blood cell-derived MP levels rise above the normal threshold weeks prior to when common clinical parameters indicate an LVAD-associated thrombotic event, suggesting a role for this predictive biomarker.

ASY 25.3 | Analysis of Platelet Polyphosphate

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Background: Platelet polyphosphate is an inorganic procoagulant polymer of orthophosphate units that is stored in dense granules and is released upon platelet activation. Polyphosphate has been shown to have a critical function for thrombosis making the polymer an attractive biomarker.

Aims: Here, we describe an assay to measure polyphosphate on human platelets and show that procoagulant platelets expose long chain polyphosphates on their surfaces.

Methods: Recombinant Escherichia coli-expressed exopolyphosphatase deletion mutant PPX_Δ12 that lacks the enzymatic domain was labeled with fluorescent Alexa488 dye and was used as a polyphosphate probe in flow cytometry.

Results: PPX_Δ12-Alexa488-signal dose-dependently increased with synthetic long-chain polyphosphate binding to platelets. Both exopolyphosphatase treatment and polyphosphate pre-incubation abolished PPX_Δ12-Alexa488 binding to polyphosphate on platelets. In contrast, short-chain polyphosphate that was found in the supernatant of activated platelets, did not bind to platelet surfaces. Ion exchange chromatography revealed that platelets contain two pools of polyphosphate of 60-100 and >500 phosphate units chain length, respectively. Stimulation of platelets with thrombin receptor agonist Trap6, and P2Y12 receptor activator ADP increased polyphosphate accumulation on platelet surfaces and PPX_Δ12-Alexa488 signal in a dose-dependent manner indicating that long chain polyphosphates are retained on activated platelet surfaces.

Conclusions: This study indicates that long-chain polyphosphate is exposed on platelet plasma membranes while short chain molecules are released into the supernatant. Our assay presents a promising

diagnostic assay to measure polyphosphates on human platelets and in platelet-rich plasma. Future investigations will aim to determine if polyphosphate can be used as a novel biomarker of thrombosis.

validation of these results in a larger population would open the possibility of implementing such a marker in clinical practice.

ISCIII y FEDER (P14/00253), Fundación Séneca 19873/GERM/15

ASY 25.4 | NETosis Markers Predict Adverse Events in Anticoagulated Patients with Atrial Fibrillation

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Background: Thrombogenesis in atrial fibrillation (AF) is multifactorial. Adverse cardiovascular events (ACEs) occur in ~4% of AF patients undergoing oral anticoagulation therapy. Recently, the relationship between neutrophil extracellular trap (NET) formation and thrombogenesis has been described.

Aims: To investigate plasma cell free DNA levels (cfDNA) and neutrophil elastase (NE) as prognostic biomarkers of ACE in anticoagulated patients with AF.

Methods: We included 248 healthy volunteers (59% male, median age 46, range 18-95) and 485 AF patients (53% male, median age 76, range 71-81) with stable anticoagulation for 6 months and a follow-up of 8 years. ACEs were stroke (ischaemic/embolic), ACS, acute HF and global or vascular death. cfDNA was measured with Sytox Green. NE activity was determined by ELISA. Data were analyzed using Cox regression.

Results: In controls, cfDNA was uniform up to 75 years, increasing significantly above that age [< 75 (n =221): 50ng/mL±0.02 vs. ≥75 (n =26): 80ng/mL±0.01, p< 0.001]. Therefore, we selected patients < 75 years [n=336, 62% males, age 69(63-72)]. cfDNA was higher in patients than in controls: 70 ng/mL±0.05 (p< 0.05). During follow-up, 50 patients suffered from thrombosis/vascular events; 27 stoke/embolism; 73 global death. cfDNA levels were not associated with ACEs. NE activity, which showed a significant correlation with cfDNA in patients (rho=0.261; p< 0.001), was also higher in patients than in controls (median 37.8 *vs.* 27.4 ng/ml; p< 0.001). Morever, NE activity was significantly associated with: combined ACE [2.2 (1.24-3.99), p=0.007], stroke [2.6 (1.21-5.56), p=0.01], global death [2.2 (1.35-3.61), p=0.001] and vascular death [5.3(1.26-22.33), p=0.02].

Conclusions: Besides providing prognostic information in AF patients, NE plasma activity might also influence the occurrence of ACE. Thus,

ASY 38.1 | Plasma miRNA Expression as Risk Marker of Stroke in Patients with Atrial Fibrillation

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Background: Atrial fibrillation (AF) is a highly prevalent disorder that contributes to increased mortality and morbidity. The use of oral anticoagulation (OAC) reduces stroke risk, even using risk stratification scales such as CHA2DS2-VASc score, AF patients with OAC suffer from stroke. Thus, searching new biomarkers is needed to improve the clinical management of these patients. In recent years miRNAs, have become important players in cardiovascular biology.

Aims: To evaluate the expression of plasma miRNAs in AF patients with OAC and to investigate their prognostic value for the risk of stroke.

Methods: From 789 AF patients with stable OAC for 6 months (INR=2-3) with a median follow-up of 2888 days, we randomly selected 10 patients, who had suffered from stroke and 10 age and sex-matched patients who did not developed stroke during the same follow-up. Samples were obtained when AF was diagnosed. MiRNAs were purified using Nucleo Spin miRNA Plasma Kit (Macherey-Nagel). *Plasma focus miRNAs PCR panel V4* (Exiqon) was used to evaluate miRNA expression and GraphPad Prism 5 was employed to analyze data.

Results: Using *t*-test Sidak-Bonferroni, we observed that miR-328 and miR-22-3p, had up-regulated levels in stroke patients compared to the no-stroke group (*p*-value< 0.1; fold>1.5). Confirmation of the arrays in the same cohort was performed with individual qRT-PCRs. Only miR-22-3p remained statistically elevated in stroke patients (*p*-value=0.017; fold=2.13).

Conclusions: AF patients who suffered from stroke, showed high levels of miR-22-3p. This miRNA has been associated with hypertrophy and cardiac remodeling. These data suggest that miR-22-3p could be a potential prognostic marker of stroke and could provide additional information to current risk stratification schemes (such as CHA2DS2-VASc) to improve the clinical management of AF patients.

Funding: ISCIII y FEDER (P14/00253); Fundación Séneca 19873/GERM/15.

FIBRINOLYSIS & PROTEOLYSIS

ASY 07.1 | Deriving the Full Atom Structure for the Coagulation Factor XIII A_2B_2 Hetero-tetrameric Complex by Molecular Modeling Using Guiding Constraints Based on Mass Spectrometric Analysis of the Complex

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Background: The coagulation Factor XIII (FXIII) is a heterotetramer composed of two catalytic A and two protective/regulatory B subunits. The structure of this heterotetramer complex is not known.

Aims: To use cross-linking chemistry in conjunction with High-Mass MALDI mass spectrometry and nLC Orbitrap MS/MS analysis to determine inter and intra-subunit interface residues within the $FXIIIA_2B_2$ heterotetramer complex and then to use these residues as guiding constraints to generate an accurate all atom model of the complex.

Methods: The $FXIIIA_2B_2$ complex was purified from the plasma concentrate Fibrogammin by size exclusion chromatography. The intact purified complex was subjected to chemical cross-linking and High-Mass MALDI MS analysis to determine the stoichiometry and molecular weight of the intact protein complex. The cross-linked protein complex was subjected to proteolysis using five different enzymes (Trypsin, Chymotrypsin, Elastase, Thermolyse and ASP-N). The peptides generated were injected in an nLC chromatographic system coupled with an Orbitrap mass spectrometer. After MS/MS analysis, XQuest and Stavrox softwares were used to identified the interlink peptides of the complex. The residues identified were used as guiding constraints to dock a threaded model of the $FXIIIB_2$ subunit on the repaired (gaps and loops filled) crystal structure of the $FXIIIA_2$ subunit. The resulting model was subjected to classical MD simulation to check for stability.

Results: The High-Mass MALDI MS analysis allowed the detection of the intact cross-linked protein complex, its molecular weight and its stoichiometry. Post proteolysis more than 85% of the sequence of $FXIIIA_2B_2$ complex is covered by the peptide mass fingerprint. The model generated from the interface residues detected in the MS coverage is very stable as observed from a long MD simulation.

Conclusions: We have been able to generate an accurate all atom model of the $FXIIIA_2B_2$ heterotetramer based on experimental data.

ASY 07.2 | Reciprocal Inter-tissue Regulation of Factor XIII-A and -B Subunits Determines Factor XIII Levels in Plasma

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Background: Plasma coagulation factor XIII (FXIII) is composed of two zymogen FXIII-A subunits and two carrier FXIII-B subunits ($FXIII\text{-}A_2B_2$). FXIII-A is produced in bone marrow-derived cells, whereas FXIII-B is synthesized by hepatocytes. FXIII-B stabilizes FXIII-A; consequently, deficiency in either subunit is associated with bleeding. Interestingly, FXIII-A-deficient mice and humans have decreased levels of FXIII-B. In humans, therapeutic infusion of recombinant $FXIII\text{-}A_2$ ($rFXIII\text{-}A_2$) increases FXIII-B levels. The mechanism mediating this inter-tissue reciprocal regulation has not been defined.

Aims: Determine how FXIII-A regulates FXIII-B levels.

Methods: FXIII-B protein and mRNA were measured using Western blotting and RT-qPCR, respectively. Liver proteomes were compared using label-free quantitative mass spectrometry and KEGG pathway analysis.

Results: $rFXIII\text{-}A_2$ treatment increased FXIII-B production in two human hepatocellular carcinoma lines (HepG2 and Huh7), but did not promote cell growth or increase FXIII-B stability. *F13a*$^{-/-}$ mice infused with $rFXIII\text{-}A_2$ showed increased FXIII-B levels within 3-6 hours, and by 24 hours, FXIII-B levels were similar to levels in *F13a*$^{+/+}$ mice. *F13b* mRNA isolated from treated cell cultures and livers of infused mice was not elevated relative to untreated controls, suggesting FXIII-A did not enhance *F13b* gene expression. Proteomic profiling of liver lysates from *F13a*$^{+/+}$, *F13a*$^{-/-}$, and $rFXIII\text{-}A_2$-infused *F13a*$^{-/-}$ mice identified unique proteins that were significantly upregulated in both *F13a*$^{+/+}$ and $rFXIII\text{-}A_2$-infused *F13a*$^{-/-}$ mice relative to *F13a*$^{-/-}$ mice. Pathway analysis reveals several of these proteins regulate RNA processing.

Conclusions: FXIII-A and FXIII-B subunits show reciprocal regulation in mice and humans. FXIII-B promotes FXIII-A stability in circulation, while FXIII-A enhances FXIII-B production, likely via post-transcriptional processing. Identification of this unique regulatory mechanism exposes newly-recognized inter-tissue crosstalk critical for hemostasis.

ASY 07.3 | Plasma-, but Not Platelet-factor XIII Promotes Red Blood Cell Retention in Contracted Clots and Mediates Clot Size during Venous Thrombosis

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Background: The transglutaminase factor XIII (FXIII) plays a seminal role in venous thrombosis by crosslinking fibrin α-chains and promoting

red blood cell (RBC) retention in contracted clots. However, the contributions of plasma ($FXIII_{plasma}$)- vs. platelet ($FXIII_{plt}$)-derived FXIII to clot composition, thrombosis, and hemostasis remain undefined.

Aims: Determine the role of $FXIII_{plasma}$ and $FXIII_{plt}$ in clot contraction, composition, and size. Identify the level of FXIII deficiency that reduces thrombus weight without increasing bleeding.

Methods: Thrombin generation, FXIII activation, and fibrin crosslinking were measured in whole blood, platelet-rich plasma (PRP) and reconstituted whole blood and PRP from *F13a*$^{+/+}$, *F13a*$^{+/-}$, and *F13a*$^{-/-}$ mice by calibrated automated thrombography and western blotting. Mice were subjected to inferior vena cava thrombosis, as well as tail transection and saphenous vein bleeding assays.

Results: FXIII activation and fibrin crosslinking were delayed in *F13a*$^{+/-}$ PRP relative to *F13a*$^{+/+}$ PRP, but thrombin generation and clot contraction were similar in PRP across genotypes. In reconstituted assays, PRP containing $FXIII_{plasma}$, but not $FXIII_{plt}$, formed fibrin α-chain crosslinks. Similarly, the absence of $FXIII_{plasma}$, but not $FXIII_{plt}$, decreased RBC retention in reconstituted whole blood contracted clots, resulting in smaller clots. In vitro and in vivo, FXIII deficiency reduced thrombus weight in a gene dose-dependent manner. In hemostatic challenges, *F13a*$^{-/-}$, but not *F13a*$^{+/-}$, mice had prolonged tail bleeding times, whereas no genotype-dependent difference was observed following saphenous vein puncture.

Conclusions: FXIII deficiency results in a gene dose-dependent decrease in thrombus weight without altering thrombin generation or platelet contraction. $FXIII_{plasma}$, but not $FXIII_{plt}$, mediates fibrin crosslinking, promoting RBC retention and increased thrombus weight. Imposition of mild-to-moderate FXIII deficiency may reduce venous thrombosis without simultaneously increasing bleeding risk.

ASY 07.4 | FXIII on Cold-stored Platelet Surface Enhance Clot Contraction and Anti-fibrinolysis

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Background: Currently, platelets (PLT) for transfusion are stored at room temperature (22°C, RT) for up to 5 days, although recently FDA clarified that PLT stored in cold (4°C) for up to 72 h may be used for active hemorrhage. We have previously demonstrated that 4C-stored PLT have significantly better metabolic reserves, hemostatic responses to agonists and inhibitors, and clot strength, compared to RT-stored PLT. Recently, we discovered a novel mechanism of fibrinogen-mediated plasma-FXIII binding to 4C-stored PLT surface as the principal contributor to enhanced clot strength.

Aims: To test the hypothesis that increased FXIII activity on 4C-stored PLT surface will increase fibrin cross-linking, clot contraction, and anti-fibrinolytic activity.

Methods: Clinical clot retraction assay in normal, FXIII-deficient plasma or with FXIII inhibitor was employed along with immunohistochemistry, electron microscopy and serum analysis to investigate structure and composition of retracted clots. Image analysis was used to quantify porosity, morphological parameters, and PLT-fibrin interaction from clot histology. Lytic properties in tPa were measured by ROTEM. (n=4,*P=0.05).

Results: Clots formed by 4C-stored PLTs were heavier than those from RT-stored PLTs, (Fig. 1A) with a highly organized structure compared to a disintegrated structure in RT PLT clots (Fig. 1B). 4C-stored PLT, but not RT-stored PLT, actively interacted with fibrinogen and formed nucleating centers for fibrin formation and cross-linking yielding clots with denser, thinner and unswerving fibrin network which offer lytic resistance to tPa (Fig. 1C). Results with FXIII-deficient plasma and FXIII inhibitor corroborate that plasma FXIII binding to 4C-stored PLT surface is a key regulator of these processes.

Conclusions: We have shown that FXIII-mediated fibrin crosslinking on 4C-stored PLT surface improve contraction and mitigate fibrinolysis forming strong and stable clots necessary to combat traumatic hemorrhage.

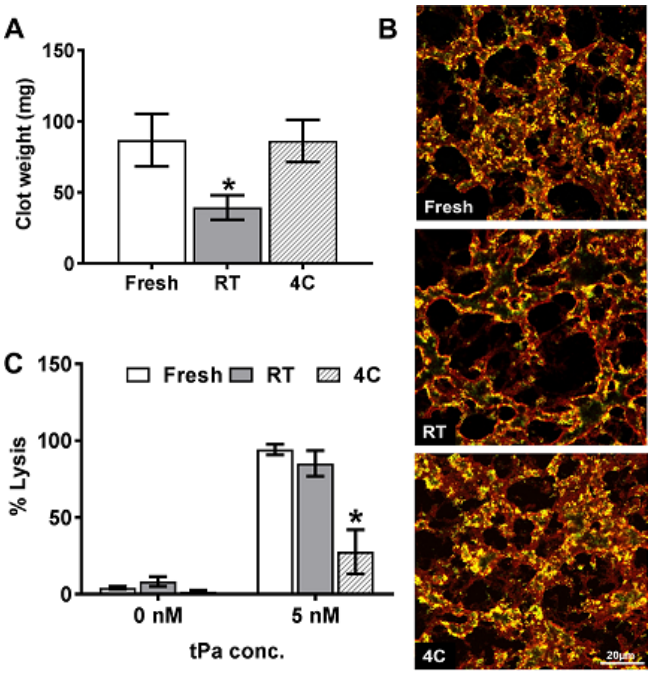

FIGURE 1 (A) Abnormal weight in RT-PLT clots (B) Structural regularity in 4C-PLT clots compared to RT(C) Fibrinolytic resistance in 4C- PLTs to tPa

ASY 15.1 | The Antithrombotic Protein C Activator E-WE Thrombin Supports Fibrinolysis by Inhibiting TAFI Activation

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Background: Thrombin contributes to clot stabilization by activating thrombin-activatable fibrinolysis inhibitor (TAFI). The thrombin

mutant, E-WE thrombin, has diminished activity toward procoagulant substrates but can still activate protein C when bound to thrombomodulin (TM), making it a potent antithrombotic enzyme. Activation of TAFI by thrombin also requires TM but the specificity of E-WE thrombin for TAFI is unknown.

Aims: The aim of this study was to investigate whether E-WE thrombin activates TAFI and to determine whether E-WE thrombin can act as a profibrinolytic agent through competitive inhibition of thrombin-mediated TAFI activation.

Methods: The ability of E-WE thrombin to activate TAFI was evaluated using Western blot and quantitative colorimetric assays to measure hippuric acid generation by activated TAFI. For both experiments, purified TAFI was incubated with wild-type (WT) thrombin and/or E-WE thrombin with or without rabbit TM for increasing times before downstream analysis. Lysis of a formed plasma clot was measured by recording the time until plasma liquefied again.

Results: Western blotting and quantitative assays showed that E-WE thrombin was a poor activator of TAFI compared to WT thrombin in the presence of TM. Whereas WT thrombin activated TAFI within minutes, we found no evidence of equimolar E-WE thrombin-mediated activation until nearly 1 hr. Furthermore, E-WE thrombin concentration-dependently inhibited thrombin-mediated activation of TAFI. Finally, when both E-WE thrombin and tPA were incorporated within plasma clots, lysis was accelerated by up to 74% compared with the addition of tPA alone.

Conclusions: Our data suggest that the human thrombin analog, E-WE thrombin, which is a selective protein C activator under development for treating acute thrombotic emergencies, also may act as a profibrinolytic agent through inhibition of TAFI activation. These data suggest that co-administration of E-WE thrombin with tPA may improve the efficacy of thrombolysis.

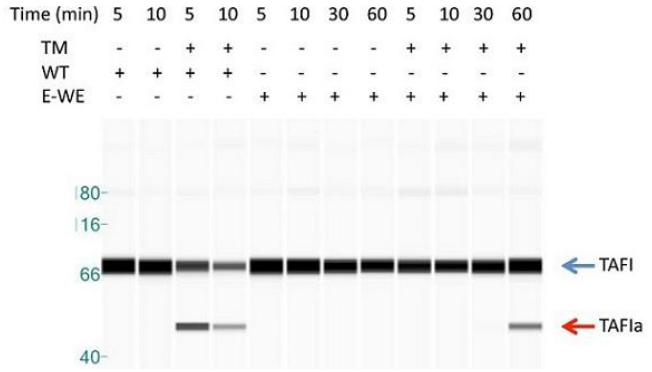

FIGURE 1 E-WE thrombin is a poor activator of TAFI compared to WT thrombin

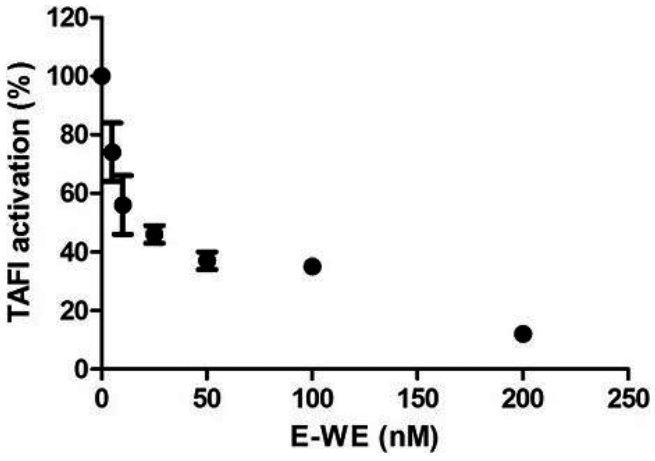

FIGURE 2 E-WE thrombin dose-dependently inhibits thrombin-mediated activation of TAFI

ASY 15.2 | Plasminogen Activator Inhibitor 1 is Retained on the Surface of Activated Platelets in a $\alpha IIb\beta_3$ and Fibrin Dependant Manner

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Background: Plasminogen activator inhibitor 1 (PAI-1) is the principal physiological inhibitor of tissue-type plasminogen activator and urokinase. Platelet a-granules harbour the primary pool of PAI-1, which is secreted upon activation.

Aims: To analyse the retention of platelet-derived PAI-1 on the activated platelet membrane.

Methods: Platelets activated with collagen ± thrombin receptor activator peptide 6 (TRAP-6) or thrombin were analysed for PAI-1 by ELISA and activity assay. Tirofiban was included to inhibit $\alpha IIb\beta_3$ or fibrin polymerisation blocked with Gly-Pro-Arg-Pro (GPRP). Flow cytometry and confocal microscopy were performed using fluorescently-labelled antibodies to PAI-1, fibrin(ogen), $\alpha IIb\beta_3$ and the α-granule marker, P-selectin. Chandler model thrombi lysis and Hemacore analysis was performed with platelet-rich plasma ± a neutralising antibody to PAI-1 and lysed with tPA.

Results: Real-time lysis of platelet-rich clots revealed a significant increase upon inhibition of PAI-1 (20 ± 13 min vs. >60 min), which was mirrored by a 3-fold increase in the lysis rate of Chandler model thrombi. The majority of PAI-1 antigen was detected in the platelet releasate, however ~20% was associated with the membrane fraction. Flow cytometry revealed an increase in membrane PAI-1 with TRAP-6 stimulation, however maximum exposure was observed with thrombin; suggesting a role for fibrin in PAI-1 retention. Tirofiban and GPRP attenuated membrane-bound PAI-1 on activated platelets. Similarly, PAI-1 antigen was reduced in the membrane fraction by tirofiban (73 ± 7%) and GPRP (64 ± 3%), as quantified by ELISA. PAI-1 was observed as a "cap" on phosphatidylserine positive platelets, co-localizing with fibrin(ogen), $\alpha IIb\beta_3$ and P-selectin. Blocking $\alpha IIb\beta_3$ and fibrin polymerisation completely abrogated PAI-1 activity associated with the membrane fraction.

Conclusions: Our data reveals a striking dependence for $\alpha IIb\beta_3$ and fibrin in modulating the retention of active, platelet-derived PAI-1 on the surface of activated platelets.

ASY 15.3 | The Signaling Lipid Sphingosine-1-Phosphate (S1P) Regulates Expression and Secretion of the Plasminogen Activator Inhibitor-1 (PAI-1) in Differentiated Fat Cells

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Background: The versatile lipid signaling molecule sphingosine-1-phosphate (S1P) is a regulator of immune functions such as utilization of immune cells and local inflammatory responses. It has also been suggested as a link between inflammation and coagulation as platelets generate and release large amounts of S1P upon activation. S1P regulates expression of the protease-activated receptors (PARs) to enhance cellular responses to thrombin in monocytes and has been implicated in mechanisms of platelet activation.

Aims: Since hyperlipidemia and obesity are typical risk factors for thrombosis, this study investigates possible effects of S1P on prothrombotic gene expression in adipocytes in vitro.

Methods: Murine 3T3-L1 fibroblasts were differentiated with MDI (methylisobutylxanthine, dexamethasone, insulin) induction medium. Gene expression of adiponectin, PAR1-4, the S1P receptors S1PR1-5 and the anti-fibrinolytic factor plasminogen activator inhibitor-1 (PAI-1) were determined by RT-PCR. PAI-1 protein was measured by Western blotting and ELISA.

Results: MDI-induced differentiation resulted in characteristic phenotypical changes as well as a 600-fold increase in adiponectin expression. Cell viability was maintained and apoptosis not significantly elevated. Expression of the PARs and S1PRs was reduced in differentiated adipocytes which fitted with cellular senescence. Incubation of adipocytes with S1P (0.3 to 10 μM) resulted in a significant upregulation of PAR-1 (2.7-fold) and PAI-1 (8-fold) mRNA. Conversely, thrombin (0.1 to 3.0 units/mL) induced expression of S1PR3, but not of the other S1P receptors. S1P also highly significantly induced PAI-1 protein expression and secretion to the culture media (about 4-fold). This was attenuated by pharmacological inhibition of S1PR2 and -3, but not by a S1PR1 inhibitor.

Conclusions: S1P regulates expression and secretion of PAI-1 in adipocytes in vitro. This mechanism may modulate the pathogenesis of thrombosis in individuals at risk such as in obesity or metabolic syndrome.

ASY 15.4 | CPU Inhibition with AZD9684: Effects on Fibrinolytic Rate in Different in vitro Models

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Background: Carboxypeptidase U (CPU, CPB2, TAFIa) is a basic carboxypeptidase able to downregulate fibrinolysis.

Aims: At this moment, little is known about the contribution of the CPU system to clot resistance in more advanced hemostatic models that include components such as blood cells and shear stress. The aim of this study is to evaluate the effect of AZD9684, a small molecule CPU inhibitor, in different *in vitro* systems with an increasing grade of complexity.

Methods: The contribution of the CPU system was evaluated in the following systems: I) standard plasma clot lysis; II) front lysis with confocal microscopy in platelet free and platelet rich plasma; III) thromboelastometry in whole blood (ROTEM); IV) microfluidic system with whole blood under arterial shear stress. All experiments were carried out in the absence or presence of AZD9684 (5 μM) in at least 5 healthy volunteers. In the experiments without inhibitor, samples were collected in order to assess proCPU (TAFI, proCPB2) consumption. Data are presented as mean ± SEM.

Results: During standard *in vitro* clot lysis in plasma, addition of AZD9684 resulted in a 22.1 ± 4.2% faster lysis. In ROTEM experiments we also found a clear acceleration of fibrinolysis. Lysis onset time decreased by 41.6 ± 3.9% in the presence of the inhibitor. Additionally, for both clot lysis and ROTEM a dose-dependent response of AZD9684 was observed and a clear consumption of proCPU was detected. Front lysis experiments in PFP resulted in a 33.6 ± 2.6% faster lysis in the presence of AZD9684. In PRP however only a tendency towards faster lysis was observed. Finally, fibrinolytic rate was accelerated by 15% in the microfluidic system when AZD9684 was added. No effect on platelet binding and DNA-content were observed.

Conclusions: Overall these experiments provide evidence that the CPU system is able to modulate fibrinolysis in complex biological systems. What the clinical significance of this observation is, requires more research.

ASY 28.1 | Fibrin Protofibril Structure at the Atomic Level

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Background: Fibrin polymerization starts when fibrinopeptides A are cleaved from the central nodule of the fibrinogen molecule. The exposed knobs 'A' then bind to the holes 'a' of the neighboring fibrin molecule, thus forming double stranded half-staggered fibrin oligomers. These oligomers elongate, spanning 10-15 monomers, thereby forming fibrin protofibrils, an important intermediate structure of fibrin polymer. Although a considerable amount of experimental effort has been expended to gather structural information about fibrin protofibrils, the atomic-level details remain elusive.

Aims: To construct the atomic structure of fibrin protofibrils and to characterize atomic-level interactions that stabilize the double-stranded half-staggered arrangement in fibrin protofibrils.

Methods: Multiscale computational modeling, which combines the full-atom MD simulations (both explicit and implicit water schemes), coarse-grained Langevin simulations. Experimental validation was performed using high-resolution atomic force microscopy imaging.

Results: The full atomic three-dimensional model of a fibrin protofibril that consists of 19 fibrin monomers was reconstructed computationally. The amino-acid residues that were not resolved by X-ray crystallography were incorporated into the structures, including the αC chain. In excellent agreement with experimental data, the structure of the protofibril shows a twist, with one strand wrapping around the other. The knobs 'A' participate in the intra-molecular interactions within each protofibril, whereas the knobs 'B' are capable of forming in both intra- and inter-protofibril non-covalent bonds with holes 'b'.

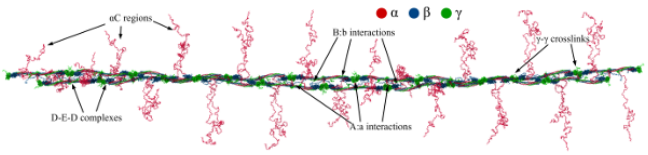

FIGURE 1 Structure of fibrin protofibril, reconstructed computationally, with the A-a and B-b knob-hole bonds, γ-γ crosslinks, and αC chains.

Conclusions: By employing our multiscale modeling approach, we have resolved and characterized in atomic detail the complete structure of fibrin protofibrils.The structures are availible for downloading in the PDB format at http://faculty.uml.edu/vbarsegov/research/fibrin.html

ASY 28.2 | Extended D-E Interaction Sites Near the Classical Knob-hole Binding Site Play an Important Role in Fibrin Polymerisation and Clot Formation

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Background: Molecular simulations indicate the presence of an extended binding interface beyond the traditional knob-hole interactions that occur when thrombin converts fibrinogen to fibrin (Kononova, JBC 2013). Within this extended binding interface, γAsp297, γGlu323 and γLys356 in the D-region of one fibrin molecule interact with βLys58, βAsp61 and βHis67 in the E-region of another, respectively. The effects of these novel electrostatic interactions on fibrin polymerisation and clot structure are unknown.

Aims: To study the role of the extended knob-hole interface in polymerisation kinetics, clot structure and clot mechanics.

Methods: Recombinant human fibrinogen γDEK variant (γD297N/E323Q/K356Q) and wild-type (WT) were produced in CHO cells and purified by affinity chromatography. Clot polymerisation kinetics were studied by turbidity. Clot visco-elastic properties were determined by magnetic tweezers. Confocal microscopy was used to study clot formation, clot structure and clot lysis.

Results: γDEK showed extended lag phase (+30%), slower clotting rate (-45%) and lower maximum OD (-41%) compared to WT. This variant produced a denser clot network (+41%) in hydrated conditions compared to WT, which resulted in slower lysis rates (-37%). Frequency dependent moduli were calculated and G' (elastic modulus), was similar at 0.1Hz but higher at 1 and 10Hz, compared to WT. G" (energy loss modulus) was increased at 0.1Hz 1Hz and 10Hz, compared to WT. The loss tangent (tanδ, visco-elasticity) was increased at 0.1Hz and 1Hz but similar at 10Hz.

Conclusions: The abolition of electrostatic interactions responsible for the extended binding interface results in altered polymerisation kinetics (prolonged protofibril formation), clot structure and viscoelastic properties. Our findings support previous molecular simulations and demonstrate that the D-E binding interface extends beyond the classical knob-hole interaction to reinforce fibrin polymerisation.

ASY 28.3 | The Factor VII Activating Protease (FSAP) Accelerates Fibrinolysis by Altering Fibrin Clot Structure

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Background: The plasma Factor VII activating protease (FSAP) affects both coagulation and fibrinolysis, although a physiologic role in haemostasis is still unclear. A pro-fibrinolytic activity is attributed to FSAP due to its ability to activate plasminogen activators. Cleavage of fibrinogen by FSAP in plasma has been reported, but the consequences for fibrinolysis are not known. Here we re-investigated the pro-fibrinolytic potential of FSAP with a particular emphasis on its interaction with fibrinogen.

Aims: To systematically investigate the influence of FSAP on clot structure and fibrinolysis.

Methods: Processing of fibrinogen by FSAP was analyzed by SDS-PAGE, Western blotting, RP-HPLC and N-terminal sequencing. Clot formation and lysis were studied by clot lysis time assay or by thrombelastometry. The clot structure was visualized by laser scanning microscopy and scanning electron microscopy. The functional characterization of fibrin clots included clot permeability and resistance to lysis by tPA.

Results: FSAP accelerated fibrinolysis independently of the activation of plasminogen activators by cleaving fibrinogen at multiple sites in the Bβ chains and within the αC-region of the Aα chains, generating a unique fibrinogen isoform. Clots of FSAP-treated plasma had a less

coarse fibrin network with thinner fibers and lower pore size, but still were less resistant to lysis by exogenous tPA. Similar observations were made in plasma after activation of endogenous FSAP. The effects of FSAP on clot structure and permeability were less pronounced in FXIII-depleted plasma, suggesting that in the αC-region important donor-and acceptor sites for FXIII cross-linking are involved.

Conclusions: Altering the fibrin clot structure in a unique way is a novel function of FSAP and is the main mechanism of its pro-fibrinolytic activity in plasma. Once activated in plasma, FSAP assumes a protective function in the vasculature by rendering fibrin clots more susceptible to lysis and reducing the risk of thrombosis.

ASY 28.4 | Relevance of Fibrinogen α-chain Cross-linking in Clot Formation, Structure, and Stability

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Background: FXIII plays a major role in stabilising clots by cross-linking fibrin γ- and α-chains. Previous studies using a fibrinogen (FGN) mutant (γ3X), unable to cross-link γ-chains, indirectly revealed that α-chain cross-linking may play a role in increasing clot stiffness and decreasing clot lysis rate. We have now produced a FGN mutant (α4X) where all 4 glutamine residues previously identified as substrate for α-chain cross-linking by FXIII have been mutated.

Aims: The aims of this study were to directly investigate the relevance of fibrin α-chain cross-linking in fibrin clot formation, structure and stability.

Methods: FGN wild-type (WT), γ3X (γQ398N/Q399N/K406R) and α4X (αQ221N/Q237N/Q328N/Q366N) were produced in CHO cells and purified by affinity chromatography. Cross-linking of α- and γ-chains was analysed by SDS-PAGE. Fibrin formation and lysis were studied by turbidity. Microscale fibrin viscoelasticity was analysed by magnetic tweezers. ANOVA was used to analyse the data.

Results: SDS-PAGE showed that whilst α- and γ-chains were cross-linked within 15 and 5 minutes (respectively) for WT, α-chain cross-linking was largely delayed (120min) for α4X whilst γ-chain cross-linking was abolished for γ3X. Preliminary data showed that the addition of FXIII increased the time to half lysis for WT and γ3X, but not for α4X. Magnetic tweezers showed that in the absence of FXIII, clot stiffness (G', storage modulus) was decreased for both α4X and γ3X, compared to WT. When FXIII was added, G' was significantly increased for WT and γ3X, but unchanged for α4X.

Conclusions: Our data show that fibrin α-chain cross-linking is a key player in reducing lysis and increasing clot stiffness. This novel FGN variant provides a welcome tool for the study of (patho)physiological role of fibrin α-chain cross-linking by FXIII *in vitro* and *in vivo*.

HEMORRHAGIC DISORDERS, HEMOPHILIA

ASY 01.2 | Fitusiran, an Investigational RNAi Therapeutic Targeting Antithrombin for the Treatment of Hemophilia: Interim Results from a Phase 2 Extension Study in Patients with Hemophilia A or B with and without Inhibitors

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Background: Hemophilia is a bleeding disorder characterized by a defect in the ability to generate sufficient thrombin for effective hemostasis. Fitusiran is a subcutaneously (SC) administered investigational RNA interference (RNAi) therapeutic targeting antithrombin (AT) as a means to improve thrombin generation (TG) and promote hemostasis in patients with hemophilia A or B with and without inhibitors. Interim data from the Phase 1 study showed fitusiran was generally well tolerated and administration of monthly fitusiran led to dose-dependent AT lowering, TG increase, and decrease in bleeding frequency (Pasi KJ, et al. *Haemophilia*. 2016, 22(Suppl 4). Pasi KJ et al. *Blood*. 2016, 128: 1397. Ragni MV et al. *Blood*. 2016, 128: 2562.).

Aims: To report interim safety, pharmacodynamics (PD), and clinical activity of fitusiran from the Phase 2 extension study.

Methods: The Phase 2 open label extension study (NCT02554773) included patients with hemophilia A or B with and without inhibitors, previously dosed in the Phase 1 study. Patients received monthly, fixed SC doses of fitusiran, 50 mg or 80 mg.

Results: As of Oct 2016, 23 patients were enrolled in the study. Previously reported data showed that fitusiran was generally well tolerated, with no serious adverse events related to study drug and no thromboembolic events. Once-monthly subcutaneous dosing achieved dose-dependent AT lowering of ~80% and TG levels approaching the lower end of normal range. Exploratory post-hoc analysis of bleed events showed median ABR=1 in patients without inhibitors and median ABR=0 in patients with inhibitors. Bleed events

were successfully managed with either replacement factors (patients without inhibitors) or bypassing agents (patients with inhibitors). Updated safety, tolerability and clinical activity, including the management of bleed events, will be presented.

Conclusions: Emerging clinical data suggest that fitusiran-mediated lowering of AT may be a promising investigational approach for promoting hemostasis in hemophilia.

ASY 01.3 | Musculoskeletal Ultrasound for Intra-articular Bleed Detection: A Highly Sensitive Imaging Modality Compared to Conventional Magnet Resonance Imaging

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Background: There is an increasing demand for musculoskeletal ultrasound (MSKUS) to detect hemophilic joint bleeding, but there is uncertainty regarding the blood detection concentration threshold, and if magnetic resonance imaging (MRI) would be more accurate.

Aims: We studied intra-articular blood detection by MSKUS in comparison to MRI.

Methods: Blood concentrations of 0, 5, 10, 25, 50, 75 and 100% in plasma or saline (enriched with 2 mg/mL hyaluronic acid to mimic joint fluid) were imaged at room temperature after 0-2 hrs, 5 hrs, 1 day, 3 days, and 1 week after transfer into 3-5 cc syringes, and also after injection of 3-5 cc into metatarsophalangeal joints of cadaveric pig feet. MSKUS was performed using a 9-18 MHz linear transducer, and MRI was performed at 3T using T1-weighted and T2-weighted fat-suppressed sequences. Images were reviewed by both a hematologist formally trained in MSKUS and a musculoskeletal radiologist.

Results: MSKUS permitted the detection of blood in syringes and pig joint spaces at concentrations as low as 5%, demonstrated by the presence of echogenic signals that were absent with plasma or saline only. However, there was no visual difference in echogenicity in relation to blood concentrations. As blood clot and degradation products formed over time, echogenicity patterns of the fluid changed dynamically during the 1 week time course. In contrast, no discernible differences between fluids were visible on the T1-weighted or T2-weighted fat-suppressed MRI images.

Conclusions: MSKUS is extremely sensitive to detect low concentrations of intra-articular blood at low volumes, and to discriminate between bloody and non-bloody fluid, whereas conventional MRI is not. These observations are the first of their kind, demonstrate the advantages of MSKUS over MRI to detect intra-articular blood, and should enable MSKUS for rapid bleed detection in hemophilia clinics.

ASY 01.4 | The Thunder Study: Haemophilia Epidemiology Treatment, Outcome and Unmet Needs in the UK: An Analysis from the UK National Haemophilia Database

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Background: The Thunder study is an analysis of UK National Haemophilia Database data sponsored by Roche.

Aims: It aims to determine the current Haemophilia A (HA) and inhibitor epidemiology, treatment patterns and outcomes and to quantify unmet needs for this patient group.

Methods: All 5732 patients (pts) registered with HA in 2015 were analysed. 1428 pts with severe (1155) or moderate (273) HA using the HAEMTRACK electronic pts diary were analysed for treatment patterns and bleeding outcomes.

Results: We reported 1831, 884 and 3017 pts with severe, moderate and mild HA, respectively, of whom 96%, 71% and 25% were treated in 2015. Regular prophylaxis was used in 95% of children < 12 years (yrs), falling to 74% of pts age ≥40 yrs. A med. (IQR) of 3,591 IU/Kg/year (2,194-5,247) and 1,321 IU/kg/year (250-3,642) in 2015 were used for severe and moderate HA, respectively. Annual usage has increased by 21% (severe) and 25% (moderate) in 5 years through a 13% (severe) and 4% (moderate) increase in pts and 8% (severe) and 21% (moderate) increase in treatment intensity. Factor VIII inhibitors were reported at some time in 406/1831 (22%) of severe HA, 94/884 (11%) of moderate HA and 62/3017 (2%) with mild HA. Inhibitors were present during 2015 in 161 (9%); 35 (4%) and 61 (2%) pts with severe/moderate/mild HA, respectively. The age-incidence of inhibitors was bimodal for both severe and moderate HA. In inhibitor-free severe HA managed with prophylaxis, median annualized bleed rate increased with age from 1.0 (IQR 0 - 4.75) (< 12 yrs) to 3.0 (1.0 - 8.0) (≥40 yrs). In severe inhibitor pts treated with prophylaxis, median ABR was 2.0 (0.25 - 8.75) (≤18yrs) and 3.0 (0.75 - 17.50) (>18yrs); Inhibitor pts on bypass therapy prophylaxis had a median ABR of 11.0 (5.0 - 20.0).

Conclusions: The increase in ABR with age in inhibitor-free patients was only partially corrected by prophylaxis. Inhibitors have a progressive adverse effect on ABR. ABR is much higher in inhibitor than non-inhibitor patients, despite prophylaxis.

ASY 12.1 | Next Generation Sequencing in Patients with No Apparent Laboratory Platelet Defect Despite Having an Extensive Bleeding History

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Background: Inherited bleeding disorders comprise a heterogeneous group of diseases that reflect abnormalities of blood vessels, coagulation proteins and platelets. Next-generation sequencing (NGS) technologies have been used previously for the rapid analysis of genes implicated in bleeding disorders and genes known to have a key role in haemostasis.

Aims: To determine the genetic aetiology of disease in a UK-wide cohort of patients with no apparent laboratory platelet defect despite having an extensive bleeding history leading to a clinical diagnosis of a platelet disorder.

Methods: Patient blood was obtained and analysed using a unique approach combining platelet phenotyping by lumi-aggregometry and genotyping by whole exome sequencing (WES), as outlined by the UK-Genotyping and Phenotyping of Platelets (GAPP) study.

Results: In the last 10 years, over 900 patients have been recruited to the UK-GAPP study. Approximately 60% have no platelet defect despite significant bleeding consistent with platelet dysfunction. To date, patients with more than one affected family member in the 'no defect' group have undergone WES (n=17). We have identified and confirmed the genetic basis of disease in two related patients; a heterozygous genetic variant (c.1611 C>A) was found in *THBD* which encodes the protein thrombomodulin and results in a stop codon and truncation of the protein (p.Cys537Stop) showing proof-of-principle of the workflow employed. In addition, novel variants in genes not previously associated with haemostasis have been discovered through our custom developed bioinformatic pipeline. The function of these genes and their encoded proteins is under investigation.

Conclusions: WES combined with platelet phenotyping is an efficient method of determining the genetic cause of disease in patients with inherited bleeding disorders. In addition, it aids in the discovery of novel genetic variants and genes not previously implicated in haemostasis helping further our knowledge of haemostasis.

ASY 12.2 | An International Prospective Cohort Study of Patients with Fibrinogen Deficiency (PRO-RBDD Study)

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Background: Management of patients with fibrinogen deficiency is challenging because the minimum amount of fibrinogen to prevent bleeding is unknown and thromboembolism may occur during substitution therapy.

Aims: To evaluate the incidence of bleeding episodes in patients with fibrinogen deficiency in a 3-year observational prospective study.

Methods: Data on 151 patients were collected in a web-based database at baseline and every 6 months by 17 HTCs worldwide.

Bleeding incidence of any type of bleeding episode and cumulative incidence of the first bleeding requiring replacement therapy were calculated in patients with available data on both antigen and activity levels.

Results: Activity and antigen levels were available on 98 patients (55 females/43 males), of whom 83 are currently on follow up. Twenty-one (25%) patients were afibrinogenemic, 17 (20%) hypofibrinogenemic, 38 (46%) dysfibrinogenemic and 7 (9%) hypodysfibrinogenemic. Patients were followed up for a median of 992 days (IQR:898-1098, min-max:298-1397).

Prophylaxis regimen with fibrinogen concentrate was used in one third of afibrinogenemic patients (7/21) with dosages range of 50-666 mg/Kg/month. Prophylactic treatment reduced the median number of bleeding events per year [from 1 (min-max: 1-3) to 0.4 (min-max: 0-1.25)]. Two allergic reactions and no thrombotic events were reported. Two deliveries (1 vaginal, 1 cesarean) and 15 surgeries (3 major, 10 minor, 2 dental) were performed successfully with no bleeding or thrombotic events, using fibrinogen concentrate or antifibrinolytics.

Conclusions: This observational study showed that the bleeding incidence decreased accordingly to plasmatic fibrinogen levels with the highest in afibrinogenemia and the lowest in dysfibrinogenemia. Preliminary data on prophylaxis in afibrinogenemia showed a reduction of bleeding episodes. A larger group of patients and a longer follow up period are required to find the optimal target level to prevent spontaneous major bleeding.

TABLE 1 Shows results in patients on on-demand therapy. [Bleeding incidence in patients on on-demand therapy]

	Afibrinogenemia	Hypofibrinogenemia	Dysfibrinogenemia	Hypodysfibrinogenemia
Bleeding incidence (patient-year-1)	0.73 (95% CI 0.48-0.97)	0.25 (95% CI 0.12-0.42)	0.12 (95% CI 0.06-0.21)	0.05 (95% CI 0. -0.22)
Bleeding cumulative incidence of the first bleeding treated with replacement therapy at 1400 days of follow up	0.43 (95%C I 0.11-0.64)	0.30 (95% CI 0.05-0.48)	0.06 (95% CI 0.0-0.13)	0.14 (95% CI 0.0-0.37)

ASY 12.3 | Studying Adaptor Protein 3 Dependent Trafficking to Weibel-Palade Bodies Using Hermansky-Pudlak Syndrome Type 2 Blood Outgrowth Endothelial Cells

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Background: Weibel-Palade bodies (WPBs) are endothelial secretory organelles that contain Von Willebrand factor (VWF), P-selectin and CD63 and play a crucial role in hemostasis. WPBs belong to the lysosome-related organelles (LROs), which also includes platelet dense granules, melanosomes and lytic granules. Trafficking of CD63 to WPBs is dependent on the Adaptor Protein 3 complex (AP3). Mutations in the *AP3B1* gene, which encodes for the AP3β3 subunit of the AP3 complex, result in Hermansky-Pudlak syndrome 2 (HPS2). HPS2 is a rare genetic disorder that leads to a bleeding diathesis as a result of abnormal platelet dense granule formation, but secretory organelle defects in HPS2 are not restricted to platelets.

Aims: The objective is to investigate the role of AP3-dependent mechanisms in trafficking of proteins to WPBs.

Methods: Blood outgrowth endothelial cells (BOECs) were isolated from an HPS2 patient with compound heterozygous mutations in *AP3B1*. We used mass spectrometry and subsequent analysis using Maxquant and Perseus to perform whole proteome analysis of HPS2 BOECs versus healthy control BOECs. We generated clonal AP3B1 knockout BOEC lines using CRISPR/Cas9 genome editing of cord blood BOECs. Morphology of WPBs in AP3B1$^{-/-}$ and HPS2 BOECs was studied using confocal microscopy.

Results: HPS2 BOECs contain elongated WPBs that are entirely devoid of CD63, but still contain P-selectin. Whole proteome analysis revealed that apart from AP3β3 also the AP3μ1 subunit of the AP3 complex was depleted from HPS2 BOECs, while AP3δ1 and AP3σ1 subunits were unaffected. Interestingly, several other proteins involved in trafficking and exocytosis were also downregulated in HPS2 BOECs, including the WPB SNARE vesicle associated membrane protein 8 (VAMP8).

Conclusions: *Ex vivo* HPS2 patient-derived BOECs and CRISPR/Cas9-engineered AP3B1$^{-/-}$ BOECs have provided new insights in AP3-dependent trafficking mechanisms in endothelial cells.

ASY 12.4 | Involvement of OCRL, a Phosphoinositide 5-phosphatase, in Cytoskeleton Reorganization and Megakaryocyte Maturation

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Background: Lowe syndrome (LS) is a rare genetic disorder that results from loss-of-function mutations in *OCRL1*. The protein OCRL, a phosphatidylinositol-4,5-bisphosphate 5-phosphatase that contains also a RhoGAP homology domain, is involved in reorganization of the actin cytoskeleton. We previously reported an increased bleeding risk in LS patients that was associated with abnormalities in primary haemostasis. Despite a moderate thrombocytopenia in LS patients, no data however exist regarding the role of OCRL in megakaryocyte (MK) maturation and platelet formation.

Aims: To get further insights into the mechanisms underlying the thrombocytopenia present in Lowe patients by studying MK maturation.

Methods: CD34+ haematopoietic progenitor cells, isolated from blood of 6 controls and 6 patients, were differentiated into MK. CD41+/CD42b+ MK were quantified by FACS. MK morphology was monitored using transmission or immunofluorescence microscopy. Similar experiments were made with normal MK transfected with siRNA to silence OCRL expression.

Results: At D10 of MK differentiation, no difference in CD41+/CD42b+ cell numbers was noted between patients and controls. Control MK formed large proplatelet extensions on D12, and isolated platelets were observed from D14. In LS patients, the number of MK bearing proplatelets was about half the control value. Control MK showed the typical structure of proplatelets with

numerous actin filaments and microtubule extensions with buds. Despite an increase level of F-actin, these structures were less developed for LS patients, and an increased basal phosphorylation of myosin light chain (P-MLC) was noted with abnormal punctuated labelling. Similar results were obtained with MK silenced for OCRL with siRNA.

Conclusions: These results support a role of OCRL in MLC phosphorylation and actin organization accompanying MK maturation and proplatelet formation. Thus, OCRL deficiency in LS causes alterations in cytoskeleton organization that may participate to the defect of platelet production.

ASY 23.1 | Product Type and the Risk of Inhibitor Development in Nonsevere Hemophilia A Patients

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Background: Inhibitor development is a major complication of treatment with factor VIII concentrates in nonsevere hemophilia. It has been suggested that plasma-derived factor VIII concentrates elicit fewer inhibitors than recombinant factor VIII concentrates, however recent studies in severe hemophilia A patients have shown conflicting results. We designed a case-control study that aims to investigate the clinical risk factors and genetic risk factors for inhibitor development in nonsevere hemophilia A patients.

Aims: We investigated whether the type of factor VIII concentrate was associated with inhibitor development in nonsevere hemophilia A patients.

Methods: This nested case-control study includes 75 inhibitor patients (cases) and 223 controls, selected from a source population of the INSIGHT study, including all nonsevere hemophilia A patients (FVIII:c 2-40%) that have been treated with FVIII concentrates in 33 European and 1 Australian center. Cases and controls were matched for date of birth and cumulative number of exposure days (CED) to FVIII products. A conditional logistic regression model was used to calculate both unadjusted and adjusted odds ratios; the latter adjusted for the following a-priori specified confounders.

Results: We did not find an increased risk for inhibitor development for any type of FVIII concentrate; either when comparing recombinant FVIII concentrates to plasma-derived FVIII concentrates (adjusted odds ratio 1.1, 95% confidence interval 0.4-2.8) or for specific types of FVIII concentrates.

Conclusions: In this nested case-control study including nonsevere hemophilia A patients, the type of FVIII concentrate was not associated with the development with inhibiting anti-FVIII antibodies.

ASY 23.2 | Risk Factors for the Progression From Low- to High-titers In 260 Children with Severe Hemophilia A and Newly Developed Inhibitors

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Background: Inhibitors mainly develop in children with severe hemophilia A during the first 50 exposure days and are classified in low- and high-titer according to the peak titer ever reached with a cut off of 5 BU/ml. At the first positive titer, the real nature of the antibody may be unclear, since some low-titers progress to high-titers afterwards.

Aims: The aim of this study was to investigate potential risk factors of the progression towards high-titers in children with severe hemophilia A and newly diagnosed inhibitors.

Methods: This study was a follow-up study of the PedNet Registry and included 260 children with severe hemophilia A and inhibitors, born between 1990 and 2009 and consecutively recruited from 31 hemophilia centers. Clinical and laboratory data were collected from the date of first positive inhibitor test for at least a 3-years follow-up. Immune tolerance induction (ITI) was defined as any regular FVIII replacement regimen of at least 45 IU/kg FVIII given for at least 3 times/week. ITI regimens were defined as daily or non-daily based on injection frequency. High-dose ITI was defined by a FVIII dose given ≥ 100 IU/kg.

Results: At first positive inhibitor test, 49% (n=127) had low-titer inhibitors, however, 50% of them progressed to high-titers following FVIII re-exposure, so only 25% maintained low-titers. Factor VIII gene (*F8*) mutation type was known in 247 patients (95%), including 202 (82%) null mutations. The progression to high-titer inhibitors was associated with null *F8* mutations (OR 2.6, 95%CI 1.0-6.5), family history of inhibitors (OR 7.2, 95%CI 1.8-28.4) and the use of high-dose ITI (OR 3.9, 95%CI 1.5-10.0).

Conclusions: These results suggest that high-dose ITI should be avoided as initial strategy in patients who develop low-titer inhibitors.

ASY 23.3 | Antigen-specific Regulatory T Cells Generated by Factor VIII-CAR Retrovirus Ttransduction Suppress Anti-factor VIII Immune Responses

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Background: The antibody formation to factor VIII (FVIII; F8 in constructs) is a major challenge for effective treatment for hemophilia A (HemA). In our previous studies, the small number of expanded FVIII-specific regulatory T cells (Tregs) from polyclonal $CD4^+$ splenocyte exerted better suppressive function compared with non-specific Tregs.

Aims: In order to obtain the stable source of antigen-specific T cells, we adopted chimeric antigen receptor (CAR) approach to generate antigen-specific Tregs and examined their suppressive function.

Methods: The CAR construct included high-binding anti-FVIII variable region (scFV) linked to signaling and costimulatory moieties of immune receptors and murine Foxp3 (F8CAR-mFoxp3). Murine $CD4^+$ T cells were transduced with retrovirus carrying F8CAR-mFoxp3 transgene to generate engineered Tregs. *In vitro* suppressive assay was performed using engineered Tregs and FVIII-specific effector T cells (Teffs) from FVIII-primed HemA mice. In addition, FVIII-specific CFSE was performed using F8CAR-Tregs and F8CAR retrovirus transduced $CD4^+$ T cells (F8CAR-Teffs). Subsequently, we adoptively transferred $1x10^6$ F8CAR-Tregs into HemA mice to examine their suppressive function *in vivo*.

Results: The engineered F8CAR-mFoxp3 Tregs showed F8CAR and Foxp3 expression by flow cytometry analysis. In both *in vitro* tests, the engineered Tregs exerted FVIII-specific suppressive function towards Teffs from two different sources. One day after adoptive cell transfer, the treated mice were injected with FVIII plasmid hydrodynamically. F8CAR-Foxp3-Tregs more significantly decrease the production of anti-FVIII antibodies overtime compared to control untransduced T cells and polyclonally expanded FVIII-specific Tregs.

Conclusions: F8CAR-mFoxp3 Tregs exerted superior suppressive activities towards FVIII-specific immune responses in both in vitro and in vivo tests, indicating the potential of F8CAR-mFoxp3 engineered cells to modulate anti-FVIII immune responses.

ASY 23.4 | Stabilin-2 Deficiency or Competitive Inhibition Reduces Human FVIII Immunogenicity in a VWF-dependent Manner

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Background: Inhibitory antibodies against factor VIII (FVIII) remain the most significant complication in hemophilia A (HA). To date, no receptor has been described to influence FVIII immunogenicity *in vivo*. The scavenger receptor stabilin-2 (Stab-2) contributes to the clearance of human von Willebrand factor (VWF) and FVIII.

Aims: To assess the role of Stab-2 on FVIII immunogenicity.

Methods: Association of VWF with Stab-2 in the spleen was assessed using immunofluorescent staining of human pdVWF-infused VWF KO mice. Wild-type (WT) and Stab-2 knockout (KO) C57Bl/6 mice were infused weekly with 2 IU (~50 IU/kg) of human plasma-derived FVIII/VWF (pdFVIII; VWF:FVIII ratio of 2.4:1) or human recombinant FVIII (rFVIII). FVIII antibodies and inhibitors were assessed by ELISA and one-stage Bethesda assay at day 28.

Results: Infused pdVWF was found to associate with CD31 and Stab-2-expressing cells in the spleen.

Compared to WT mice, Stab-2 KO mice treated with pdFVIII showed a lower incidence of αFVIII IgG (30% vs 90%; p=0.02; n=10) and lower aFVIII antibody titres (p=0.003). Stab-2 KO mice also displayed a decreased incidence of inhibitors (≥0.6 BU) compared to WT KO mice (10% vs 50%; p=0.14) and higher inhibitor titres (p=0.051). All mice developed αhuman VWF IgG, but titres were lower in Stab-2 KO mice (p=0.003). Stab-2 KO mice exposed to rFVIII exhibited a less pronounced decline in the incidence of αFVIII IgG (40% vs 80% respectively; p=0.17; n=10) and inhibitors (20% vs 50%; p=0.35) compared to WT mice.

Concurrent infusion of pdFVIII with 100 μg of hyaluronic acid (a major Stab-2 ligand) in WT mice decreased the incidence (10% vs 90%; p=0.001; n=10) and titre of αFVIII IgG (p=0.0008). No significant differences were observed in the titre of αVWF IgG.

Conclusions: Stab-2 plays a role in the immunogenicity of human FVIII in a VWF-dependent manner. Inhibiting Stab-2-VWF/FVIII interaction may be an effective method to prevent inhibitors in hemophilia A patients.

ASY 27.1 | A Sparse Sampling Method for FVIII Population Pharmacokinetic (PK) Analyses in Males with Severe Hemophilia A (SHA)

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Background: Standard PK assessments are demanding for patients with SHA, requiring a 72 hr washout and minimum of 5 timed blood samples at baseline and following FVIII infusion (ISTH Guidelines, 2001). A sparse sampling method is an attractive alternative.

Aims: The aim of this study was to identify a practical sparse sampling method using a population PK program (myPKFiT™) built from pre-licensure PK data of a recombinant FVIII (rFVIII) concentrate.

Methods: 27 inhibitor negative males with SHA (FVIII< 1%) receiving a standard half-life rFVIII were pooled from 2 studies (median age 12 yrs, range 5-34). Subjects were consented and studies approved by the research ethics boards. 50 IU/kg rFVIII was infused after a 72 hr washout. FVIII activity levels were measured pre-infusion and at 1, 9, 24 and 48 hrs post-infusion (5-point PK, study 1). Study 2 (6-point PK) had an added 3 hr post-infusion sample. Study 2 also included a 2-point PK taken 1 week later (no washout): a sample was taken at the start of a clinic visit 24 hrs after self-infusion at home (15-50 IU/kg), followed by an in-clinic infusion of 25 IU/kg and 3 hr post-infusion sample. PK parameters clearance (Cl), volume of distribution at steady state (Vss), terminal half-life (t1/2) and time to 1% (tt1%) were calculated using the PK program. Intra-class correlations (ICCs) were used to compare the sparse sampling methods to the full 5 or 6 point PK.

Results: Table 1 shows an overview of PK parameters derived from the 5 sampling time points. It was noted, in males < 18 yrs (n=23), Cl was strongly negatively (r=−0.73, p< 0.01) and t1/2 strongly positively (r=0.78, p< 0.01) correlated with age. Table 2 shows the agreement between full sampling PKs and sparse sampling methods.

Conclusions: There is substantial/almost perfect agreement for all PK parameters between the sparse sampling methods and full 5 or 6 point PKs. In an outpatient setting, the 24 and 3 hr sampling method is the most practical option for obtaining a PK profile estimate in a single clinic visit.

TABLE 1 Overview of PK parameters derived from the 5 sampling time points

PK parameters	median (range), n=27
Cl (dL/hr/kg)	0.03 (0.02-0.05)
Vss (dL/kg)	0.5 (0.3-0.6)
t1/2 (hrs)	12.4 (8.2-16.1)
tt1% (hrs)	79.0 (52.0-110.0)

ASY 27.2 | Factor VIII Concentration and the Risk of Spontaneous Bleeding Following Treatment with rFVIII (Turoctocog Alfa) in Patients with Severe Haemophilia A

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Background: Turoctocog alfa, a B-domain truncated recombinant FVIII, showed favourable safety and efficacy in adults/adolescents (guardian™1) and children (guardian™3) with severe haemophilia A. The

TABLE 2 ICC agreements between full sampling PKs and sparse sampling methods.

	PK parameters	24 & 3 hrs, ICC (95% CI)	1 & 9 hrs, ICC (95% CI)	1 & 24 hrs, ICC (95% CI)
5 point PK (n=27) vs	Cl	n/a	0.97 (0.94-0.99)	0.93 (0.85-0.97)
	Vss	n/a	1.00 (1.00-1.00)	1.00 (1.00-1.00)
	t1/2	n/a	0.90 (0.80-0.95)	0.92 (0.83-0.96)
	tt1%	n/a	0.90 (0.80-0.96)	0.91 (0.82-0.96)
6 point PK (n=13) vs	Cl	0.81 (0.41-0.95)	0.98 (0.94-0.99)	0.90 (0.72-0.97)
	Vss	0.68 (0.13-0.91)	0.90 (0.70-0.97)	0.90 (0.70-0.97)
	t1/2	0.81 (0.48-0.96)	0.95 (0.83-0.98)	0.89 (0.68-0.97)
	tt1%	0.78 (0.34-0.94)	0.95 (0.83-0.98)	0.87 (0.63-0.96)

recently completed guardian™2 trial is a large extension of the guardian™1 and guardian™3 phase 3 trials.

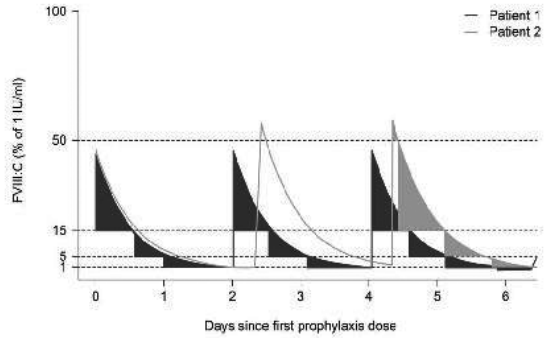
Aims: To investigate the relationship between FVIII concentration (FVIII:C) and annualised bleeding rate (ABR) for spontaneous bleeds.

Methods: A population pharmacokinetic (PK) model derived from the guardian™ trials was combined with patients' diary-recorded dosing data. Each patient's time on prophylaxis was categorised into five clinically meaningful groups of predicted FVIII:C (Figure, 0-1%, >1-5%, >5-15%, >15-50% and >50%) calculating exposure time, mean FVIII:C and number of bleeds. Bleeding data were used to estimate ABRs for each category using negative binomial regression and predictions of a parametric model. Relationships between ABR and mean FVIII:C were evaluated for the overall dataset by trial phase (guardian™1 and 3 [main phase] + guardian™2 [extension phase]) and age (adults/adolescents [≥12 years] versus children [0-< 12 years]).

Results: Patient numbers and patient years of exposure (PYE) in the populations analysed were: main (n=170; PYE=84.78) versus extension phase (n=185; PYE=665.61), adults/adolescents (n=133; PYE=504.88) versus children (n=54; PYE=245.51), and overall (n=187; PYE=750.40). Patients were predicted to reach FVIII:C levels associated with non-severe haemophilia for 86.6% of the time. Spontaneous bleed ABR decreased as FVIII:C increased (Table) evident in both the main and extension phases, with lower ABRs in the extension phase. The FVIII:C-response relationship was apparent for adults and children, although children had lower ABRs than adults.

Conclusions: Bleed risk data offer insight into tailoring prophylactic regimens. Clinicians should consider the association between PK, age, and bleed risk when designing prophylaxis strategies.

Figure: Patients' time spent in different FVIII:C activity ranges predicted from dosing diaries



The first week of prophylaxis is illustrated for two representative patients on thrice weekly turoctocog alfa dosing. Predicted profiles of FVIII:C activity were calculated from the patients' diary information on doses and timing of doses. Both patients exhibited a dosing interval pattern in their first week of 2-2-3 days, although the last two doses were delayed and higher for Patient 2 compared to Patient 1.

Triangles represents the area under the curve (AUC) of FVIII:C contributing to the mean FVIII:C value for each range. The horizontal span of each triangle defines the time spent in the relevant FVIII:C range. Both the AUC and time spans were calculated across dosing intervals and patient years.

FIGURE 1

TABLE 1

Table: FVIII concentration ranges and spontaneous ABR

FVIII concentration range (mean)	Patient years	Total number of spontaneous bleeds	Mean spontaneous ABR (negative binomial estimate, 95% CI)				
			Analyses population				
			Overall	Main	Extension	Adults / Adolescent	Children
0–1% (0.4%)	101	324	4.19 (3.28–5.39)	5.79 (4.02–8.41)	3.25 (2.40–4.48)	5.53 (4.18–7.36)	2.03 (1.29–3.26)
>1–5% (2.7%)	203	374	2.80 (2.18–3.62)	4.54 (3.20–6.56)	1.70 (1.27–2.33)	3.44 (2.66–4.49)	1.28 (0.64–2.77)
>5–15% (9.2%)	180	361	2.48 (1.92–3.24)	3.74 (2.49–5.76)	1.72 (1.28–2.34)	3.15 (2.38–4.22)	0.85 (0.48–1.54)
>15–50% (29.0%)	205	200	1.06 (0.80–1.41)	1.48 (0.91–2.43)	0.86 (0.63–1.19)	1.33 (0.99–1.82)	0.37 (0.19–0.76)
>50% (71.0%)	62	14	0.24 (0.12–0.46)	0.63 (0.14–1.92)	0.19 (0.09–0.37)	0.40 (0.20–0.74)	0.03 (0.00–0.15)

ABR, annualised bleeding rate; CI, confidence interval; FVIII, Factor VIII.

ASY 27.3 | Current PK Tools Differ in Prophylactic Dosing Advice of Factor VIII Concentrate in Hemophilia A Patients

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Background: Patients with severe and moderate hemophilia A (HA) are often treated prophylactically with factor VIII (FVIII) concentrate. Individualization of prophylaxis can be achieved by pharmacokinetic (PK)-guided dosing. Currently three PK tools are available for calculating the FVIII dose to ensure the FVIII concentrations above the target FVIII trough level.

Aims: To compare the estimated PK parameters and FVIII doses generated by the current PK tools.

Methods: Twenty-seven patients with HA (FVIII< 0.05 IU/ml) underwent individual PK profiling after a FVIII bolus of 50 IU/kg (Advate®) followed by three FVIII measurements at 4, 24 and 48 hours. The PK was analyzed by application of (1) Bayesian analysis in NONMEM®-software using population model reported by Bjorkman et al. 2012, (2) myPKFiT PK tool, and (3) WAPPS-Hemo portal. For each patient individual PK parameters clearance (CL) and volume of distribution at steady-state (Vss) were estimated by (1) and (2). The terminal half-life was produced by all methods, whereas FVIII dosing advices were calculated by (1) and (2) aiming for trough levels >0.01 IU/ml.

Results: Age (mean±SD) of the patients was 40.8±20.6 year with a body weight of 80.4±17.0 kg. Method (2) did not produce individual PK parameters for five patients. V_{ss} estimated by (2) was significantly higher than by (1) and CL estimated by (2) was lower than by (1) (Table 1). The three methods produced different estimates for terminal half-life; values were 10.6±2.1h (1), 12.5±1.9h (2) and 11.4±1.9h (3) (p< 0.001). Due to the shorter half-life (1) produced significantly higher doses for severe HA patients while dosing every 48 hours (mean difference: 721 IU, p=0.01).

Conclusions: Currently available PK tools show significant variation in individual PK parameters estimates and consequently a significant difference in the advised prophylactic dose. Prospective validation of

TABLE 1 Individual PK parameters and FVIII doses generated by the current PK tools

	(1) Bayesian analysis using NONMEM		(2) MyPKFiT		(3) WAPPS-Hemo portal		
	Mean	SD	Mean	SD	Mean	SD	P-value
Volume of distribution at steady state (ml)	4028	824	4170	665	NA	NA	0.038
Clearance (ml/h)	274	66	266	56	NA	NA	0.051
Elimination half-life (h)	10.6	2.1	12.5	1.9	11.4	1.9	<0.001
Dose of FVIII - each 48 hour	1949	957	1229	402	NA	NA	0.010

PK tools is crucial to attain a uniformed approach towards PK-guided dosing of FVIII prophylaxis.

ASY 27.4 | Preoperative Screening for Bleeding Disorders: A Comprehensive Laboratory Assessment of Clinical Practice

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Background: Patients with mild bleeding disorders (MBDs) are at risk of perioperative bleeding, but preoperative screening for MBDs remains challenging.

Aims: We aimed to 1) assess and compare the prevalence of haemostatic deficiencies in patients with and without reported bleeding symptoms on a preoperative questionnaire, consisting of guideline-proposed bleeding questions, and 2) appraise the diagnostic value of the ISTH-Bleeding Assessment Tool (BAT), and laboratory screening tests for the identification of patients with haemostatic deficiencies.

Methods: Patients without known bleeding disorders were included. All patients completed the ISTH-BAT and underwent haemostatic testing (Table 1). Haemostatic deficiencies were defined as coagulation, von Willebrand Factor (vWF) or fibrinolysis factor levels below reference range (usually < 50-60%) or ≥2 abnormal LTA curves. Informed consent was obtained and the medical ethics committee approved the study.

Results: In 21 of 240 (8.8%) patients reporting bleeding symptoms, mild haemostatic deficiencies were found, including 7 coagulation factor deficiencies, 10 platelet function abnormalities and 4 low vWF levels. In comparison, 10 of 95 (10.5%) patients not reporting bleeding symptoms had a deficiency. The prevalence of deficiencies was not different between the groups after adjustment for age, gender, blood type (p=0.48). The ISTH-BAT could not identify patients with haemostatic deficiencies (ROC-curves: women AUC 0.51 (95%CI 0.34-0.67), p=0.94. Men AUC 0.55 (95%CI 0.39-0.72), p=0.51), while the aPTT, PT and PFA had high specificity but low sensitivity to detect haemostatic deficiencies (Table 2).

Conclusions: The prevalence of haemostatic deficiencies in both patients with and without reported bleeding symptoms was 9-10%. This indicates

TABLE 1 Haemostatic confirmatory and screening assays

	Coagulation	vWF	Platelet function	Fibrinolysis
Confirmatory tests	Fibrinogen, factor II, V, VII, VIII, IX, X, XI, XII, XIII	vWF antigen and activity	Light transmission aggregometry (LTA); agonists: arachidonic acid, TRAP, Collagen 1 and 4 µg/mL, Epinephrine, Ristocetin, ADP 5 and 10 µmol/L	Tissue Plasminogen Activator, Plasminogen Activator Inhibitor, α2-antiplasmin
Screening tests	activated Partial Thromboplastin Time (aPTT), Prothrombin Time (PT), Thrombin Time (TT)	Platelet Function Analyser (PFA)	PFA	Euglobulin Lysis Time

TABLE 2 Diagnostic performance of screening assays for detecting haemostatic deficiencies in the confirmatory assays

	All patients (n=335), abnormalities n (%)	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
aPTT (> 32 sec)	3 (0.9)	5.9 (0.3-15)	99 (99-100)	33 (1.8-87)	95 (95-96)
PT (> 11.5 sec)	5 (1.5)	63 (32-63)	100 (99-100)	100 (50-100)	99 (98-99)
TT (> 21 sec)	0	NA	100	NA	100
PFA-epi (Closure Time > 160 sec)	19 (5.7)	10 (1.8-30)	93 (93-95)	8.7 (1.5-2.7)	94 (94-95)
PFA-ADP (Closure Time > 118 sec)	23 (6.9)	5.0 (0.3-24)	94 (94-96)	5.3 (0.3-25)	94 (94-95)
Euglobulin lysis time (<40 min)	1 (0.3)	0 (0-91)	99.7 (99-100)	0 (0-91)	99.7 (99-100)

that the guideline-based questionnaire could not differentiate between preoperative patients with and without deficiencies, while the discriminative power of the aPTT, PT, PFA and ISTH-BAT was also limited.

MANAGEMENT OF THROMBOEMBOLISM

ASY 10.1 | Mortality in Patients with Intracerebral Hemorrhage Associated with Vitamin K Antagonists, Direct Oral Anticoagulants, Antiplatelets or No Antithrombotic Therapy

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Background: Whether mortality is increased in patients with intracerebral hemorrhage (ICH) associated with vitamin K antagonists (VKAs), direct oral anticoagulants (DOACs), or antiplatelets (APs) remains unclear.

Aims: To assess the risk for death in patients with ICH associated with VKAs, DOACs, or APs or no antithrombotic treatment.

Methods: This is a multicenter cohort study in consecutive patients admitted for ICH while on treatment with VKAs, DOACs, APs or no antithrombotic. Cerebral CTs were centrally evaluated by a panel unaware of antithrombotic treatment. Primary outcome was death at 30 days.

Results: 481 patients with ICH associated with VKA (163 patients), DOAC (54 patients), AP (97 patients) or no antithrombotic treatment (167 patients) were included in the study (Table 1).

At multivariate analysis, age (HR 1.04 per year; 95% CI 1.02-1.07), GCS ≤8 at admission (HR 5.47; 95% CI 3.58-8.35) and previous stroke (HR 1.96; 95% CI 1.21-3.18) were independent predictors of death. No association was found between antithrombotic therapy and death (HR 1.07, 95% CI 0.57-2.01 for VKAs; HR 1.18, 95% CI 0.58-2.44 for DOACs; HR 1.06, 95% CI 0.53-2.10 for APs) after adjusting for differences among the study groups.

Baseline cerebral CT was available for central reading in 339 patients (83 VKA, 35 DOAC, 80 AP and 141 no antithrombotic patients). In these patients, ICH volume at admission ≥30 mL (HR 2.78; 95% CI 1.54-5.02) was an independent predictors of death.

TABLE 1 Clinical features of patients, according with treatment

	No Antithrombotics=167	APs=97	VKAs=163	DOACs=54
Age,mean±SD	68±14	76±10	79±9	80±9
Hypertension, n (%)	128 (77)	86 (89)	126 (77)	36 (67)
Renal failure, n (%)	6 (4)	14 (14)	29 (18)	7 (13)
Diabetes, n (%)	25 (15)	24 (25)	36 (22)	15 (28)
Previous stroke, n (%)	3 (2)	18 (19)	50 (31)	16 (30)
Heart failure, n (%)	4 (5)	11 (11)	34 (21)	5 (7)
Vascular disease, n (%)	2 (1)	25 (26)	47 (29)	7 (13)
GCS ≤8, n (%)	13 (8)	11 (11)	34 (21)	11 (20)
Trauma, n (%)	11 (7)	11 (11)	52 (32)	11 (20)

TABLE 2 Death at 30 days occurred in 90 patients (19%). [Death at 30 days and radiological features, according with treatment]

	No antithrombotics	APs	VKAs	DOACs
Death at 30 days, n (%)	18/167 (11)	16/97 (16)	39/163 (24)	17/54 (31)
DEEP, n (%)	82/161 (38)	45/89 (50)	39/88 (44)	14/37 (38)
LOBAR, n (%)	65/161 (40)	37/89 (41)	38/88 (43)	20/37 (54)
POSTERIOR FOSSA, n (%)	14/161 (9)	7/89 (8)	11/88 (12)	3/37 (8)
Volume at admission >30 mL, n (%)	38/141 (27)	26/80 (32)	27/83 (32)	15/35 (43)
Volume at admission, mean±SD; median; IQR	24.85±33; 11.6; 29.1	30.02±40; 12.4; 32.3	34.48±47; 12.8; 38.7	42.26±52; 18.3; 51.1
Intraventricular extension, n (%)	38/141 (27)	32/80 (40)	26/83 (31)	11/35 (31)
Volume expansion ≥6 mL or 33%, n (%)	28/134 (21)	17/74 (23)	22/73 (30)	8/29 (27)

Baseline and 48-hours CTs were available for central reading in 310 patients (73 VKA, 29 DOAC, 74 AP or 134 no antithrombotic patients). In these patients, volume expansion (>6 mL or 33%) (HR 2.31; 95% CI 1.20-4.45) was independent predictor of death.

Conclusions: GCS, ICH volume at admission and volume expansion were predictors of death in ICH patients regardless of the associated antithrombotic therapy.

ASY 10.2 | Rates, Management and Outcome of Bleeding Complications during Apixaban and Edoxaban Therapy in Daily Care - Results from the Dresden NOAC Registry (NCT01588119)

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Background: Apixaban and edoxaban (A/E) are approved for treatment and prevention of thromboembolic diseases but, in contrast to rivaroxaban and dabigatran, detailed data on the rates, management and outcome of bleeding complications in daily care are scarce.

Aims: To evaluate rates, management and outcome of bleeding complications during A/E therapy.

Methods: Bleeding events during A/E therapy were evaluated using data from a prospective, non-interventional oral anticoagulation registry of daily care patients. All A/E patients enrolled in the *Dresden NOAC registry* were assessed and bleeding events which occurred during A/E exposure (during or within 3 days after last intake) were evaluated.

Results: Between December 1st 2012 and November 30th 2016, 3419 patients were enrolled into the registry. Of these, 677 patients (19.8%) received apixaban and 292 patients (8.5%) received edoxaban. The mean duration of follow-up was 521.7±355.9 days (median 512 days; IQR 184/822 days). The mean duration of A/E exposure was 451.8±360.7 days (median 275 days; IQR 135/791 days). During follow-up 405 patients (41.8%) reported 672 bleeding events during or within 3 days after last intake of A/E (69.9% minor, 48.9% clinically relevant non-major (CRNM) and 7.4% major according to ISTH definition).

The main driver for classification as "ISTH major bleeding" was "transfusion of ≥ 2 units of red blood cells" (16/33), followed by "drop of haemoglobin > 2g/dl" (15/33), "critical organ bleeding" (13/33) and "fatal bleeding" (3/33). Most cases of major bleeding (66.7%) could be treated conservatively but in 11 cases (33.3%) surgical or interventional treatment was needed.

Following bleeding, all-cause mortality at 90 days were 1.7% (7/405) for all bleeding and 2.5% (5/198) for CRNM and 20% (6/30) for major bleeding, respectively.

Conclusions: In daily care, bleeding complications are frequent also with apixaban/edoxaban treatment. However, bleeding rarely manifests as major bleeding and such cases can mostly be managed by transfusion only.

ASY 10.3 | Prevention of Exsanguination under Apixaban Anticoagulation Using Andexanet Alfa in a Polytrauma Model

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Background: Life-threatening bleeding requires prompt reversal of factor Xa inhibitors. Andexanet alfa is being investigated for reversal of their anticoagulation effects.

Aims: The ability of andexanet to reverse bleeding in an apixaban anticoagulated porcine trauma model was investigated.

Methods: After ethical approval, male pigs (n=15) were given apixaban for 3 days (20 mg daily), the sham group (n=5) received placebo. A standardized polytrauma by blunt liver injury and bilateral femur

TABLE 1 Severity and management strategies of apixaban-/edoxaban-related bleeding complications

672 bleeding events in 405 patients	Conservative (no treatment or compression / tamponade / transfusion)	Surgery or intervention	Red blood cell transfusion	Vitamin K supplementation	Fresh frozen plasma transfusion	Prothrombin complex concentrate	Recombinant Factor VII
Minor 375/672 (55.8%)	375/375 (100.0)	0	0	0	0	0	0
NMCR 264/672 (39.3%)	225/264 (85.2)	39/264 (14.8)	2/264 (0.8)	0	0	0	0
Major 33/672 (4.9%)	22/33 (66.7)	11/33 (33.3)	16/33 (48.5)	0	4/33 (12.1)	3/33 (9.1)	0
TOTAL	622/672 (92.6)	50/672 (7.4)	18/672 (2.7)	0	4/672 (0.6)	3/672 (0.4)	0

fractures was inflicted. 12 min post trauma animals were randomized (n=5 group) to a single andexanet bolus (1000 mg), a bolus (1000 mg) + infusion (1200 mg over 2 h) regimen, or vehicle (control). Blood loss (BL) and hemodynamics were monitored over 5 h or until time of death and analyzed by ANOVA (mean±SEM).

Results: Apixaban anti-fXa levels were 183±26 ng/mL with no differences between anticoagulated groups prior to injury. BL in the sham animals was 494±24 mL 12 min after injury (Total BL 651±39 mL at 5 h, 100% survival). Anticoagulation with apixaban significantly increased BL 12 min after injury (873±25 mL, p< 0.01). Controls exhibited a total BL of 3913±235 mL with 100% mortality (mean survival time 165 min). Treatment with a bolus or bolus + infusion of andexanet was associated with a significant reduction in BL and 100% survival (Fig. 1a). 2 h after injury, apixaban anti-fXa levels in bolus animals were 99±45 ng/mL, while the levels were 17±6 ng/mL in the bolus + infusion animals (Fig. 1b). Hemodynamic parameters (e.g., cardiac output) and markers of shock (e.g., lactate) recovered to pre-trauma levels in andexanet treated groups. Clinically and macroscopically no adverse events were observed.

Conclusions: In this study, andexanet safely reversed anticoagulation effects of apixaban, reduced BL, and decreased mortality induced by severe trauma under anticoagulation. The bolus alone had a similar impact as the bolus + infusion regimen in this lethal porcine model.

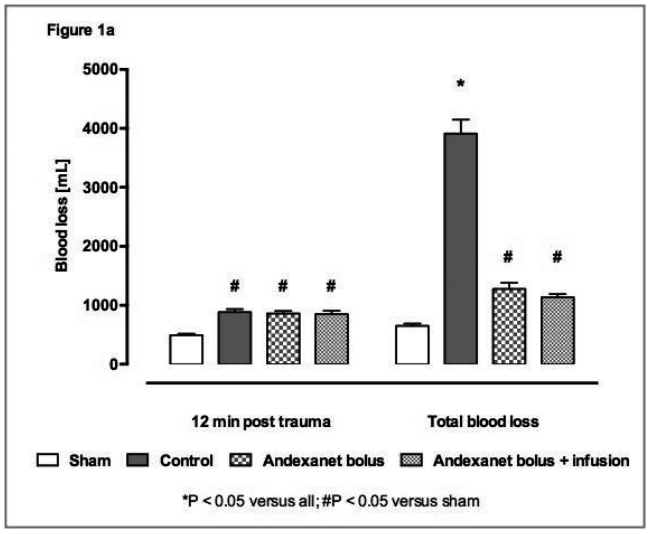

FIGURE 1A Blood loss [mL]

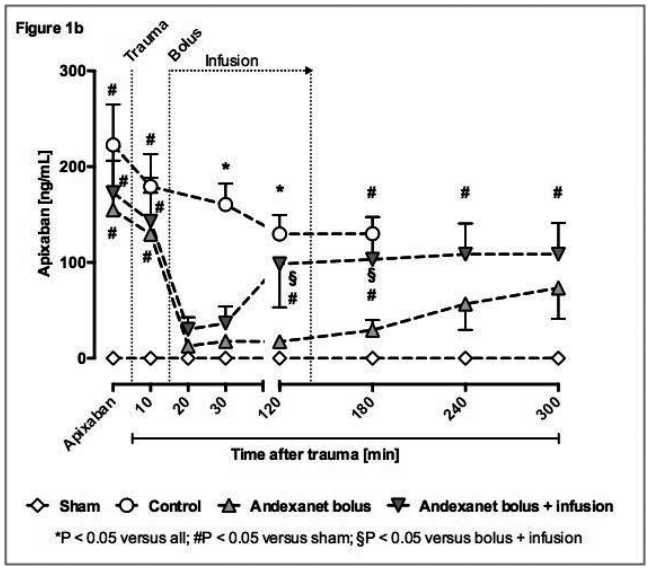

FIGURE 1B Apixaban concentration [ng/mL]

ASY 10.4 | Reduction of Hepatic Factor XII Expression in Mice by ALN-F12 Inhibits Thrombosis without Increasing Bleeding Risk

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Background: Plasma coagulation Factor XII (FXII) plays a crucial role in contact activation, ultimately regulating both the kallikrein-kinin system and the intrinsic pathway of coagulation. A growing body of evidence suggests that inhibition of FXII, and its proximal substrate Factor XI (FXI), can prevent thrombosis. Given FXII does not appear to modulate hemostasis, targeting FXII appears to be a promising strategy for the prevention of pathological thrombus formation without the hemostatic risks typically associated with anticoagulants. To this end, a subcutaneously administered investigational RNAi therapeutic targeting F12 mRNA (ALN-F12) was developed.

Aims: To investigate the thromboprotective and hemostatic effects of FXII reduction by ALN-F12 in rodent thrombosis and hemostasis models.

Methods: A single dose of ALN-F12 was subcutaneously administered to C57Bl/6 mice. After reaching steady state FXII reduction, the impact on hemostasis (saphenous vein and tail tip transection bleeding models) and thrombosis (ferric chloride and laser injury induced thrombosis models) was evaluated. A siRNA targeting Factor XI (FXI-siRNA) was included for comparison.

Results: Administration of ALN-F12 resulted in dose-dependent reductions of F12 mRNA and plasma FXII protein. Further, ALN-F12 led to dose dependent reductions in platelet and fibrin accumulation in the thrombosis models evaluated in this study. At 10 mg/kg ALN-F12, the top dose level evaluated, this resulted in >95% reduction of plasma FXII and ~10 fold reduction in fibrin deposition. Similarly, administration of FXI-siRNA also led to dose dependent reductions in FXI as well as platelet and fibrin accumulation; >95% FXI reduction resulted in ~5 fold reduction in fibrin deposition. Finally, hemostasis models showed that >95% reduction of plasma FXII or FXI had no impact on bleeding time or blood loss.

Conclusions: Reduction of plasma Factor XII by ALN-F12 provided thromboprotective effects with no increased bleeding risk in rodent models of thrombosis and hemostasis.

ASY 14.1 | Two Years versus Six Months of Oral Anticoagulation after a First Episode of Unprovoked Proximal Depp Vein Thrombosis: the PADIS DVT Multicenter, Double-blind, Randomized Trial (Clinical Trials. Gov Number NCT00740493)

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Background: Patients with unprovoked deep vein thrombosis (DVT) have a high risk of recurrent venous thromboembolism (VTE) after stopping anticoagulation; however, the optimal duration of anticoagulation remains uncertain.

Aims: To assess the benefits and risks of extended anticoagulation after a first episode of unprovoked DVT initially treated during 6 months.

Methods: In a multicenter, randomized, double-blind, controlled trial, we compared an additional 18 months of warfarin with placebo in patients with a first episode of unprovoked proximal DVT initially treated during 6 months. After stopping study treatment, all patients were followed up for an additional period of 2 years. Primary outcome was the composite of recurrent VTE or major bleeding during the 18-month treatment period. Secondary outcomes included the composite outcome during the entire study period, deaths not caused by VTE or major bleeding and the components of the composite outcome. All outcomes were centrally adjudicated.

Results: A total of 104 patients were included and analyzed on an intention-to-treat basis. During the treatment period, the composite outcome occurred in 0 of 50 patients in the warfarin group and in 16 of 54 patients (29.6%) in the placebo group (hazard ratio [HR], 0.03; 95% confidence interval [CI], 0.01-0.09; p< 0.001). During the entire study period of 42 months, the composite outcome occurred in 14 (36.8%) patients in the warfarin group and in 17 (31.5%) in the placebo group (HR, 0.72; 95%CI, 0.35-1.46; p=0.36) (Figure). There were 1 major bleeding in the warfarin group and 3 deaths unrelated to study outcome (1 in the warfarin group and 2 in the placebo group). Of the 31 episodes of recurrent VTE, 27 (87.1%) were DVT and 28 (90.3%) were unprovoked.

Conclusions: After 6 months of anticoagulation for a first episode of unprovoked proximal DVT, extending anticoagulation for an additional 18 months was not associated with a long-term reduction in the risk of recurrent VTE or major bleeding after stopping anticoagulation.

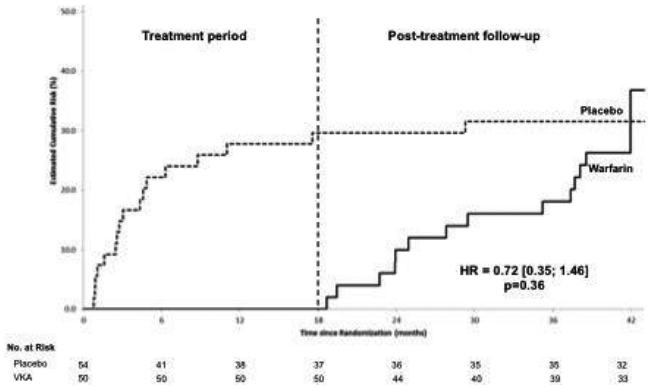

FIGURE 1 Cumulative risk of the composite outcome (recurrent VTE or major bleeding) over the entire study period

ASY 14.2 | Odds of Pharmacologic Prophylaxis by Venous Thromboembolism and Bleeding Risk Groups

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Background: Guidelines for venous thromboembolism (VTE) prevention recommend risk assessment for both VTE and bleeding.

Aims: To evaluate the current practice of pharmacologic prophylaxis by VTE and bleeding risk groups.

Methods: Trained abstractors from 51 hospitals participating in the Michigan Hospital Medicine Safety collaborative collected elements of the Padua VTE risk and IMPROVE bleeding risk scores of hospitalized medical patients. Those admitted to intensive care, on therapeutic anticoagulation or admitted with VTE were excluded. A Padua score of ≥ 4 and an IMPROVE score of ≥7 were classified as high risk. Rates of pharmacologic prophylaxis during the admission by VTE and bleeding risk groups were compared. VTE and major bleeding event rates were also assessed. Major bleeding was defined using ISTH

TABLE 1 Prophylaxis, Major Bleed and VTE Rates by Risk Group

VTE risk	Bleed risk	Total Subjects	Prophylaxis	Major Bleed	VTE	Major Bleed or VTE
High	High	2678 (3.4%)	1111 (41.5%)	215 (8.0%)	13 (0.5%)	225 (8.4%)
High	Low	14156 (17.9%)	10588 (74.8%)	194 (1.4%)	48 (0.3%)	236 (1.7%)
Low	High	4137 (5.2%)	1473 (35.6%)	401 (9.7%)	7 (0.2%)	405 (9.8%)
Low	Low	58093 (73.5%)	37404 (64.4%)	919 (1.6%)	70 (0.1%)	979 (1.7%)

TABLE 2 Odds of Receiving Pharmacologic Prophylaxis by Risk Group

Comparison	OR (95% CI)	P-value
High risk bleed vs. Low risk bleed	0.28 (0.27, 0.30)	<.0001
High risk VTE, low risk bleed vs. All others	2.09 (2.01, 2.19)	<.0001

criteria. All hospitals used VTE risk assessment tools; however none formally assessed bleeding risk.

Results: Of 79,064 patients, 50,576 (64%) received pharmacologic prophylaxis. Major bleeding events were more common than VTE (Table 1). Patients at high risk of bleeding were less likely to receive pharmacologic prophylaxis, regardless of VTE risk, OR 0.28 (Table 2). Odds of receiving pharmacologic prophylaxis was highest (OR 2.09) in patients with high VTE and low bleeding risk, representing 17.9% of the cohort. Of those receiving pharmacologic prophylaxis, 39,988 (79.1%) were either low risk for VTE or high risk for bleeding. Odds of Receiving Pharmacologic Prophylaxis was calculated from logistic mixed effects models with hospital level random intercepts.

Conclusions: Despite the lack of hospital-supported formal bleeding risk assessment tools, providers tend to use less pharmacologic prophylaxis in patients at increased risk of bleeding. In congruence with guidelines, patients at high risk for VTE and low risk of bleeding were twice as likely to receive pharmacologic prophylaxis. However, four out of five patients received anticoagulant prophylaxis despite having low VTE risk or high bleeding risk.

ASY 14.3 | VTE in Acute Leukemia: Improved Survival with Anticoagulation

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Background: Venous thromboembolism (VTE) has long been regarded as the second leading cause of death in cancer patients. Treatment with anticoagulation (AC) can be complicated in cancer patients owing to therapeutic side effects and thrombocytopenia (TCP). Contraindications to VTE treatment are prominent in acute leukemia (AL) patients, where antineoplastic treatment expectedly causes significant myeloid and end-organ toxicity. Due in part to its lethality and rarity, therapeutic outcomes of VTE in patients with AL and severe TCP have not been looked at in detail.

Aims: Delineate the differences in VTE treatment outcomes in patients with AL+TCP.

Methods: Approved by the Institutional Review Board with consent waiver, we retrospectively identified 74 AL+TCP patients from 2002 to 2016 with acute pulmonary embolism (PE;21), lower extremity proximal deep venous thrombosis (DVT;44), or both (9) treated with only AC (23), only inferior vena cava filter (IVCF) (21), or observation (OB) (30). We excluded those with both IVCF and AC from analysis. χ^2 or Kruskal-Wallis tests were used to examine associations between groups. Cox regression model was used to evaluate the effect on overall survival (OS; VTE to death or last follow-up).

Results: There were no significant differences in age, sex, performance status, VTE recurrence, clinically relevant bleeding (CRB), or cause of demise, and no IVCF complications. A statistical difference between index event and treatment modalities was found, with predominant use of IVCF in patients with DVT (16) or both (3) vs PE (2) (p=0.02). AC had a strikingly significant improvement in OS, while IVCF was nearly indistinguishable from OB (Figure 1). On multivariate analysis, AC was associated with a 71% lower hazard of death compared to OB (Table 1).

Conclusions: Treating AL-associated VTE with AC was associated with increased survival, without increased CRB. IVCFs did not lead to

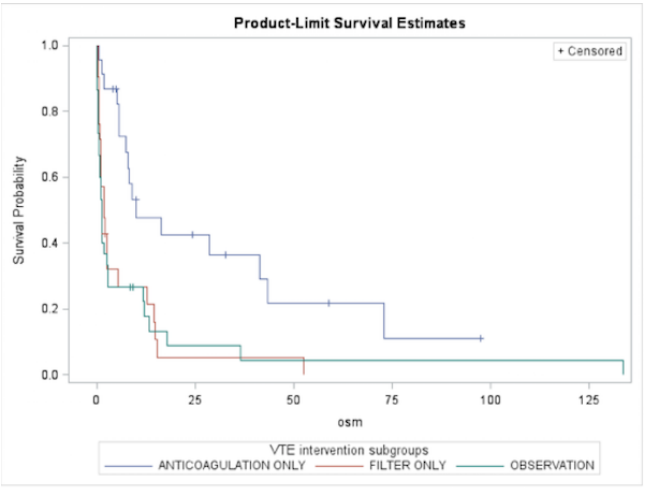

FIGURE 1 Kaplan-Meier Estimates Stratified by Intervention

TABLE 1 Multivariate Cox model for OS

Parameter		Hazard Ratio	95% Confidence Limits		p-value
Intervention	Anticoagulation vs. observation	0.29	0.14	0.58	0.0004
	Filter vs. observation	0.66	0.36	1.22	0.19
History of VTE	Yes vs. no	1.77	1.01	3.09	0.05
Index event	PE vs. Both	0.26	0.1	0.72	0.009
	DVT vs. Both	0.55	0.22	1.35	0.19

complications or an increased rate of death by sepsis, or provided no statistical benefit compared to observation.

ASY 14.4 | Incidence of Fatal Thromboembolic and Bleeding Events in over Ninety Year-old Patients on Treatment with Oral Anticoagulants for Non-valvular Atrial Fibrillation

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Background: The net clinical benefit of oral anticoagulants (OAC) in very elderly patients with non-valvular atrial fibrillation (AF) remains to be defined.

Aims: To evaluate the clinical features associated with type of OAC treatment and the clinical outcome in patients with AF and more than 90 year of age.

Methods: Consecutive patients with age ≥ 90 years treated with either direct oral anticoagulants (DOACs) or vitamin K antagonists (VKAs) for non-valvular AF were included in the study.

Results: Overall, 235 patients were analyzed: mean age was 91.8±1.8, female gender 63%. Previous ischemic stroke or TIA, chronic heart failure and renal creatinine clearance lower than 50 ml/min were reported in 27.2, 35.3, 15.3% respectively.

168 (72.5%) patients were treated with DOACs (dabigatran 19%, rivaroxaban 46% and apixaban 35%) and 67 (28.5%) with VKAs. Twenty-three out of 168 (13.7%) were prescribed with the standard dose of DOACs. The mean CHA_2DS_2-VASc score was 5.07±1.3 and 4.37±1.3 (p=0.001) and the mean HAS-BLED score was 2.64±1.2 and 2.27±1.1 (p=0.026) in patients receiving DOACs and VKAs, respectively. Previous stroke (OR 3.7 95% CI 1.6-8.2), female gender (OR 2.4 95% CI 1.4-4.4) and previous bleeding (OR 4.2 95% CI 1.8-9.8) were independently associated with DOAC prescription. Mean $CHADS_2$, CHA_2DS_2-VASc and HAS-BLED were similar in patients prescribed with standard and reduced doses of DOACs. In patients receiving VKAs mean TTR was 68.8±22.8%.

After a mean follow-up of 483±375 days, 46 patients died (21%). Fatal bleedings occurred in 4 patients, fatal myocardial infarction in 2 and fatal ischemic stroke in one (8.9, 4.3 and 2.2% of all deaths). Fatal thrombotic and bleeding events occurred in 4 and in 2% of patients receiving DOACs and VKAs (p=ns).

Conclusions: A considerable proportion of very elderly patients treated with DOACs receive a standard dose. DOAC patients have a higher thrombotic and bleeding risk compared to VKA patients. No differences were found in terms of fatal events between DOAC and VKA patients.

ASY 22.1 | Sex-specific Incidence Rates of Deep Vein Thrombosis and Pulmonary Embolism in The Netherlands

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Background: The incidence of venous thrombosis (VT) differs between men and women but its distribution over the ages is uncertain, as well as whether the presenting location (deep vein thrombosis, DVT or pulmonary embolism [PE]) differs between the sexes.

Aims: To study sex-specific incidence rates stratified by VT location in a large population.

Methods: Data on all VT patients who were treated with vitamin K antagonists between 2008-2013 were collected at 3 anticoagulation clinics in 2 Dutch provinces serving 1.5 million people. The Dutch Statistics Netherlands provided total numbers of inhabitants by age and sex per postal code. Person-years (py) were calculated based on these numbers corresponding with the geographical service areas of the anticoagulation clinics. Incidence rates (IR) were estimated with 95% confidence intervals (CI) for DVT, PE (with or without DVT) and total VT events. Incidence rate ratios (IRR) in men and women were adjusted for age by Mantel-Haenszel methods.

Results: 7373 VT (3707 DVT [50.2%] and 3666 PE [49.8%]) events occurred in 7 472 400 py at a rate of 9.9 per 10 000 py (95%CI 9.6-10.1), IR 10.5 per 10 000 py (95%CI 10.0-10.9) for women and 9.2 per 10 000 py (95%CI 8.9-9.5) for men. VT incidence was higher in women than in men until the age of 50. At ages 50-70 VT incidence

TABLE 1 Incidence rates (IR) and incidence rate ratios (IRR) with 95%CI for DVT and PE in men and women per age group

Age group and sex		Events (n), PE/DVT	Person years (n)	IR (95%CI), DVT/PE	IRR (95%CI), DVT/PE
Men 15-50 yrs	DVT/PE	462/421	2254325	2.1 (1.9-2.2)/ 1.9 (1.7-2.1)	1.1 (1.0-1.2)
Men 50-70 yrs	DVT/PE	831/696	1018525	8.2 (7.6-8.7)/ 6.8 (6.3-7.4)	1.2 (1.1-1.3)
Men >70 yrs	DVT/PE	413/502	352550	11.7 (10.6-12.9) / 14.2 (13.0-15.5)	0.8 (0.7-0.9)
Women 15-50 yrs	DVT/PE	738/636	2283025	3.2 (3.0-3.5)/ 2.8 (2.6-3.0)	1.2 (1.1-1.3)
Women 50-70 yrs	DVT/PE	517/593	1031875	5.0 (4.6-5.5)/ 5.8 (5.3-6.2)	0.9 (0.8-1.0)
Women >70 yrs	DVT/PE	746/816	531250	14.0 (13.1-15.1) / 15.4 (14.3-16.4)	0.9 (0.8-1.0)

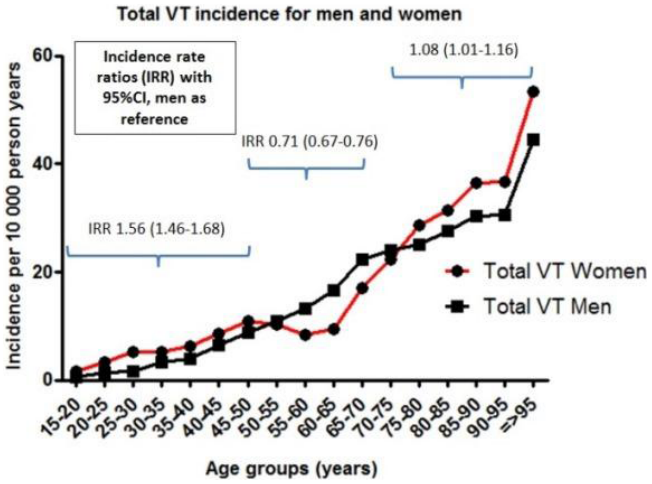

FIGURE 1 Total venous thromboembolism (VT) incidence for men and women separately

was higher in men. From 70 years onwards VT incidence was again higher in women (Figure 1). In women, DVT incidence was higher than PE incidence for ages 15-50, IRR 1.2 (95%CI 1.1-1.3) (Table 1), while for ages 50-70 and >70, DVT incidence was lower than PE, IRR 0.9 (95%CI 0.8-1.0) and 0.9 (0.8-1.0), respectively. In men DVT incidence was higher than PE incidence for both age groups 15-50 and 50-70, IRR 1.1 (95%CI 1.0-1.13) and 1.2 (95%CI 1.1-1.3), respectively, and was lower at 0.8 (95%CI 0.7-0.9) >70 years.

Conclusions: The incidence and presenting VT location differ between men and women among age groups for which the explanation is yet unknown.

ASY 22.2 | Incidence Rate and Factors Influencing Occurrence of Unprovoked Venous Thrombosis during Long Term Period of Follow-up in Women with Previous Pregnancy Related Thromboembolism

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Background: Venous thromboembolism (VTE) complicates 0.5-2.2 per 1000 deliveries. Women with pregnancy related thrombosis are at increased risk of venous thromboembolism in next pregnancies. However, little is known about long term risk of unprovoked venous thrombosis in women with previous pregnancy related thrombosis.

Aims: Determine the incidence rate and try to identify risk factors for occurrence of unprovoked VTE in women with pregnancy related index VTE

Methods: We conducted a retrospective analysis of 223 consecutive women that developed VTE during pregnancy or puerperium (6 weeks after the delivery) from January 1985 to January 2015, and who were referred to our institution. We evaluated age, gestational age at time of thrombosis, localization and massiveness of VTE, history of spontaneous pregnancy loss, presence of congenital thrombophilia and family history as risk factors for development of unprovoked VTE after pregnancy related thrombosis. Time to event analysis was done with Kaplan-Meier estimates and Cox proportional hazards modeling.

Results: After the median follow-up of 9 years, in 223 consecutive woman with pregnancy related thrombosis, 22 recurrent unprovoked VTE episodes were documented with incidence rate of 10,3 cases per 1000 person-years. Two women (9,1%) developed recurrent VTE during 2 year follow up, 4 (18,2%) during 5 years and 16 (72,7%) during more than 5 years of follow up. Cox regression model didn't identify any of the investigated factors as unfavorable in terms of occurrence of unprovoked thrombosis. Only positive thrombophilia testing was close to reaching statistical significance (X^2 = 3.486, p=0,062).

Conclusions: After initial pregnancy related thrombosis the rate of spontaneous VTE during long term period is about 1% per year. Carriers of hereditary thrombophilia who experienced pregnancy related thrombosis may be at slightly increased risk than non-carriers to develop unprovoked thrombosis during long term period.

ASY 22.3 | Aspirin, Heparin or Both to Improve Live Birth in Women with Antiphospholipid Syndrome and Recurrent Pregnancy Loss

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Background: Aspirin and heparin are widely used to prevent pregnancy complications in women with antiphospholipid syndrome

(APS), although the evidence underlying this approach is limited. We are currently updating a previous systematic review (Empson et al, 2005, Cochrane Database of Systematic Reviews). Here, we report the evidence on the outcome live birth. We will report other predefined outcomes on the congress.

Aims: To systematically review the evidence from randomized controlled trials that compared aspirin, heparin, or both on the effect of live birth in women with APS and recurrent pregnancy loss.

Methods: A systematic literature search updating the previous search (Empson 2005) was done in Embase and PubMed on the 29th of March 2016. Randomized controlled trials in women with APS and recurrent pregnancy loss (2 or more) assessing the effect of aspirin, heparin (low-molecular-weight heparin [LMWH] or unfractionated heparin [UFH]) or both versus aspirin or placebo with live birth as outcome were included. Studies in which the laboratory criteria of APS were not met were excluded. Studies underwent critical appraisal and effects were pooled in a random effects model.

Results: We found 939 articles of which 27 remained after title and abstract screening. Full text review yielded 9 studies. Five studies with a total of 398 women were included. We compared aspirin plus heparin (LMWH or UFH) versus aspirin alone. The pooled odds ratio (OR) for live birth was 2.28 (95%CI: 1.24-4.18) in favour of aspirin plus heparin compared to aspirin (Figure 1). There was significant heterogeneity between the subgroups of LMWH and UFH (OR for aspirin plus LWMH versus aspirin 1.16, 95%CI: 0.58-2.29; OR for aspirin plus UFH versus aspirin 3.75, 95%CI: 2.04-6.09; test for subgroup differences: I^2 84.2%, p 0.01;).

Conclusions: Heparin plus aspirin compared with aspirin improves live birth in women with APS and recurrent pregnancy loss. This effect is driven by studies that investigated UFH and not LMWH.

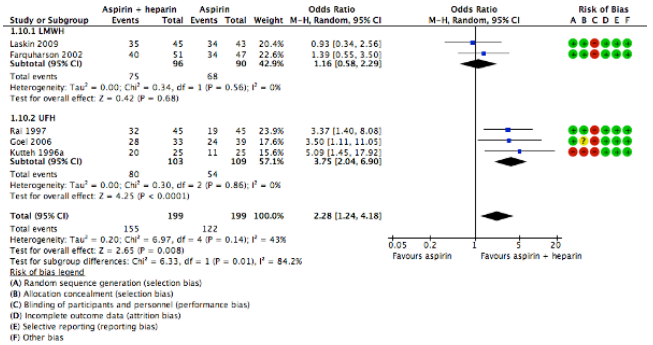

FIGURE 1 Aspirin plus heparin (LMWH or UFH) versus low-dose aspirin for live birth as outcome

ASY 22.4 | Induced Delivery and Neuraxial Anesthesia in Pregnant Women Using Thromboprophylaxis: Data from the Highlow Study

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Background: Antepartum and postpartum thromboprophylaxis with low-molecular-weight heparin (LWMH) is indicated in most women with a personal history of venous thromboembolism (VTE) to prevent recurrence. The optimal dose of LMWH is unknown and both low and intermediate doses are suggested. A higher dose may be more efficacious but may increase the risk of (peripartum) bleeding and affect the possibility to receive neuraxial anesthesia (NA). NA is contraindicated when time since last injection is less than 12 hours for low or less than 24 hours for intermediate dose LMWH. Delivery may be induced to assure the possibility of NA.

Aims: To explore the practices regarding inducing delivery and applying NA in women using antepartum LMWH.

Methods: The ongoing Highlow study (NCT **01828697**) is a multicenter, multinational randomized controlled trial comparing efficacy and safety of two doses of LMWH. We compared the type of delivery

TABLE 1 Distribution of type of delivery and NA by dose LMWH

Variable	Low dose LMWH	Intermediate dose LMWH	OR (CI 95%)
Total, n	78	83	
Spontaneous delivery, n (%)	34 (43.6)	43 (51.8)	1.18 (0.69-2.06)
Induced delivery, n (%)	43 (55.1)	35 (42.2)	
Time between last injection and delivery in hours, median (range)	29.3(4.7- 312.1)	35.0 (0.8-149.7)	
Type of anesthetic			
None, n (%)	30 (38.5)	32 (38.6)	
Neuraxial anesthesia, n (%)	32 (41.0)	31 (37.3)	
• Netherlands n (% of delivery per country) • France, n (% of delivery per country) • Ireland, Norway and Belgium n (% of delivery per country)	16/49 (33), 13/19 (68), 3/10 (30)	16/55 (29), 10/15 (66), 5/12 (42)	
Other, n (%): • Due to time interval • Due to preference for other type of anesthesia	16 (20.5), 1 (1.3) 15 (19.2)	19 (22.9) 6 (7.2), 13 (15.6)	5.64 (0.66-47.90)

TABLE 2 Distribution of type of anesthetic by type of delivery

Variable	Spontaneous delivery	Induced delivery	OR (CI 95%)
Total, n	77	79	
Time between last injection and delivery in hours, median (range)	27.4 (0.8-222.1)	38.4 (7.5-312.1)	
Type of anesthetic			
None, n (%)	36 (46.28)	24 (30.4)	
Neuraxial anesthesia, n (%)	22 (28.6)	40 (50.6)	1.77 (0.97-3.25)
Other, n (%)	19 (24.7)	15 (19.0)	
Due to time interval	4 (5.2)	3 (3.8)	

and type of anesthesia between the two doses of LMWH and between countries. Data from 161 patients who have delivered were used for this analysis; updated results will be presented at the Congress.

Results: There was no difference in spontaneous and induced delivery between LMWH doses. NA was applied in 41.0% of women on low dose and 37.3% of women on intermediate dose (Table 1). In women who used another type of anesthesia, this was more often due to a short time interval since last injection in the low than the intermediate dose (1.3% versus 7.2%, OR 5.64, 95% CI 0.66-47.90). In induced deliveries, NA was applied more often than in spontaneous deliveries (Table 2). Use of NA varied substantially between countries (Table 1).

Conclusions: Both spontaneous and induced delivery is applied regardless of LMWH dose. Women using intermediate dose LMWH seem more likely to not receive NA due to a short time interval since last dose. There is substantial variation in NA practice between countries.

ASY 35.1 | No Further Reduction in the Number of Required CT Pulmonary Angiograms When Implementing the ADJUST (Age Adjusted) D-dimer Cut-off Level in the YEARS Algorithm

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Background: The YEARS algorithm was designed to simplify the diagnostic work-up of pulmonary embolism (PE) and to reduce the number of necessary computer tomography pulmonary angiography (CTPA) scans (*Figure 1*). A recent outcome study confirmed the safety and efficacy of YEARS (*2016 ESC abstract 5727*). Another strategy to reduce the number of CTPAs is the age-adjusted D-dimer cut-off in patients aged 50 or older. A combination of both diagnostic strategies might save additional CTPAs.

Aims: To investigate whether the age adjusted cut-off for D-dimer in patients aged 50 or older provides incremental diagnostic value to YEARS in patients with suspected PE, i.e. whether the number of required CTPAs will diminish further after implementation of this D-dimer cut-off without jeopardizing safety.

Methods: We performed a post hoc analysis of the YEARS study to compare the number of required CTPAs for the two D-dimer cut-off scenarios.

Results: Using YEARS, 1651 patients (48%) were managed without CTPA; PE was diagnosed in 456 (13%) patients at baseline and 18 (0.52%) venous thrombo-embolism (VTE) during 3-month follow-up in patients with initial normal testing (*Figure 1*). When applying the age adjusted D-dimer cut-off, 1497 patients (43%) would have been managed without CTPA for an absolute difference of 4.4% (95%CI 2.1-6.8) (*Table 1*). However, 4 PE would have been missed at baseline in patients with ≥ 1 YEARS item (*see arrow in figure 1*).

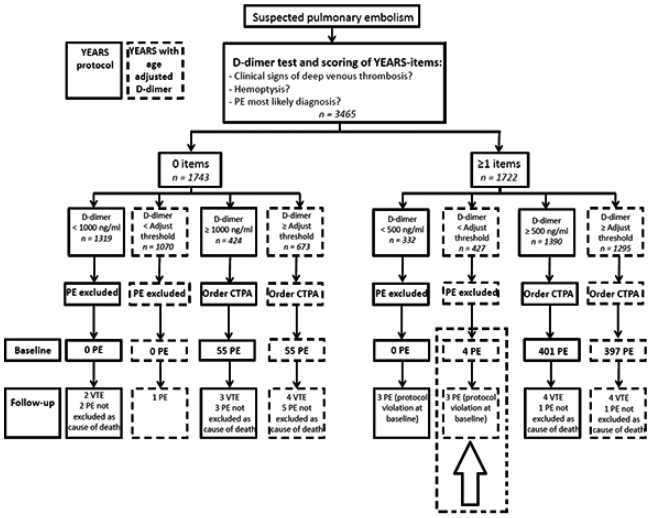

FIGURE 1 Flowchart of YEARS algorithm for both strategies

TABLE 1 Overview of patients managed without CTPA and failure rate

	Number of patients managed without CTPA	Failure rate
YEARS protocol	1651/3465 (48%)	18/3465 (0.52%)
Years combined with age adjusted D-dimer	1497/3465 (43%)	22/3465 (0.64%)
Total difference between both protocols	+ 154 CTPAs	+ 4 failures

Conclusions: In our cohort, there was no added value of implementing the ADJUST (age adjusted) D-dimer cut-off level into the YEARS algorithm due to both an increased required number of CTPAs for excluding PE and an increased 3-month VTE failure rate.

ASY 35.2 | Mean Bilateral Proximal Extension of the Clot - A New Score for Risk Stratification of Pulmonary Embolism

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Background: Risk stratification is routinely performed to decide the management of patients with pulmonary embolism (PE). Various tools have been proposed and validated for risk stratification oF PE including clinical, biochemical, radiological and echocardiographic parameters. Mean bilateral proximal extension of the clot (MBPEC) is a simple CT radiological score that was derived and validated in a small cohort of PE patients (Ghanima et al. J Inter Med 2007; 261).

Aims: To validate the score by investigating in a larger cohort of patients the association between MBPEC and the severity of PE as determined by various clinical (Pulse rate (PR), Systolic Blood pressure (SBP), PESI score), biochemical (troponin), management associated (ICU admission and thrombolysis) and CT radiological (right/Left ventricular ratio (RV/LV)) parameters.

Methods: MBPEC score is estimated by calculating the mean value of the largest affected vessel [subsegmental= 1, segmental= 2, lobar= 3, main pulmonary artery= 4] in each lung. Patients were identified from Østfold Thrombosis Registry, Norway. Only patients who were alive and provided written consent were included in this study. The study was approved by the ethics committee. ANOVA or Kruskal Wallis test were used for continuous variables and Chi square test for categorical variables.

TABLE 1 Level or the distribution of various pe severity markers according to the mbpec score

MPEC score	1 n=25 n=25	2 n=67	3 n=65	4 n=98	P-value
PR /min	80	85	85	91	0.017
SBP mmHg (mean)	134	141	142	140	0.497
SaO2% (mean)	95	96	96	94	0.004
PESI score (mean)	66	66	67	75	0.050
Troponin T/I (n +/-)	2	2	4	37	0.000
D-dimer mg/l (mean)	2	2.6	5.3	9	0.000
RV/LV ratio (mean)	1	1	1.1	1.3	0.000
ICU admission (N)	2	6	8	35	0.000
ICU admission (N) Thrombolysis (N)	0	0	2	8	0.006

Results: 255 patients with PE were identified; 152 (59%) were males. Mean age was 59 years. MBPEC score was 1 in 25 (10%), 2 in 67 (26%), 3 in 65 (25%) and 4 in 98 (38%). Table shows the level or the distribution of various PE severity markers according to the MBPEC score. Troponin T or I were available in 153 patients only (16, 38, 31, 68 in score 1-4).

Conclusions: Higher MBPEC score was associated with increased severity of PE in most studied parameters including PESI score, ICU admission and thrombolysis. The score can be a potentially valuable tool for risk stratification of PE. Prospective outcome studies are needed to evaluate its prognostic value in clinical practice.

ASY 35.3 | Disease Prevalence Dependent Failure Rate in Diagnostic Management Studies in Suspected Deep Vein Thrombosis

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Background: Current guidelines state that the standard against which deep vein thrombosis (DVT) diagnostic management studies should be evaluated is a failure rate of < 1.3% (upper limit 95%CI 4.4%), i.e. the percentage of patients with a VTE during 3 months of follow-up despite a normal venogram. However, the disease prevalence in DVT studies has decreased over the past decade. Bayes' theorem states that disease prevalence and failure rate are connected, implying that the diagnostic standard of future diagnostic management studies should be changed accordingly.

Aims: To evaluate the association of DVT prevalence and diagnostic failure rate in published studies on diagnosis of DVT.

Methods: Systematic review and meta-analysis selecting all high-quality diagnostic studies in suspected acute DVT from 1990 on, including ≥ 100 consecutive patients with a follow-up period of ≥ 3 months, using an appropriate diagnostic standard, i.e. (an algorithm consisting of) a validated clinical decision rule combined with a highly sensitive D-dimer test, venography, compression ultrasound (CUS) (whole leg or 2 point CUS) or duplex ultrasound.

Results: Fifty-one studies including 31.511 patients were selected, with a mean baseline DVT prevalence of 20% (95%CI 19.6-20.5, range 1.2-47%) and a failure rate of 0.83% (95%CI 0.73-0.93, range 0-2.8%). Disease prevalence decreased over the years with 2.55% per 5 year (R^2 0.066, p< 0.001; Fig. 1). The mean failure rate decreased with lower disease prevalence in individual studies (R^2 0.121, p< 0.001; Fig. 2), with an absolute 1.0% lower DVT prevalence leading to a mean 0.020 percentage points decrease in failure rate.

Conclusions: We have confirmed the trend of decreasing DVT prevalence and diagnostic failure rates in DVT diagnostic studies. A DVT prevalence-dependent diagnostic safety threshold for future

diagnostic studies should be considered to prevent validation and implementation of new diagnostic strategies with insufficient sensitivity to safely rule-out DVT.

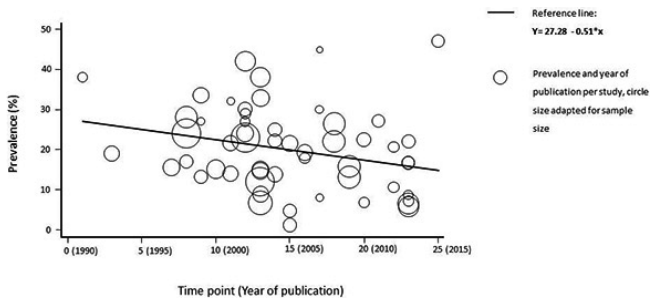

FIGURE 1 Decrease of disease prevalence over the last years

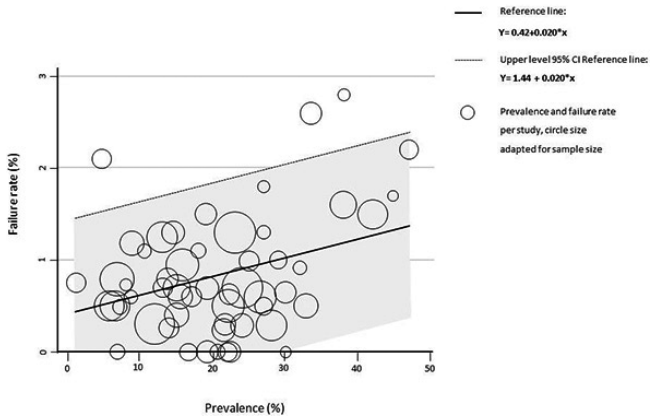

FIGURE 2 Prevalence versus failure rate in high quality deep vein thrombosis diagnostic management studies

ASY 35.4 | Clinical characteristics and management of 10,329 patients with a confirmed diagnosis of venous thromboembolism: the GARFIELD-VTE registry

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Background: The aim of venous thromboembolism (VTE) treatment is prevention of the acute and chronic complications of deep vein thrombosis (DVT) and pulmonary embolism (PE). The treatment of VTE has evolved with the introduction of direct oral anticoagulants (DOACs). GARFIELD-VTE is a global prospective registry designed to characterize the epidemiology and to observe management and outcomes of VTE patients.

Aims: To describe the baseline characteristics of patients enrolled from 2014 to 2016.

Methods: Interim data on patient demographics, risk factors, and diagnostic and treatment strategies were collected at 410 sites in 28 countries.

Results: Of the 10,329 patients eligible for analysis, 61.7% had DVT and 38.3% had PE. Mean (SD) age was 58.4 y (16.9), and 49.7% were female. Provoking factors included: surgery (12.3%), hospitalization (11.8%), trauma (7.7%), acute illness (5.6%), known thrombophilia (2.8%) and in women, oral contraceptives (10.0%), pregnancy (3.6%) and hormone replacement therapy (2.8%). A total of 2057 patients had either a history of cancer (10.8%) or active cancer (9.1%), and 3.7% had renal insufficiency. DVT was diagnosed using compression ultrasonography in 95.4% and PE by CT scan in 91.5%. DVT commonly involved the lower limb (89.9%), on the left side (53.6%) and 6.6% were bilateral. Upper limb involvement occurred in 8.4%. The most proximal artery involvement in PE patients was: main (29.5%), lobar (29.5%), segmental (31.3%) or sub-segmental (9.7%). Anticoagulant (AC) treatment within ±2 wks of diagnosis included: parenteral AC only (17.6%), parenteral AC followed by vitamin K antagonists (VKA) (28.3%), VKA only (3.7%), and DOACs (50.5%). In addition, 4.4% received thrombolytic therapy; 2.1% underwent surgical/mechanical interventions; and 34.8% of patients received graduated compression stockings.

Conclusions: Our data indicate that there is wide heterogeneity among VTE patients necessitating an individualized approach to therapy.

ASY 36.1 | Direct Oral Anticoagulants for Pulmonary Embolism: Importance of Anatomical Extent

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Background: Studies in pulmonary embolism (PE) have used direct oral anticoagulants (DOACs) with or without a heparin lead-in. In

practice, the anatomical extent of PE is often considered when deciding whether to use a heparin lead-in.

Aims: To

1). evaluate if PE patients benefit from initial heparin;
2). describe patient characteristics in the phase III DOAC studies;
3). investigate whether the anatomical extent of PE correlates with N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, cause of PE, and recurrence rate.

Methods:

1). We performed an indirect meta-analysis comparing the risk of recurrence in DOAC-treated patients with or without a heparin lead-in relative to that in patients given heparin with vitamin K antagonists (VKAs).
2). To compare the PE studies, we extracted available information on the characteristics including anatomical extent and causes of PE.
3). We used the Hokusai-VTE study to correlate the anatomical extent of PE at baseline with NT-proBNP levels, causes of PE and recurrent venous thromboembolism (VTE). Hokusai-VTE was approved by the institutional review boards; informed consent was obtained.

Results: The meta-analysis included 11,539 PE patients. The relative risk of recurrent VTE with DOAC treatment versus VKA was 0.76 (95% confidence interval [CI] 0.6-1.1) with heparin lead-in and 1.05 (95%CI 0.8-1.5) without heparin. In the DOAC studies the proportion of patients with extensive PE varied from 24% to 47%. In Hokusai-VTE, NT-proBNP was elevated in 4% of patients with limited disease and in over 60% with extensive PE. Cause of PE and anatomical extent were not related. Recurrence rates increased from 1.6% with limited extent to 3.2% with extensive disease in the edoxaban group, and from 2.4% to 3.9% in the warfarin recipients.

Conclusions: Indirect evidence suggests that heparin lead-in before DOACs may be advantageous in PE. Anatomical extent was related to elevated NT-proBNP and outcome, but not to PE cause.

ASY 36.2 | Validation of the IMPROVE Bleeding Risk Assessment Model in Hospitalized Medical Patients: A Multi-center Study

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Background: Guidelines recommend against venous thromboembolic (VTE) pharmacologic prophylaxis for hospitalized medical patients at high risk for bleeding. The IMPROVE risk assessment model was developed to predict bleeding up to 14 days from admission.

Aims: To validate the IMPROVE bleeding score using clinical data from a large population of hospitalized medical patients at multiple sites.

Methods: Trained abstractors from 51 hospitals participating in the Michigan Hospital Medicine Safety collaborative collected elements of the IMPROVE score and receipt of pharmacologic prophylaxis from patient records, and contacted patients 90 days after discharge to capture VTE and bleeding events. Patients admitted to intensive care, on therapeutic anticoagulation or admitted with VTE were excluded. An IMPROVE score ≥7 was considered high risk for bleeding. Outcomes of interest were clinically overt bleeding within 14 days of admission and ISTH defined major bleeding during hospitalization. Receiver operator characteristic (ROC) curve was generated for overt bleeding with continuous scores.

Results: Of 50,408 patients, 4,779 (9.5%) were classified as high risk for bleeding. Overt bleeding occurred in 11.4% (95% CI, 10.5-12.3%) of high risk and 2.6% (95%CI, 2.4-2.7%) of low risk patients. Major bleeding occurred in 8.5% (95%CI, 7.8-9.4%) high and 1.5% (95%CI, 1.4-1.7%) low risk patients. Test characteristics are shown in Table 1

TABLE 1 Test Characteristics of the IMPROVE Bleeding Risk Assessment Model

	All patients	95% CI	With pharmacologic prophylaxis	95% CI	Without pharmacologic prophylaxis	95% CI
Sensitivity any overt bleed	31.8%	29.6% to 34.1%	21.4%	18.4% to 24.3%	40.7%	37.5% to 43.9%
Specificity any overt bleed	91.3%	91.0% to 91.6%	94.8%	94.5% to 95.0%	85.0%	84.4% to 85.5%
Positive predictive value any overt bleed	11.3%	10.6% to 12.1%	9.0%	7.9% to 10.3%	12.7%	11.7% to 13.6%
Negative predictive value any overt bleed	97.5%	97.4% to 97.6%	98.0%	97.9% to 98.1%	96.4%	96.2% to 96.6%
Sensitivity major bleed	36.7%	33.9% to 39.7%	25.6%	21.3% to 30.3%	42.5%	38.8% to 46.1%
Specificity major bleed	91.1%	90.9% to 91.4%	94.6%	94.4% to 94.9%	84.8%	84.2% to 85.3%
Positive predictive value major bleed	8.5%	7.9% to 9.2%	5.3%	4.5% to 6.3%	10.5%	9.7% to 11.4%
Negative predictive value major bleed	98.5%	98.4% to 98.5%	99.1%	99.0% to 99.1%	97.2%	97.0% to 97.4%

and the area under the ROC curve was 0.75 for overt bleeding (Figure 1). Among patients who received pharmacologic prophylaxis, the negative predictive values were 98% (95%CI, 97.1-98.1) for overt bleeding and 99.1% (95%CI, 99.0-99.1) for major bleeding.

Conclusions: The IMPROVE score reliably predicts the risk of overt or major bleeding. It appears to be most useful in identifying patients who are less likely to bleed when given pharmacologic prophylaxis.

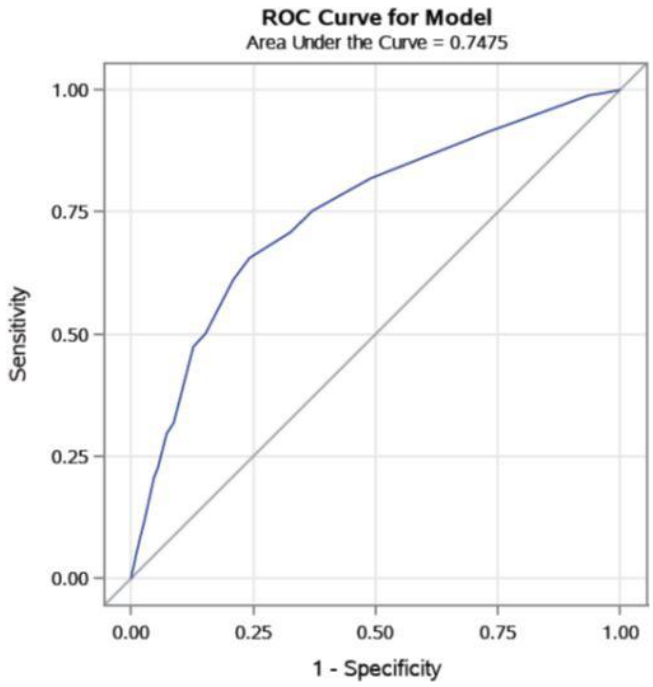

FIGURE 1 Overt Bleeding 14 Days after Admission Receiver Operator Characteristic Curve for the IMPROVE Risk Assessment Model Using a Continuous Score

ASY 36.3 | Tinzaparin for Treatment of Fetal Growth Retardation: A Randomized Multicenter-trial

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Background: Fetal growth retardation (FGR) is a leading cause of perinatal death. Placental thromboses are pivotal for developing FGR. In lack of randomized trials, there has been no evidence of low-molecular weight heparins (LMWH) for FGR treatment.

Aims: We conducted a randomized trial testing the hypothesis that LMWH (tinzaparin) increases birth weight in FGR pregnancies.

Methods: The trial was undertaken in 3 obstetric centers in Denmark during 2011 to 2016. Pregnant women with FGR (estimated fetal weight < 2.3 percentile, ≤-22%) and no chronic disease, substance abuse, no fetal malformations, or fetal chromosome anomalies were included. Informed consent was obtained upon inclusion, and the regional ethics committee approved the study. Participants were randomly allocated to Tinzaparin (Innohep®, 4500 international units daily until 37 gestational weeks) or no Tinzaparin. We used an intention to treat analysis approach including all randomized women in our statistical analyses. The trial was registered in ClinicalTrials.gov (EudraCT number: 2011-000818-20).

Funding: The Danish Council for independent Research (Grant no: 0602-02173B FSS), Aarhus University, Health, Denmark, and Central Region of Denmark. The trial drug was donated by Leo Pharma. The funders had no role in the design or conduct of the study; in the collection, analysis and interpretation of the data.

Results: In total, fifty three women consented to participate. Baseline characteristics were balanced across the groups. The Tinzaparin group (N=27) had a higher mean birth weight than the untreated women (N=26), an absolute difference 354 grams (p=0.05). The Tinzaparin group had significantly less placental infarctions (p=0.03) and gestational age at delivery tended to be higher (p=0.06).

Conclusions: Preliminary results suggest that Tinzaparin improves birth weight in FGR pregnancies probably mediated through diminished placental infarction enabling later delivery.

ASY 36.4 | Puerperium But Not Pregnancy Is a Risk Factor for Cerebral Venous Thrombosis

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Background: Pregnancy and puerperium are generally considered to be risk factors for cerebral venous thrombosis (CVT), but this has not been assessed in a controlled study.

Aims: We aimed to assess whether pregnancy or puerperium are risk factors for CVT.

Methods: We performed an unmatched case-control study. Cases were consecutive adult patients with CVT admitted to four academic hospitals between 1987 and 2015. Controls were subjects from the control population of the Dutch MEGA study (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis). We excluded men, subjects older than 50, and women who used oral contraceptives. Puerperium was defined as the first 12 weeks after delivery. We adjusted for age and history of cancer. We stratified for pregnancy vs. puerperium, and 0-6 vs. 7-12 weeks postpartum.

Results: We included 145/714 cases and 1231/6296 controls in the analysis. Cases were younger (median 38 vs. 41 years) and more often had cancer (14% vs. 4%) than controls. A total of 38/145 (27%) cases and 82/1231 (7%) controls were pregnant or postpartum. Pregnancy

TABLE 1 Effect of pregnancy or postpartum period on the risk of cerebral venous thrombosis

	CVT cases (n=145)	Controls (n=1231)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Pregnancy or postpartum	38/145 (26%)	82/1231 (7%)	5.0 (3.2-7.7)	3.8 (2.4-6.0)
Pregnant	12/145 (8%)	64/1231 (5%)	1.7 (0.9-3.1)	1.2 (0.6-2.3)
Postpartum	26/145 (18%)	18/1231 (2%)	15.5 (11.6-56.6)	19.3 (8.5-43.7)
Postpartum 0-6 weeks	23/145 (16%)	9/1231 (1%)	25.6 (11.6-56.6)	19.3 (8.5-43.7)
Postpartum 7-12 weeks	3/145 (2%)	9/1231 (1%)	2.9 (0.8-10.7)	1.7 (0.4-6.6)

or puerperium was associated with an increased risk of CVT (adjusted odds ratio 3.8; 95% confidence interval 2.4-6.0). This association was fully attributable to an increased risk during puerperium (10.7; 5.5-20.6). There was no association between pregnancy and CVT (1.2; 0.6-2.3). Women had the highest risk of CVT during the first 6 weeks after delivery (0 to 6 weeks: 19.3; 8.5-43.7 and 7 to 12 weeks: 1.7;0.4-6.6)).

Conclusions: Puerperium is a strong risk factor for CVT, in particular during the first 6 weeks. We did not find an association between pregnancy and CVT.

PATHOGENESIS OF THROMBOEMBOLISM

ASY 06.1 | IgM Antibodies against Oxidation-specific Epitopes Identify Patients with a Lupus Anticoagulant at Risk for Thrombotic Complications: The Vienna Lupus Anticoagulant and Thrombosis Study

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Background: The pathomechanisms leading to an increased risk of arterial and venous thrombotic complications in patients (pts) with the lupus anticoagulant (LA) are incompletely understood. Low levels of IgM antibodies against oxidation-specific epitopes (IgM-OSE-Abs) have recently been identified as risk factors for coronary events and stroke in several human populations.

Aims: To quantify the relationship between IgM-OSE-Abs, LA laboratory phenotype, and thrombotic outcomes in pts with the LA.

Methods: In this prospective cohort study, we examined clinical and laboratory characteristics of 171 pts with persistent LA (Table 1), and 37 healthy controls. 141 of the 171 LA-positive pts were followed-up for symptomatic arterial (n=16 events) and/or venous thrombosis (n=15 events; median follow-up: 9.5 years; 10-year risk of arterial+venous thrombosis: 24.6% (95%CI: 17.0-33.0)). IgM-OSE-Abs were determined with a chemiluminescent ELISA, and normalized for total serum IgM. Specifically, we investigated IgM antibodies against MDA-LDL and CuOx-LDL.

Results: LA-positive patients had lower levels of MDA-LDL abs than healthy controls (p=0.008, Table 1). MDA-LDL abs were inversely correlated with LA-specific autoantibodies, such as IgM aCL (rho=-0.32, p=0.0002), and IgM aβ2GPI (rho=-0.32, p=0.0003). Ten-year thrombotic risk was 48.6% in the 14 patients that had CuOx-LDL < the 10th percentile of the CuOx-LDL distribution, and 22.4% in the 130 pts ≥ this cut off (48.6% vs 22.4%, Gray's test p=0.018, Figure **1**). This association was more pronounced for arterial (Hazard ratio (HR)=3.9, 95%CI: 1.2-12.3, p=0.021) than for venous events (HR=1.6, 0.4-6.8, p=0.554).

Conclusions: We demonstrate a critical link between IgM-OSE-Abs and prospective thrombotic risk in LA-positive patients, which suggests that OSE-specific immune responses are protective modulators of thrombotic burden and potential biomarkers for thrombotic risk stratification in patients with the LA.

TABLE 1 Baseline characteristics of the study population - Cross-sectional analysis (n=208)]

Variable	LA-positive (n=171) Median [25th-75th percentile], or Absolute count (percent)	Healthy controls (n=37) Median [25th-75th percentile], or Absolute count (percent)	p for difference
Age (years)	42.1 [32.3-60.1]	44.9 [39.4-55.3]	0.486
Female gender	137 (80.1%)	28 (75.7%)	0.545
Prior history of thrombosis	110 (64.3%)	0 (0.0%)	<0.0001
Established Anti-Phospholipid-Syndrome (APS)	124 (72.5%)	0 (0.0%)	<0.0001
aβ2GPI IgM (MPL)	6.6 [3.1-15.6]	3.0 [2.3-4.2]	<.0001
aCL IgM (MPL)	9.0 [3.7-23.9]	3.9 [3.1-6.5]	0.0001
"Triple positivity"	57 (45.2%)	0 (0.0%)	<0.0001
MDA-LDL	325 [254-434]	388 [323-463]	0.008
CuOx-LDL	407 [300-539]	396 [333-469]	0.732

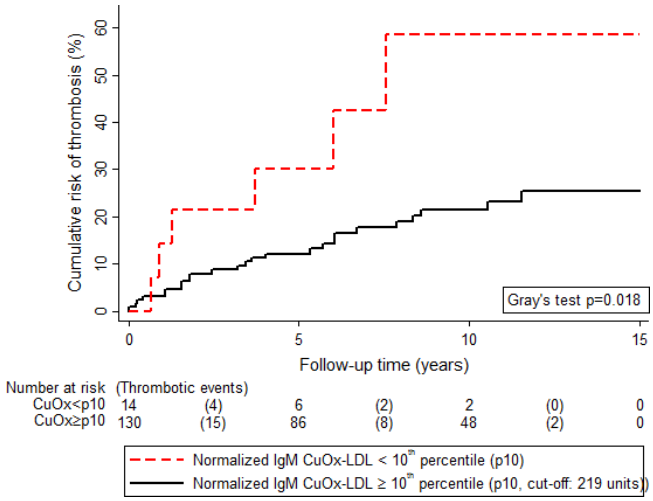

FIGURE 1 Cumulative risk of thrombosis in patients with a Lupus Anticoagulant according to baseline levels of IgM-OSE-Abs against CuOx-LDL

ASY 06.2 | Differences in the Platelet Proteome Between Lupus Anticoagulant Positive Individuals with or without Thrombotic Manifestations and Healthy Controls

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Background: Patients with lupus anticoagulants (LA) are at high risk to develop thromboembolism (TE), however, not all individuals with LA experience TE. Whether platelets play a role for the development of thrombosis in patients with LA is still not clear. Several studies have shown that LA/antiphospholipid antibodies interact with platelets, leading to platelet activation and aggregation.

Aims: We investigated whether there is a difference in the platelet proteome between LA-positive individuals with TE (LA+TE+), LA-positive individuals without TE (LA+TE-) and healthy controls. Identification of thrombotic biomarkers provide new mechanistic insights into the role of platelets in diseases with a high thrombotic risk.

Methods: Platelets from 30 LA+TE+ patients, 17 LA+TE- patients and 47 matched healthy controls were isolated and analyzed for differences in the platelet proteome with two-dimensional differential in-gel electrophoresis (2D-DIGE). Differentially expressed proteins were identified by mass spectrometry.

Results: Significant differences in the expression of eight proteins (Fig 1) were observed between LA+TE+ individuals, LA+TE- individuals and healthy controls, whereas no differences between LA+TE- patients and healthy controls could be detected. Most of the changed proteins correspond to platelet activation and degranulation, including protein disulfide isomerase (Fold change 1.1, p=0.00013) and proteasome activator PA28 (Fold change 1.14, p=0.0037). In addition, leukocyte elastase inhibitor, involved in the cross talk with neutrophils, was downregulated by 10% (p=0.014) in LA+TE+ patients compared to healthy controls (Fig 2).

Conclusions: Our findings correspond to platelet activation and degranulation in LA-patients with thrombosis, suggesting an indirect and direct involvement of platelets in thrombotic complications of this pathology. The reduced abundance of leukocyte elastase inhibitor expression might ease increased formation of neutrophil extracellular traps and thus add to the thrombotic risk.

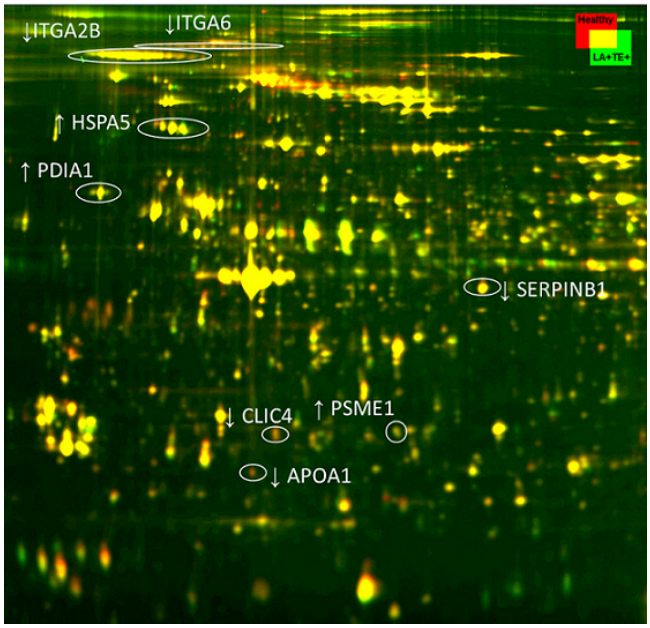

FIGURE 1 Representative 2D-DIGE Gel for the platelet proteome in the pH range 4-7

			LA+TE+/Healthy		LA+TE+/LA+TE-		LA+TE-/Healthy	
Spot No.	Gene name	Protein name	T-test	Fold change	T-test	Fold change	T-test	Fold change
1334	PDIA1	Protein disulfide isomerase	0.00013	1.1	<0.0001	1.16	0.95	0.96
864	HSPA5	78 kDa glucose-regulated protein	0.0009	1.12	<0.0001	1.2	0.76	1.01
2780	PSME1	Proteasome activator PA28	0.0037	1.14	0.0065	1.16	0.08	1.09
315	ITGA6	Integrin alpha-6	0.014	0.88	0.0028	0.77	0.58	1.03
317			0.022	0.89	0.0034	0.8	0.72	1.02
323			0.022	0.89	0.011	0.85	0.44	0.96
313			0.03	0.89	0.011	0.82	0.61	1.03
1965	SERPINB1	Leukocyte elastase inhibitor	0.014	0.9	0.075	0.93	0.7	0.98
2786	CLIC4	Cloride channel protein 4	0.017	0.89	0.37	0.96	0.68	0.98
3166	APOA1	Apolipoprotein A-I	0.03	0.87	0.05	0.83	0.11	0.9
361	ITGA2B	Integrin alpha-IIb	0.022	0.92	0.21	0.95	0.79	1.01

FIGURE 2 Proteins differentially regulated between LA+TE+, LA+TE- and healthy controls. Abbreviations: LA lupus anticoagulant, TE thromboembolism

ASY 06.3 | Antiphospholipid Antibody Induced Cellular Signal Transduction Depends on Antigen Specificity

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Background: The antiphospholipid syndrome (APS) is characterized by venous and/or arterial thrombosis and severe pregnancy morbidity associated with persistently elevated titers of antiphospholipid antibodies (aPL).

Aims: In the present study we analyzed cellular effects of three human monoclonal aPL with different antigen specificity and compared them with IgG isolated form APS patients.

Methods: Mouse monocytes or human platelets were stimulated with three human monoclonal aPL IgG: HL5B (anticardiolipin - aCL), rJGG9 (anti-b2-glycoprotein-1 - anti-b2GPI) and HL7G (aCL+anti-b2GPI). Gene expression in mouse monocytes was measured by qRT-PCR, platelet aggregometry was performed on an APACT 4004 aggregometer.

Results: Using monocytes from different knock-out mice we could show that two distinct signaling pathways were activated by HL5B and rJGG9. HL5B was dependent on the previously described signaling pathway via endosomal NADPH-oxidase (NOX) 2. rJGG9 signaling was fully dependent on the presence of LDL-receptor related protein 8 (LRP8). HL7G activated both signaling pathways. Deficiency of TLR2 or TLR4 had no effect on HL5B but reduced cellular activation by rJGG9. While HL5B had no effect on platelets, rJGG9 and HL7G strongly induced platelet aggregation. IgG-fractions from APS patients most often activated cells like HL7G (aCL + anti-b2GPI). Some patient IgG were restricted to either the aCL or the anti-b2GPI-pattern of cellular responses.

Conclusions: We show for the first time that aCL and anti-b2GPI aPL induce completely different cellular signal transduction pathways. This is also reflected in platelet reactivity to different aPL. All patient IgG fractions tested follow one or the other pattern observed with the three monoclonal aPL.

ASY 06.4 | Complement-dependent Monocyte TF Activation Triggers Antiphospholipid Antibody Internalization and ROS Signaling

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Background: Pathogenic effects of antiphospholipid antibodies (aPL) are linked to complement activation. β2-glycoprotein-independent aPL amplify inflammation and coagulation by inducing NADPH oxidase2 (NOX2) translocation into endosomes and reactive oxidant species (ROS) production required for TNF and tissue factor (TF) upregulation. Although complement is known to convert monocyte TF to a procoagulant molecule, the underlying mechanisms remain incompletely understood.

Aims: We tested the hypothesis that aPL cause complement-dependent signaling through TF and coagulation activation on monocytes.

Methods: We studied human and mouse monocytic cell activation by aPL by single stage clotting assay, induction of genes and ROS, and molecule tracking by confocal microscopy.

Results: Quiescent monocytes were found to express low levels of cryptic cell surface TF that was rapidly activated by aPL dependent on complement C3 and protein disulfide isomerase(PDI). Blockade of this pathway or of subsequently generated thrombin and thrombin-dependent protease activated receptor 1 cleavage prevented aPL internalization, ROS production and TNFα induction in mouse and human monocytic cells. In addition, blockade of TF and thrombin prevented NOX2 internalization into $EEA1^{+}$ endosomes. Mice lacking the TF cytoplasmic domain ($TF^{\Delta CT}$ mice) are protected from ROS production and pregnancy loss induced by aPL. Although aPL induced TF procoagulant activity normally in $TF^{\Delta CT}$ monocytes, NOX2 internalization, ROS production and inflammatory cytokine production was completely absent. Although ROS production can contribute to TF procoagulant conversion e.g. in the context of inflammasome activation, inhibition of endosomal ROS production did not prevent aPL-induced TF procoagulant activity.

Conclusions: Our results demonstrate an unexpected pathway that connects complement and PDI generation of procoagulant monocyte TF to aPL ROS and pro-inflammatory signaling of cofactor-independent aPL.

ASY 18.1 | Citrullinated Histone H3, a Biomarker of Neutrophil Extracellular Trap Formation, is Associated with Increased Risk of Venous Thromboembolism in Patients with Cancer

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Background: Neutrophil extracellular traps (NETs) are decondensed chromatin fibers which might play a role in the prothrombotic state of patients with cancer. Cancer mouse models showed that NETs were associated with venous thrombus formation and it is well known that patients with cancer have a high risk to develop venous thromboembolism (VTE).

Aims: The aim of this project was to investigate if NET markers (citrullinated histone H3 (H3Cit), cell free DNA (cfDNA), and nucleosomes could be used to predict the risk of VTE in patients with cancer.

Methods: In 946 patients with newly-diagnosed cancer or progression after remission H3Cit, cfDNA, and nucleosomes were determined from blood samples drawn at study inclusion. Patients were followed prospectively for 2 years until occurrence of symptomatic VTE or death.

Results: VTE occurred in 89 patients, and 352 patients died during follow-up. Patients with elevated H3Cit (i.e. $>75^{th}$ percentile of its

distribution, n=236) experienced a higher cumulative incidence of VTE (2-year risk=15.6%) as compared to patients below this cut-off (2-year risk=8.2%, n=710, Gray's test p=0.002, Figure 1). In a competing risk regression analysis, a 100ng/mL increase in H3Cit was associated with a 13% relative increase in the risk of VTE (subdistribution hazard ratio (SHR) =1.13, 95%CI: 1.04-1.23, p=0.005). This association prevailed after adjustment for high- and very-high-VTE risk tumor sites, D-dimer, and soluble P-selectin. The association between elevated nucleosomes and cfDNA with risk of VTE was time-dependent with associations with higher risk of VTE only during the first 3-6 months (Figure 2).

Conclusions: Biomarkers of NETs predict the occurrence of VTE in patients with cancer, indicating an important role of NET-formation in the pathogenesis of cancer-associated thrombosis.

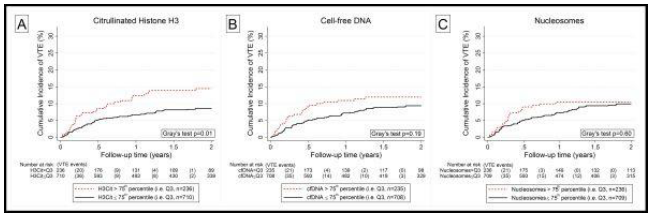

FIGURE 1 Cumulative incidence of VTE according to baseline H3Cit, cfDNA and nucleosome levels

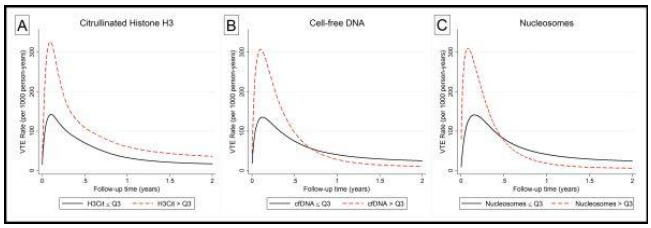

FIGURE 2 Predicted time-dependent VTE rates over 2 years of follow-up according to baseline levels of H3Cit, cfDNA and nucleosomes

ASY 18.2 | Elucidation of the Role of Neutrophils in Human Pancreatic Cancer-associated Thrombosis Using a Humanized NSG Mouse Model

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Background: Patients with pancreatic cancer have a high incidence to develop deep vein thrombosis (DVT). NETs (Neutrophil extracellular traps) that are formed by neutrophils enhance DVT. The white counts, however, are not necessarily elevated in the pancreatic cancer patients who develop DVT as compared to those without DVT.

Aims: To investigate whether neutrophils contribute to cancer-associated thrombosis in a humanized mouse model.

Methods: NSG (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) mice with functionally defective neutrophils were used as neutropenia models and SCID mice were used representing neutrophil-rich controls. A moderately differentiated human pancreatic cancer cell line, AsPC-1, established from the ascites of a metastatic cancer patient, was orthotopically inoculated into NSG and SCID mice. At 3 weeks after inoculation, mice were sacrificed and analyzed.

Results: We found that the tumor volumes in the SCID and NSG mice were comparable (502.3±157.8 and 478.1±365.5 mm^3; mean ± SD, n=7 in each group). The levels of thrombin-antithrombin (TAT) complex and cell-free DNA (cfDNA) were significantly elevated in tumor-bearing SCID mice (TAT complex: 5.18±4.67 ng/mL; cfDNA: 4.18±1.25 ng/mL) but not in tumor-bearing NSG mice (TAT complex: 1.77±0.51 ng/mL; cfDNA: 1.66±0.15 ng/mL) as compared with non-tumor bearing controlled mice respectively.

Conclusions: We have demonstrated that the levels of the TAT complex and cfDNA in tumor-bearing NSG mice did not increase as those observed in the SCID mice. The difference may be due to the lack of functionally competent neutrophils in NSG mice. Functional competence of neutrophils was associated with the TAT and cfDNA levels in our human pancreatic cancer mouse models. Whether neutrophils play a major role in TAT complex formation and/or cfDNA elevation through the formation of NETs in human pancreatic cancer mouse models will be further addressed.

ASY 18.3 | Neutrophils Prothrombotic Characteristics during Myeloproliferative Neoplasms

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Background: Thrombosis is the most frequent complication during evolution of myeloproliferative neoplasms (MPN) but the events causing these clotting abnormalities remain unclear. Recently, clinical studies have identified leukocytosis as a risk factor for thrombosis and neutrophils are now recognized as important actors of thrombosis, especially by their capacity to emit neutrophil extracellular traps (NET) when activated.

Aims: To assess if JAK2V617F neutrophils are more activated than JAK2WT neutrophils, emit more NET, thus promoting thrombosis.

Methods: We first studied MPN patients and quantified: 1) neutrophils activation markers, 2) plasma levels of free DNA and MPO-DNA complex. In a second part, we used *PF4-iCreERT2;JAK2$^{V617/WT}$* mice with expression of JAK2V617F in hematopoietic cells. We quantified: 1) NET emission after neutrophils activation, 2) plasma levels of circulating DNA and matrix metallopeptidase 9 (MMP9), 3) pulmonary thrombus formation.

Results: In MPN patients, we found: 1) increased neutrophils CD11b expression, 2) increased neutrophils TF expression in patients with history of thrombosis, 3) increased plasma levels of free DNA in all patients and increased plasma levels of MPO-DNA complex in patients with history of thrombosis. In *PF4-iCreERT2;JAK2*$^{V617F/WT}$ mice, we observed: 1) proliferation of all hematopoietic lineage, secondary to presence of JAK2V617F in neutrophils, platelets and red blood cells, 2) increased NET formation after neutrophils activation, 3) increased plasma level of free DNA and MMP9, 4) increased pulmonary thrombus formation.

Conclusions: Our results show that neutrophils are hyperactivated during MPN, in a patient cohort, and in a mouse model with JAK2V617F expression in neutrophils. Increased NET formation in PF*4-iCreERT2;JAK2*$^{V617F/WT}$ model, associated with increased thrombus formation suggests an important role of neutrophils in thrombus formation during JAK2V617F positive MPN.

ASY 18.4 | Mice Lacking the Novel Venous Thromboembolism Susceptibility Gene Slc44a2 Have Normal Hemostasis, but Reduced Fibrin Accumulation upon Vascular Injury

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Background: Recently SNPs located within the *SLC44A2* gene were identified to be susceptibility loci for venous thromboembolism (VTE) by two independent GWAS studies. *SLC44A2* encodes the Solute Carrier-Like Family 44 Member 2 (SLC44A2) protein which has not been previously linked to VTE and therefore is of interest to study in order to identify novel mechanisms of VTE pathogenesis.

Aims: Characterize *Slc44a2* knockout (KO) mice from a hemostasis perspective.

Methods: Lung and liver isolated from *Slc44a2* wild type or KO mice were used to determine expression of several coagulation genes by qPCR in addition to measuring changes in fibrin deposition by immunoblot (n=7). Differences in thrombin generation by plasma (n=7) and *ex-vivo* analysis of platelet aggregation and activation were also evaluated (n=10). Finally, we used intravital microscopy cremaster arteriole thrombosis model to distinguish variations in platelet and fibrin recruitment upon vascular damage induced by laser injury.(3 mice/group; 8-10 independent injuries per mouse).

Results: In KO mice, a significant increase in lung *Plat* transcript levels was observed (p=0.018) with no differences found in *F3*, *Vwf*, *F2r*, *F8*, *Thbd*, *Tfpi* or *Serpine1*. In the liver, expression of *Fga*, *F2*, *F5*, *F11* and *Proc* was not altered by the loss of *Slc44a2*. No differences in fibrin deposition and tissue factor triggered thrombin generation were found. Interestingly, platelet analysis revealed an increased induction of P-selectin upon thrombin stimulation in KO mice, however no other sizeable changes in activation were recorded. Upon laser injury, KO mice had reductions in fibrin accumulation ($p < 0.001$) whereas platelet recruitment remained unaffected.

Conclusions: In general *Slc44a2* KO mice without a thrombotic stimuli do not present obvious defects in hemostasis however, our initial characterization has uncovered a deviant response upon vascular injury, possibly due to an effect of *Slc44a2* on thrombus fibrin turnover.

ASY 29.1 | Variety of Activators and Intracellular Sources Determine the Structural Diversity of Platelet-derived Microparticles

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Background: Platelet-derived microparticles (MPs) comprise the major population of circulating blood MPs that play an important role in hemostasis and thrombosis. Despite numerous studies on the (patho)physiological roles of platelet MPs, their structural composition and the basis for heterogeneity remain largely unknown.

Aims: To study the intracellular origin and structural diversity of MPs derived from human platelets activated by various stimuli.

Methods: MPs released by isolated quiescent human platelets or platelets stimulated with arachidonic acid, ADP, collagen, thrombin, or calcium ionophore A23187 were studied with flow cytometry, scanning and transmission electron microscopy.

Results: The structure, dimensions, and intracellular origin of MPs depend on the cell-activating stimulus. The most common structural groups include a vesicle surrounded by a membrane or multivesicular structures. Thrombin, unlike other stimuli, induced formation of MPs not only from the outer plasma membrane but also from intracellular membranous structures comprising the endomembrane system, including the open canalicular system. Some vesicular particles contained organelles, such as mitochondria, glycogen granules, and vacuoles, indicating their cytoplasmic origin. The size of platelet MPs varied from 30 nm to 500 nm, but the size distributions depended on the nature of the cell-activating stimulus with the smallest MPs formed by thrombin, a strong platelet activator.

Conclusions: The study has shown that the structure of platelet MPs depends on whether they originate from the plasma membrane or the endomembrane system. Based on their detailed structure, platelet microvesicles are classified as homogenous rounded particles surrounded by one single membrane, multivesicular structures, and vesicles containing cellular organelles. The results obtained show that the structural diversity of platelet-derived MPs is based on the nature and strength of cell-activating stimulus and their intracellular origin.

ASY 29.2 | Tissue Factor-Positive Microvesicles from a Human Pancreatic Tumor Grown Orthotopically Enhance Venous Thrombosis in Mice

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Background: Patients with pancreatic cancer have elevated levels of circulating microvesicle (MV) tissue factor (TF) activity, and there is an association between MV TF activity and venous thromboembolism. We showed that injection of exogenous TF-positive MVs (TF+ MVs) derived from a human pancreatic cancer cell line (BxPc-3) increased thrombosis in an inferior vena cava (IVC) stenosis mouse model.

Aims: Determine if endogenous human TF+ MVs derived from a human pancreatic tumor grown orthotopically enhance thrombosis in an IVC stasis mouse model.

Methods: We used the human pancreatic cancer cell line BxPc-3 because it expresses a high level of TF. Tumors were growth subcutaneously or orthotopically in nude mice. Tumor-bearing mice and controls were subjected to IVC ligation and thrombi harvested after 48 hours. Thrombus formation was also assessed using ultrasound at 3, 6, 24 and 48 hours after surgery. In a second experiment, mice with orthotopic tumors were treated with either an inhibitory anti-human TF antibody (HTF-1) or control IgG (2.86 mg/kg i.v.) just before surgery and 24 hours after surgery.

Results: Mice with subcutaneous BxPC-3 tumors (0.50 - 1.08 g) had a non-significant increase in the thrombus compared with sham controls (20.81±7.12 mg, n=8 vs 18.53±4.09 mg, n=13, *P*=0.4249). In contrast, mice with orthotopic BxPC-3 tumors (1.13 - 3.14 g) had significantly larger thrombi compared with sham controls (30.74±3.80 mg, n=8 vs 19.23±3.71 mg, n=9, *P*=0.0006). Importantly, administration of HTF-1 significantly reduced thrombosis in mice with orthotopic tumors compared with IgG (*P*=0.0152).

Conclusions: Our studies show mice containing orthotopic BxPc-3 tumors have increased thrombosis compared with control mice. The enhanced thrombosis was abolished with an antibody against human TF, which suggests that TF+ MVs released from the tumor increase venous thrombosis in this mouse model.

ASY 29.3 | SerpinE2 Deficiency Accentuates Coagulation and Inflammation and Promotes Pulmonary Fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is a devastating form of interstitial lung disease associated with extracellular matrix deposition. SerpinE2/protease nexin-1 (PN-1) is a tissue serpin with both anticoagulant and antifibrinolytic properties and has been shown to be overexpressed in lungs during IPF.

Aims: To determine the role of PN-1 in the development of pulmonary fibrosis.

Methods: We compared bleomycin-induced pulmonary fibrosis on the survival of Wild-Type (WT) and PN-1-deficient mice (PN-$1^{-/-}$), and of irradiated mice reconstituted with bone marrow cells from *PN-1*$^{-/-}$ or *Par4*$^{-/-}$ or *PN-1*$^{-/-}$/*Par4*$^{-/-}$ or WT mice. Bronchoalveolar lavages (BAL) of the mice were analysed by counting platelets and leucocytes at the hemocytometer, by measuring thrombin activity using a specific chromogenic substrate and by quantification of TGFβ_1, platelet factor 4, myeloperoxidase and D-Dimers using ELISA.

Results: PN-1 deficiency in mice was associated with a significant increase in mortality resulting from more severe fibrosis after bleomycin challenge. Active TGFβ_1, a profibrotic growth factor, was detectable only in BAL from bleomycin-treated *PN-1*$^{-/-}$ mice. These data indicate a protective role of PN-1 in lungs. When the direct thrombin inhibitor argatroban was administered to the mice, a significant improvement of the survival of PN-$1^{-/-}$ mice treated with bleomycin was observed. PN-1 deficiency was also associated with a marked increase in active thrombin, and an accumulation of platelets and neutrophils in BAL. Bone marrow transplantation experiments showed that protective PN-1 was derived from the hematopoietic cell compartment. Compound deficiency of PN-1 and the thrombin receptor PAR-4, in hematopoietic cells, abolished the mortality associated with PN-1 deficiency.

Conclusions: Prevention of thrombin signalling by PN-1 present in hematopoietic cells, constitutes an important endogenous mechanism of protection against lung fibrosis and associated mortality in mice.

ASY 29.4 | Von Willebrand Factor Regulates Deep Vein Thrombosis in a Mouse Model of Diet-induced Obesity

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Background: Obesity is associated with increased von Willebrand factor antigen (VWF:Ag), factor VIII activity (FVIII:C) and risk of deep vein thrombosis (DVT). VWF may regulate thrombosis by localizing FVIII, platelets and leukocytes to the site of thrombus formation.

Aims: To evaluate the role of VWF in DVT in a diet-induced obese (DIO) mouse model.

Methods: DVT was induced by inferior vena cava (IVC) ligation in DIO C57BL/6 mice and lean littermates. Thrombi were weighed and longitudinal sections were analyzed by IHC 24 hours post stenosis.

Results: DIO mice had increased VWF:Ag (1.3-fold, p=0.003), FVIII:C (1.4-fold, p=0.006), circulating granulocytes (1.15-fold, p=0.006) and erythrocytes (1.05-fold, p=0.0002) after 2 weeks on a high-fat diet compared to controls. DIO mice had larger thrombi after 2 weeks (1.75-fold, n=32, p=0.0497) and 10 weeks (1.28-fold, n=39, p=0.24)

on diet than controls. DVTs from DIO mice were comprised of more red thrombus (71.6% vs. 61% in controls, p=0.01) that correlated with DVT size (r^2=0.8, p=0.006). High-intensity VWF staining co-localized with white thrombus (leukocytes and platelets) and low-intensity VWF staining with red thrombus. Quantitative IHC showed that DVTs from DIO mice had increased VWF (1.4-fold, p=0.03) and total leukocytes (1.5-fold, p=0.098) but not fibrin or platelets. In DIO mice and controls, DVT size correlated with VWF (r^2=0.43, p=0.002), leukocytes (r^2=0.41, p=0.003), fibrin (r^2=0.69, p=0.0002) and platelets (r^2=0.48, p=0.006). ADAMTS13 KO DIO mice did not have increased DVT size. VWF KO DIO mice were protected from developing DVT (55% decreased incidence) with DVT size decreased by 89% (p=0.003). Treatment of DIO mice with a polyclonal anti-VWF antibody decreased DVT incidence by 35% and size by 73% (p=0.005).

Conclusions: DIO mice exhibit increased venous thrombogenicity that may be related to the role of VWF in platelet/leukocyte recruitment and FVIII binding. VWF is integral to DVT formation in this model and may be a novel therapeutic target.

ASY 37.1 | Adverse Pregnancy Outcomes in Women with Persistent Lupus Anticoagulant - Experiences from the Prospective Vienna Lupus Anticoagulant and Thrombosis Study (LATS)

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Background: Lupus anticoagulant (LA) positivity is a known risk factor for pregnancy complications.

Aims: To prospectively analyze pregnancy complications in women with persistently positive LA.

Methods: In the ongoing prospective Vienna Lupus Anticoagulant and Thrombosis Study (LATS) we followed 165 (female=136, 82%) patients over a median follow-up (FU) period of 9.2 years. Eighty-two women (60%) were of child-bearing age (≤45 years) at study inclusion.

Results: During FU, we observed 34 singleton pregnancies in 21 women (Table 1).

One induced abortion and one tubular pregnancy, each, were not included in further analysis. Pregnancy complications occurred in 16 women during 22 pregnancies (65%), including 17 spontaneous abortions (< 10^{th} week of gestation (WOG): n=11, 10^{th}-24^{th} WOG: n=6) and 5 deliveries ≤34^{th} WOG (4 due to preeclampsia/HELLP syndrome). Ten pregnancies were uneventful with live births ≥36^{th} WOG. Neither a prior history of pregnancy complications (Odds ratio (OR)=5.3, 95%CI: 0.3-82.8, p=0.23) nor a prior history of thrombosis (OR=2.0, 95%CI: 0.2-16.6, p=0.52) were significantly associated with pregnancy complications during FU. Women with pregnancy complications had higher baseline levels of anti-β2-GPI IgG antibodies than those without pregnancy complications (median: 53.5 vs. 23.7 GPL, p=0.06). Pregnancy complications according to treatment are listed in Table 2. The combination of low molecular weight heparin (LMWH) with low dose aspirin (100 mg qd) led to the most favorable pregnancy outcome.

Conclusions: The risk for pregnancy complications is extremely high in women with persistent LA. This finding urges a more precise identification of patients at risk for pregnancy complications and improved treatment options.

TABLE 1 Baseline characteristics of the 21 women with at least one pregnancy during follow up

Variable	Median [25th-75th percentile], or absolute count (percent)
Age at entry (years)	28 [24-29]
Diagnosis of APS	18 (86%)
Prior history of arterial or venous thrombosis	16 (76%)
Prior history of pregnancy complications	11 (52%)
aPTT-LA (seconds)	99 [86-118]
aβ2GPI IgM (MPL)	7.7 [3.0-15.8]
aβ2GPI IgG (GPL)	50.0 [8.2-84.2]
aCL IgM (MPL)	6.4 [3.8-16.7]
aCL IgG (GPL)	40.8 [16.4-104.3]

TABLE 2 Treatment modalities and pregnancy outcomes of 32 pregnancies

	LMWH	LMWH+ low dose aspirin	VKA or Rivaroxaban	No therapy
Total number of pregnancies	11	13	4	4
Early abortion (<10th WOG)	5	1	3	1
Late abortion (10th-24th WOG)	3	2	1	1
Live birth ≤34th WOG	2	3	0	0
Live birth >34th WOG	1	7	0	2
Live birth vs total	3/11	10/13	0/4	2/4

ASY 37.2 | Identification of Gene-environment Interactions in Pregnancy-related Venous Thromboembolism

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Background: Pregnancy and the postpartum period are major risk factors for venous thromboembolism (VTE), with 4-fold increased odds of VTE and a VTE incidence of 200 per 100,000 women-years. Pregnancy-related VTE accounts for approximately 10% of all maternal deaths.

Aims: Our aim is to identify genetic variants associated with VTE in pregnancy using the Mayo Clinic VTE study.

Methods: Of 758 female VTE cases in the study (all of non-Hispanic European ancestry), 318 had a VTE event during the considered procreative age interval of 18 to 45 years; 634 of these women had genome-wide 1000G imputed genotype data. We used this data to perform a genome wide association (GWA) analysis to identify genetic variants associated with pregnancy-related VTE (gene-environment interaction) using Cox proportional hazards modeling, adjusted for age, stroke/MI and state of residence.

Results: None of the common genetic variants known to be associated with VTE (i.e., *F5* rs6025 [Factor V Leiden], *ABO* rs8176719 [ABO non-O blood type], and *F2* rs1799963 [prothrombin G20201A]) reached genome-wide significance in this analysis, indicating that there is a different genetic mechanism for pregnancy-related VTE. Only two intragenic single nucleotide polymorphisms (SNPs) reached genome-wide significance: *PURB* chr7.44909852.D (HR=0.41; p=3.34E-08) and rs10215876 (HR=0.40; p=1.15E-08); two additional intragenic SNPs, *LINGO2* rs4878679 (HR=0.63; p=3.31E-07), and *RDXP2* chrX.rs2191549 (HR=0.52; p=4.91E-07) reached borderline genome-wide significance. All four SNPs were validated using internal cross-validation. We are in the process of validating our results using pregnant females with VTE from the MEGA study.

Conclusions: We observed that the main genetic risk genes involved in VTE, F5, ABO and F2, are not genome-wide significant for pregnancy-related VTE, indicating that the genes, PURB, LINGO2 and RDXP2, are potential candidate genes for provoked VTE in pregnant women.

ASY 37.3 | Cancer and Venous Thromboembolism in Pregnancy

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Background: Hospitalized patients with cancer are at high risk of developing venous thromboembolism (VTE), and this risk increases with pregnancy. No studies have assessed the risks of VTE in women with cancer during pregnancy and hospitalization.

Aims: The goal of this study was to apply a thromboprophylaxis protocol with a VTE risk score for hospitalized pregnant women with cancer and to evaluate the effect on maternal morbidity and mortality.

Methods: This was a longitudinal, interventional and prospective study of hospitalized pregnant women diagnosed with cancer. Patients were classified as low risk or high risk according to a VTE risk score. The high-risk group received thromboprophylaxis with low-molecular-weight heparin (LMWH) unless the patient had a contraindication for anticoagulation, such as active bleeding or a high bleeding risk. The collected data were descriptively analysed to identify the profile of pregnant women and type of cancer using percentages and absolute values. One patient could have undergone more than one evaluation.

Results: The data of 87 cases (61 patients) were descriptively analysed: 64 (73.5%) were classified as high-risk and 55 (86%) received enoxaparin; 46 (52.8%) had breast cancer, 10(11.49%) cervical cancer of the uterus and 14 (21.9%) had metastatic cancer. The main risk factors for VTE were chemotherapy (within 6 months) - 39/64 (61%) and age ≥ 35 years (24 - 37%). No patient exhibited VTE, adverse effects of anticoagulation or death up to three months after hospitalization.

Conclusionss: Most pregnant women with cancer had a high risk for VTE at the time of hospitalization. Breast cancer was the most prevalent and recent chemotherapy was the main risk factor for anticoagulation. The application of a thromboprophylaxis protocol and determination of a VTE risk score for these patients was effective for the prevention of maternal morbidity and mortality due to VTE.

ASY 37.4 | Type of Combined Contraceptives, Factor V Leiden Mutation and Risk of Venous Thromboembolism: A Case-Only Study

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Background: Combined hormonal contraceptives (CHC) and Factor V Leiden mutation (FVL) increase the risk of venous thromboembolism event (VTE). Whether the risk level of VTE differs with the progestin-type of CHC in women with FVL as compared to non-carriers remains to be determined.

Aims: Using Data from Contraception and Recurrent Venous Event (COREVE) study, we estimated the interaction between types of CHC and FVL on VTE risk.

Methods: We conducted a case-only study (a tool for testing gene-environment interactions) in women with first documented VTE who were referred to our hemostasis Unit between 2000 and 2009. FVL screening was performed in all women. Under the assumption of independence between genotype and exposure, this methodology is equivalent to comparing the risk of VTE associated with CHC use in FVL carriers with the risk of VTE associated with CHC use in non-carriers.

Results: Among 2613 women, 415 had a FVL (15.9%). At the time of the VTE, 803 (30.7%) were non-users and 1810 (69.3%) used CHC. The interaction between CHC use and presence of FVL on VTE risk was statistically significant (1.37; 1.06-1.77 95% CI).

The comparison between the progestin types of CHC showed that CHC containing drospirenone (n=98) or cyproterone acetate (n=326) had a higher interaction in FVL carriers than in non-carriers (2.09 (1.23-3.53) and 1.79 (1.26-2.56) respectively). In addition, interactions

were higher in drospirenone or cyproterone acetate group as compared with 1st (norethisterone), 2nd (lévonorgestrel) or norgestimate CHC users (p=0.02 and p=0.03 respectively). The results were similar when we restricted the population to women without familial history of VTE or women with idiopathic VTE.

Conclusions: Our results suggest that CHC containing drospirenone or cyproterone acetate may increase risk of VTE more in FVL carriers that in non-carriers, even among women without familial history of VTE.

ASY 38.2 | Identification of a microRNA Profile for Predicting the Thrombotic Risk of Biliopancreatic Cancer Patients

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Background: Venous thromboembolism (VTE) is a common complication of cancer increasing mortality and morbidity, and new biomarkers to identify cancer patients with high VTE risk are needed. microRNAs (miRs) are small non-coding RNAs that regulate protein expression. miRs seem to regulate cancer progression and VTE.

Aims: To find a miR profile to identify biliopancreatic cancer patients with high VTE risk.

Methods: 125 biliopancreatic cancer patients were prospectively recruited and followed for two years. Blood was drawn at diagnosis and every three months. The miR expression level was studied in plasma of 5 selected patients who developed VTE and in 5 who did not, at inclusion and right before the VTE event, with the Serum/plasma Focus microRNA PCR Panel V4 (Exiqon). Statistical analysis was performed using R (v3.2.3). Next, we validated the VTE predictive model in a different subset of 32 biliopancreatic cancer patients.

Results: We adjusted an elastic net logistic regression model for VTE risk using the miR expression levels at inclusion. This predictive model included 7 miRs with fold-changes ranging from 1.51 to 4.28 and achieved an *AUC* of 0.95 in the validation cohort (95% CI: 0.87-1.00; p < 0.001). Moreover, comparing the miR expression level at inclusion and right before the VTE event with the Paired T-test, we identified a profile of 7 dysregulated miRs that might prompt the VTE. We have identified the cancer- and VTE-related target proteins of these miRs.

Conclusions: A profile of 7 miRs may allow us to estimate the risk of VTE in biliopancreatic cancer patients at diagnosis. This could promote a closer follow-up and a personalized thromboprophylaxis in high-risk patients. We have also identified 7 dysregulated miRs right before the VTE that could shed light on the mechanism triggering a VTE in biliopancreatic cancer patients. ISCIII-FEDER (PI12/00027, RIC RD12/0042/0029, PIE13/00046, PI14/00079, PI14/00512, FI14/00269, CPII15/00002), GVA (PrometeoII/2015/017), SETH, OBEL Family Foundation (26145).

PEDIATRICS

ASY 26.1 | Unravelling Pediatric Pulmonary Embolism: Outcomes and Severity Predictors

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Background: Pulmonary embolism (PE) is increasingly recognized in children, Little is known about pediatric massive and submassive PE in children (M/SMPE).

Aims: Main: To describe pediatric PE-related outcomes according to PE severity [M/SMPE vs non-MPE (NPE)].

Secondary: To explore the role of PE severity scores in children as outcome predictors.

Methods: Patients aged 0-18 years with objectively confirmed PE diagnosed between 2003-2016 were included. Hypotension/shock/death within 24h of presentation defined MPE, whereas right ventricular dysfunction defined SMPE. Main outcomes were death and combined unfavourable outcomes (pulmonary hypertension, recurrence and progression). Pulmonary Embolism Severity Index (PESI) and simplified PESI (sPESI) were calculated using age-adjusted parameters. Group comparisons used Wilcoxon rank sum, Chi-square or Fisher exact tests, as appropriate. Associations between outcomes and predictors were tested with logistic regression. The institutional review board approved the study.

Results: 95 cases of PE were reviewed; 26/95 (27%) were classified as M/SMPE.

M/SMPE patients were younger [median age, years (25th-75th percentile) 12 (0.8-15) vs NPE 15 (10-16), p=0.04].

TABLE 1 Patients characteristics divided by PE groups

Characteristics	NPE n=69	M/SMPE n=26	*P value*
Age, years [median, (25th-75th percentile)]	15 (10-16)	12 (0.8-16)	0.04
Male:female ratio	1.09	1.17	0.88
Underlying conditions			0.03
Cancer	19 (27%)	3 (11%)	
Heart disease	9 (13%)	9 (34%)	
Inflammatory/infectious conditions	15 (22%)	2 (8%)	
No underlying condition	4 (6%)	0 (0%)	
Central venous catheter	29 (42%)	13 (50%)	0.49

Treatment modalities included observation [M/SMPE 4%, NPE 10%], anticoagulation [M/SMPE: 35%, NPE 81%] or invasive treatment [embolectomy/local-/systemic-thrombolysis (M/SMPE 61%, NPE 9%; p< 0.001]. M/SMPE was associated with PE-related death (12% vs 0%, p=0.02) and unfavourable outcomes (31% vs 10%, p=0.01).

Cardiac disease and M/SMPE were associated with unfavourable outcomes in univariable logistic regression. In a multivariable analysis, only M/SMPE associated with unfavourable outcomes (OR 4.2 (95% CI 1.2-14.4)).

Finally, PESI was not associated with death or unfavourable outcomes, but sPESI was associated with death (p=0.02).

Conclusions: Despite more aggressive therapy, M/SMPE led to a higher morbidity and mortality than NPE in children. However, PESI and sPESI may not be applicable to predict pediatric PE outcomes.

ASY 26.2 | Congenital Antithrombin Deficiency Increases 43-fold the Risk of Pediatric Thrombosis and 200-fold the Risk of Neonatal Thrombosis. Results from 468 Patients with Antithrombin Deficiency from 176 Unrelated Families

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Background: Pediatric thrombosis is rare: 0.14/10,000 children and 5.1/100,000 births. Deficiency of endogenous anticoagulants increases the risk of pediatric thrombosis, but the low number of cases limits the conclusions on clinical characteristics of these patients. Antithrombin (AT) deficiency is the strongest thrombophilia, but there are few studies in small cohorts evaluating the role of AT deficiency on pediatric thrombosis.

Aims: To define the prevalence of pediatric thrombosis in carriers of congenital AT deficiency and to describe their clinical features.

Methods: We performed an observational retrospective multicentric study of 468 subjects (0-85 years, y) with congenital AT deficiency from 176 unrelated families. Genetic, functional and biochemical analysis were done.

Results: 28 cases had pediatric thrombosis, 6% of our cohort. Two periods with high risk of thrombosis were identified: adolescence (16-18y, N=13) in whom localizations and risk factors are common in adults (deep venous thrombosis or pulmonary embolism, and contraceptives, surgery or pregnancy); and newborns (< 30 days, N=5) with idiopathic thrombosis in unusual localizations.

Cerebral sinovenous thrombosis was frequent (N=5, 2 neonates & patients with 2, 5 and 18y).

Thrombosis was very severe, with fatal consequences (N=5, including a neonate homozygous for p.Leu131Phe); serious sequelae (psychomotor delay or amputation, N=5); and recurrence overtime (N=5).

Type I deficiency was predominant (75%).

AT levels in available neonates were very low (11-20%).

Conclusions: Our data support the high risk of pediatric (43-fold) and particularly neonatal thrombosis (200-fold) associated to congenital AT deficiency. The low levels of AT in neonates probably exacerbates the reduced anticoagulant capacity associated with the deficiency, making this moment of high thrombotic risk. Our results support the screening of AT deficiency (mainly if it is type I) in children of affected families.

Funding: PI15/00079; CB15/00055, ISCIII and FEDER;9873/GERM/15, F.Séneca

ASY 26.3 | A Polymorphism in the Transcriptional Repressor Growth Factor Independence-1 Gene GFI1^{36N} is Associated with DVT Risk in Pediatric Oncology Patients: Results from a Genome-wide Association Analysis

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Background: Pediatric cancer patients are at increased risk for deep vein thrombosis (DVT). Investigation of genomic loci contributing to DVT risk may help to identify novel mechanisms for risk and to manage affected patients. Genome-wide association studies (GWAS) are a valuable tool in an unbiased approach to identifying unique genetic factors.

Aims: Identification of susceptibility loci for DVT in pediatric oncology patients using GWAS.

Methods: A population based nested case control study was conducted in 7 Canadian centers. A total of 411 survivors of pediatric cancer were recruited, 127 with DVT (cases) and 284 without DVT (controls) during treatment for cancer in childhood. DNA isolates from probands were hybridized to Illumina marker Infinium PsychArrays and 308496 markers remain for analysis. Genomic inflation factor lambda is 1.035 indicating no serious inflation. Association analysis was performed using logistic regression assuming an additive model adjusted for age, blood group, cancer type, chemotherapy.

Results: The SNP rs34631763 was significantly associated with DVT (p = 3.32 E-7). The SNP rs34631763 on chromosome 1 is an exonic

missense variant within the transcriptional repressor Growth Factor Independence 1 (GFI1) gene which generates a protein with asparagine ($GFI1^{36N}$) in place of a serine ($GFI1^{36S}$) at position 36. GFI1 is primarily important in hematopoietic stem cells lymphoid and myeloid differentiation and has been shown to be involved with development of acute lymphoblastic leukemia. $GFI1^{36N}$ variant is associated with risk for developing acute myeloid leukemia in adults.

Conclusions: The increased incidence of the $GFI1^{36N}$ variant in pediatric solid tumor and leukemia patients who developed DVT during cancer treatment suggests a novel role for GFI1 in regulation of genes involved with development of DVT. Future directions include replicating these results in an independent patient cohort and assessing pathways for GFI1 mediated transcriptional repression of genes involved with DVT.

ASY 26.4 | Haemostatic Monitoring and Thresholds for Blood Product Replacement Therapy during Treatment of Acute Lymphoblastic Leukaemia in Children and Young People Vary between Centres in the UK

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Background: Bleeding is a frequent cause of morbidity and can contribute to mortality in children and young people treated for acute lymphoblastic leukaemia (ALL). Recommendations vary in terms of frequency of coagulation testing and thresholds for blood product replacement therapy.

Aims: This study aimed to determine current UK practice in relation to coagulation testing and blood product replacement therapy during treatment of ALL in young people.

Methods: A survey was sent using a web-based tool, SurveyMonkey.com, to the 28 centres participating in the Medical Research Council UKALL 2011 trial for individuals aged 1-24 years.

Results: The survey was completed by investigators at 23 centres. 96% have a local protocol for blood product replacement therapy in young people with ALL. 21/23 centres (91%) routinely perform coagulation screening at the time of ALL diagnosis. 6/23 centres (26%) routinely monitor coagulation tests during induction chemotherapy. During the period of asparaginase activity, fibrinogen is supplemented in 9 centres (39%) prior to insertion of a CVAD and 6 centres (26%) prior to lumbar puncture. Threshold for supplementation of fibrinogen is < 1.0 g/L in all cases. The replacement product chosen is cryoprecipitate in 7, plasma in 1 and fibrinogen concentrate in 2. Threshold for platelet transfusion varies, particularly prior to lumbar puncture, insertion of a central venous access device (CVAD) and in the patient with bleeding.

During therapeutic anticoagulant therapy, interruption of anticoagulation or transfusion of platelet concentrate is considered necessary when platelet count is < 20 x 10^9/L in 1 centre (4%), < 30 x 10^9/L in 6 (26%) and < 50 x 10^9/L in 16 (70%).

Conclusions: This survey has identified significant variation in practice in relation to the frequency of coagulation testing and thresholds for blood product replacement therapy in children and young people with ALL highlighting the need for future research and evidence-based guidance.

PLATELETS – BASIC

ASY 04.3 | Three-Dimensional Fluorescence Emission Computed Tomography (FLECT) with a Newly Generated Activated Platelet-specific Fluoroprobe: A Unique Technology for the Diagnosis of Thrombotic Diseases

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Background: Effective preclinical testing involving small animal studies is fundamental to the development of new pharmaceutical products.

TABLE 1 Thresholds for the transfusion of platelet concentrate in children and young people with acute lymphoblastic leukaemia

	Threshold for the transfusion of platelet concentrate, Number of centres (%)						
	<10 x 10^9/L	<20 x 10^9/L	<30 x 10^9/L	<50 x 10^9/L	<70-80 x 10^9/L	<100 x 10^9/L	No response
Well patient	20 (87%)	2 (9%)	0	0	0	0	1 (4%)
Febrile patient	3 (13%)	20 (87%)	0	0	0	0	0
Patient with active bleeding	0	0	4 (17%)	14 (61%)	3 (13%)	1 (4%)	1 (4%)
Prior to lumbar puncture	1 (4%)	3 (13%)	5 (22%)	13 (57%)	1 (4%)	0	0
Prior to insertion of CVAD	0	0	0	12 (52%)	9 (39%)	2 (9%)	0

Targeted molecular imaging that characterizes biological processes in living animals is important to determine the clinical translation of novel drug candidates. Especially for detection of thromboembolism, there is a strong need for sensitive preclinical imaging technologies.

Aims: We aim to demonstrate the use of a world-first three-dimensional near-infrared (NIR)-fluorescence whole-animal bioimager called FLuorescence Emission Computed Tomography (FLECT) to diagnose thromboembolism using a novel probe that is safe, easily prepared in large scale and stably stored.

Methods: We generated a novel NIR-fluoroprobe comprising a single-chain antibody ($scFv_{GPIIb/IIIa}$), which exclusively binds activated-platelets, conjugated via the combination of biological (Sortase A) and chemical (Click-Chemistry) coupling to Cy7 dye for *in vivo* detection. The ability of the FLECT scanner to detect this probe in thrombotic sites of mice was then ascertained.

Results: Upon carotid artery injury, the injected fluoroprobe bound platelet-rich thrombi and was detected by FLECT-imaging of mice. The analyzed FLECT image quantified the NIR signal and clearly localized it to the injured artery. The FLECT-detected fluorescence was verified on the excised arteries using a 2-dimensional IVIS® scanner. Next, longitudinal FLECT-imaging to monitor thrombolysis over time demonstrated a loss of detected signal in successfully thrombolyzed mice. Besides intravascular thrombosis, we have also successfully employed FLECT imaging for the diagnosis of *in vivo* pulmonary embolism, with the detection of the affected lung lobes.

Conclusions: This report showcases a novel preclinical imaging technology using a newly generated, radioactivity-free fluoroprobe in a three-dimensional *in vivo* fluorescence scanner that allows sensitive thromboembolism detection and monitoring the efficacy of antithrombotic/thrombolytic drugs.

ASY 08.1 | CLEC-2 Contributes to Hemostasis Independently of Classical HemiTAM Signaling in Mice

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Background: C-type lectin-like receptor 2 (CLEC-2) is a platelet receptor that is critical during development and implicated in thrombus stability in thrombosis and hemostasis. It is the only known platelet receptor to signal through a hemi-immunoreceptor tyrosine-based activation motif (hemITAM). Current investigations into the function of CLEC-2 *in vivo* have focused on knock-out studies; however these are unable to explore the possible signaling independent functions of the receptor indicated by its only known physiological ligand, podoplanin, being an integral membrane protein.

Aims: To investigate possible signaling independent functions of CLEC-2 in development and hemostasis and thrombosis.

Methods: We generated a knock-in mouse in which the critical tyrosine in the CLEC-2 hemITAM is replaced by an alanine (Y7A KI). This is the first approach to specifically target the hemITAM of CLEC-2 *in vivo*.

Results: Constitutive and chimeric Y7A KI mice showed signs of blood-lymphatic mixing defects despite normal CLEC-2 surface expression levels on Y7A KI platelets. In ex vivo activation studies the Y7A KI mutation abolished platelet activation responses to CLEC-2 agonists in a dominant-negative fashion. Blocking the CLEC-2 ectodomain with Fab′ fragments of the antibody INU1 destabilized Y7A KI platelet aggregate formation under flow but the presence of the Y7A KI mutation alone had no effect. This contribution of the CLEC-2 ectodomain, but not hemITAM signaling, to thrombus stability was also seen in *in vivo* hemostasis and thrombosis assays.

Conclusions: We present a novel knock-in mouse that expresses a signaling-dead CLEC-2 receptor. This work identified an as yet undescribed signaling-independent function of CLEC-2. Further studies will be required to identify the blood-borne ligand/counter receptor through which CLEC-2 mediates its adhesive role in thrombosis and hemostasis.

ASY 08.2 | Desialylation of O-glycans on GPIbα Drives Receptor Signaling and Platelet Clearance

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Background: Sialidase is involved in platelet clearance under pathological and/or physiological circumstances. While the involvement of the Ashwell-Morell receptor (AMR) in clearing desialylated platelets is established, the underlying mechanism remains unclear. It was thought that AMR mediates platelet clearance by binding the exposed galactoses in the N-glycans of platelet GPIbα. However, sialidases can induce platelet clearance in mice, and yet unlike its human ortholog murine GPIbα contains no N-glycosylation sites (NxS/T). GPIbα is highly O-glycosylated. We recently showed that ligand binding to GPIbα under physiological shear could induce unfolding of its mechanosensory domain (MSD) and subsequently GPIb-IX signaling that leads to platelet clearance. The MSD contains O-glycosylation sites.

Aims: To test whether desialylation of O-glycans in GPIbα induces MSD unfolding and platelet clearance.

Methods: Bacterial sialidase was injected into wild type (WT), $Vwf^{-/-}$, $Adam17^{\Delta Zn/\Delta Zn}$, IL4R-IbαTg (transgenic mice expressing only the IL4R-GPIbα chimera), and hTg mice (transgenic mice expressing only human GPIbα), and the effects on counts and glycans of platelets and erythrocytes measured. Sialidase-induced effects on GPIb-IX dynamics and signaling in the platelet were analyzed by flow cytometry and fluorescence microscopy.

Results: Injection of bacterial sialidase or α-2,3-sialidase significantly reduced the platelet count in WT, Vwf$^{-/-}$, Adam17$^{\Delta Zn/\Delta Zn}$, and hTg mice, but not in IL4R-IbαTg mice. Changes in PNA binding, but neither ECL nor SNA binding, correlated inversely with the change in platelet count. In vitro treatment of hTg and human platelets with sialidase induced MSD unfolding, indicated by the increased binding of an anti-MSD antibody 5G6, and GPIb-IX-mediated intracellular signaling, indicated by the filopodia formation.

Conclusions: Desialylation of O-glycans of platelet GPIbα results in unfolding of the MSD in GPIbα, which leads to GPIb-IX signaling and platelet clearance.

ASY 08.3 | Impaired GlycoproteinVI-mediated Signaling and Platelet Functional Responses in CD45 Knockout Mice

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Background: CD45 is a Receptor Protein Tyrosine Phosphatase C (PTPRC) which regulates Src Family Kinase (SFKs) activation in Lymphocytes. Although CD45 is absent from the platelet surface, proteomics studies showed that the CD45 c-terminal catalytic domain is present in platelets.

Aims: The aim of this study is to identify the presence of the CD45 c-terminal domain in platelets and characterize the functional implications of CD45 deficiency in platelets.

Methods: All experiments were performed using CD45 WT and KO mice.In order to establish the presence of CD45 in platelets, we used an established primary antibody that recognizes the c-terminal domain of CD45 for western blot analysis. Isolated murine platelets were used to perform platelet functional tests such as aggregation, secretion, and flow-cytometry. Activation of SFK, Syk, and PLCγ2 were evaluated using western blotting and the in vivo platelet functions were tested using techniques such as tail bleeding and pulmonary embolism.

Results: We observed that CD45 c-terminal domain antibody recognizes a protein of approximately 65 kDa, which is the expected size of the c-terminal 1 and 2 domains of CD45, in platelets from wild-type mice (WT), but not from CD45 Knockout (KO) mice. Platelets from CD45 KO mice displayed a selective impairment of aggregation and dense granule secretion mediated by the collagen receptor, glycoprotein VI (GPVI). CD45 KO mice show increased bleeding times, indicating an important role for CD45 in hemostasis. Using a model of pulmonary embolism, we observed prolonged time to cessation of respiration in CD45 KO mice as compared to WT mice, which is indicative of a defect in thrombus formation in CD45 KO mice. Signaling downstream of the GPVI receptor, indicated by SFK, Syk, and PLCγ2 tyrosine phosphorylation was also impaired in CD45 KO murine platelets compared to WT murine platelets.

Conclusions: We conclude that CD45 is expressed in platelets as a truncated form and regulates GPVI signaling through regulation of SFK activation.

ASY 08.4 | Visualizing Cooperative Mechanosensing of GPIb and GPIIb-IIIa on a Single Aspirated Platelet

L. Ju[1,2,3]; Y. Chen[4,5]; S. Jackson[2,6]; C. Zhu[3,4,5]

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Background: Unlike the classic model of agonist driven platelet thrombus formation, under disturbed blood flow conditions with atherosclerotic plaques, medical device intervention or pre-existing thrombi, thrombus formation is driven by shear-dependent discoid platelet aggregation. This "biomechanical thrombosis" model features a two-step cascade: rapid tethering of platelets via receptor GPIb followed by stable adhesion via GPIIb-IIIa. How these interactions are orchestrated and how they crosstalk are unclear.

Aims: To elucidate how GPIb cooperates with GPIIb-IIIa to mediate platelet adhesion and signaling under force.

Methods: We developed a novel nanotool: dual biomembrane force probe (dBFP) with concurrent force spectroscopy and Ca^{2+} imaging to control signal initiation, visualize signal transduction and interrogate signal outcome, which temporally dissects GPIb and GPIIb-IIIa behaviors (Fig. 1). A micropipette aspirated platelet first repeatedly engages a GPIb ligand (VWF-A1) under controlled force to trigger Ca^{2+}, then quickly switch (< 1min) to engage a GPIIb-IIIa ligand (FN), which allows us to visualize the activation of GPIIb-IIIa triggered by GPIb in real time. GPIIb-IIIa conformational dynamics is observed using conformation reporter antibodies.

Results: A 25pN force lasting >2s is sufficient to trigger Ca^{2+} flux by unfolding a GPIb juxamembrane domain, which activates GPIIb-IIIa to an intermediate affinity 7-fold higher than the affinity of the resting

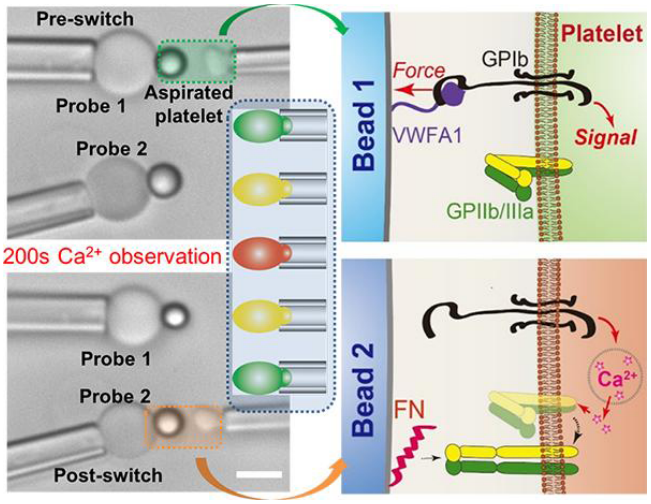

FIGURE 1 Micrograph and illustration of the dual biomembrane force probe to visualize GPIb and GPIIb-IIIa crosstalk on an aspirated platelet

state and 3.5-fold lower than that of the ADP-activated state (Fig. 2). The intermediate integrin adopts an extended-close conformation. Replacing the VWF-A1 with its 2B and 2M VWD mutants abrogates the GPIIb-IIIa intermediate activation.

Conclusions: The dBFP allows us to observe signal crosstalk between GPIb and GPIIb-IIIa on a single platelet in real-time. Force transmitted through GPIb upregulates GPIIb-IIIa to an intermediate affinity state. Our study explains how discoid platelets form aggregation independent of agonists under highly dynamic shear.

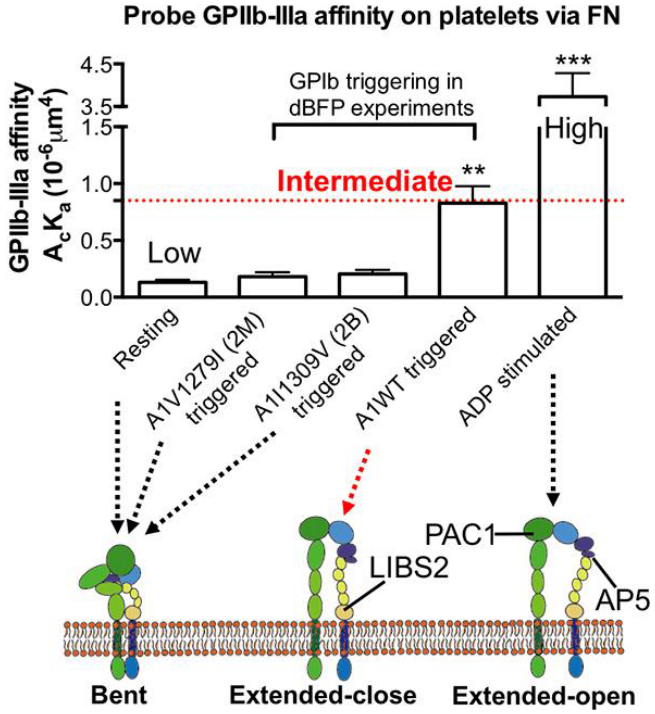

FIGURE 2 Probe GPIIb-IIIa affinity and conformation states on single aspirated platelets of different conditions

ASY 20.1 | Mutations in Tropomyosin 4 Underlie a Rare Form of Human Macrothrombocytopenia

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Background: Platelets are produced by large polyploid precursor cells called megakaryocytes. Previous genome-wide association studies (GWAS) indicated that single nucleotide variants in the gene encoding the actin cytoskeletal regulator Tropomyosin (TPM) 4 exert an effect on platelet count and volume. The function of tropomyosins in haematopoiesis has not been investigated.

Aims: We studied the role of TPM4 for platelet biogenesis and function in humans and mice.

Methods: A lookup study of *TPM4* in the BRIDGE Bleeding and Platelet Disorders (BPD) collection was performed. In parallel, a mouse line with an *N*-ethyl-*N*-nitrosourea (ENU)-induced missense mutation in *Tpm4*, as well as *Tpm4* knock-out (KO) mice were generated. Platelet function in mutant mice and in individuals carrying *TPM4* missense variants was analysed by flow cytometry, and static and flow adhesion assays. MK morphology and proplatelet formation were investigated using confocal immunofluorescence microscopy.

Results: We describe two unrelated families identified in the BPD collection that carry a variant which causes truncation of the TPM4 protein, and segregates with macrothrombocytopenia. *Tpm4* mutant and KO mice likewise exhibited dose-dependent macrothrombocytopenia. Insufficient TPM4 expression did not affect MK maturation but resulted in defective proplatelet formation in vitro. Platelet function was mildly affected, as demonstrated by the variable bleeding phenotype observed in *TPM4* patients, as well as delayed platelet spreading on fibrinogen and reduced thrombus formation on collagen under flow in vitro. Decreased TPM4 expression impacted on different cytoskeletal regulators of known importance in MKs and platelets, including Myosin IIa, Actinin 1, Filamin A and Cofilin.

Conclusions: Our findings demonstrate a non-redundant role for *TPM4* in platelet biogenesis in humans and mice, and reveal that truncating variants in *TPM4* cause a rare, dominant, Mendelian platelet disorder.

ASY 20.2 | A Human Germ Line Mutation in the Ephrin Receptor B2 Gene Associated with a Bleeding Syndrome, Affects Platelet Functions and GPVI Signaling

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Background: The ephrin (Eph) transmembrane receptor family of tyrosine kinases is involved in platelet functions but their contribution to inherited platelet disorders has never been reported.

Aims: To understand how an *EPHB2* variant impairs platelet functions, platelet signaling and proplatelet formation in two children P1 and P2 of a consanguineous family with a recurrent bleeding syndrome.

Methods: Whole exome sequencing identified a c.2233C>T mutation (missense p.R745C) of the *EPHB2* gene. Gene sequencing demonstrated that P1 and P2 were homozygous for this variant while their parents were heterozygous. Platelet functions (aggregation, secretion, adhesion), signaling (protein phosphorylation) and morphology (electron microscopy (EM)) were studied. Proplatelet formation in vitro by immunofluorescence microscopy of $CD34^+$-culture megakaryocytes (MKs) was examined.

Results: P1 and P2 exhibited a severe platelet functional defect including platelet aggregation, Ca^{++} mobilization, $\alpha_{IIb}\beta_3$ integrin activation and granule secretion induced by ADP, convulxin and thrombin, and thrombus formation on collagen under blood flow conditions. Most importantly, the phosphorylation of Syk, FcRγ and Akt, the initial steps in GPVI platelet signaling were drastically impaired. In contrast, clot retraction and platelet adhesion on fibrinogen under blood flow, were only mildly defective indicating limited effects on $\alpha_{IIb}\beta_3$ outside-in signaling. Initially normal for P1 and P2 platelet counts decreased moderately (120 $x10^9$ platelets/L) for P1 as he aged. EM showed abnormal elongated platelets with membrane extensions, while MK proplatelets, appeared moderately enlarged.

Conclusions: We conclude that EphB2 is involved in the first steps of GPVI signaling, thereby controlling platelet activation and is required for thrombus formation. Furthermore, EphB2 is involved in regulating platelet morphology and fragmentation.

ASY 20.3 | Cytokine Receptor-like Factor 3 (CRLF3): A Novel Regulator of Platelet Biogenesis and Potential Drug Target for Thrombocythaemia

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Background: Essential thrombocythaemia (ET) is characterised by a sustained circulating platelet count above 450$x10^9$/L causing an increased risk of cardiovascular events. Current therapies have unwanted side effects.

Aims: We aim to show CRLF3's role in platelet biogenesis and how it could be used as a novel therapeutic target to treat ET.

Methods: *Crlf3* knockout mice (*Crlf3*$^{-/-}$) were used in a range of *in vitro* and *in vivo* assays focusing on platelets and megakaryocytes (MKs). They were also crossbred with JAK2 V617F mice to create an ET mouse deficient of *Crlf3*.

Results: *Crlf3*$^{-/-}$ mice have an isolated and sustained 30-40% decrease in platelet count compared to wildtype (WT) controls. They have increased bone marrow MKs compare to WT controls but MK morphology and their ability to form proplatelets is preserved. *Crlf3*$^{-/-}$ platelet survival is normal and no gross functional abnormalities of platelets were demonstrated in a range of assays (response to agonists in flow cytometry, platelet spreading and *in vitro* thrombus formation). However, pre/pro-platelet structures are abnormally present in the circulation of elderly *Crlf3*$^{-/-}$ mice (see figs.). Immunohistochemistry showed increased microtubule stability of *Crlf3*$^{-/-}$ platelets compared to WT controls, especially in the pre/pro-platelet forms. Splenectomy of *Crlf3*$^{-/-}$ animals restored platelet counts to WT values.

JAK2V617F ET mice crossbred with *Crlf3*$^{-/-}$ mice showed restoration of platelet counts to WT values without affecting other blood lineages.

Conclusions: We identified a mechanism by which *Crlf3* controls platelet biogenesis. Slowed maturation of *Crlf3*$^{-/-}$ pre/pro-platelets in the peripheral circulation due to increased structural stability leads to rapid removal of these immature forms by the spleen and therefore a decrease in platelet count. The isolated effect on platelet numbers and normalisation of platelet count in ET mice deficient of *Crlf3* provides the rational for further study on CRLF3 drug targeting as a novel therapeutic strategy for ET.

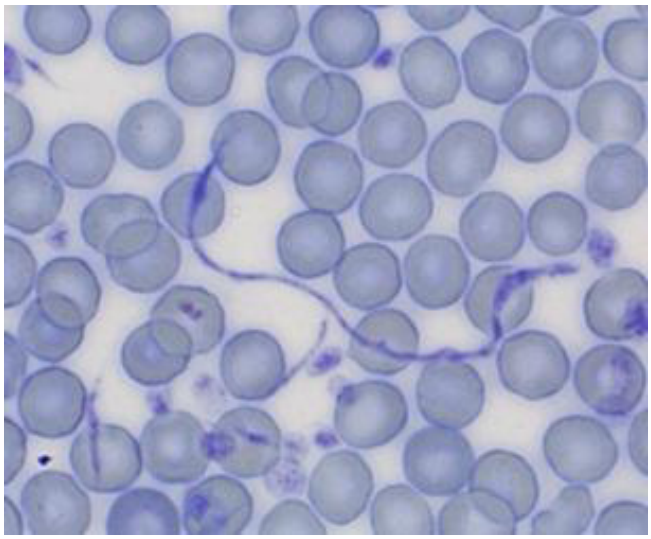

FIGURE 1 Blood smear of an elderly (>1 year) Crlf3-/- mouse stained with rapid Romanowsky. Abnormal platelets resembling pre/pro-platelets can be seen

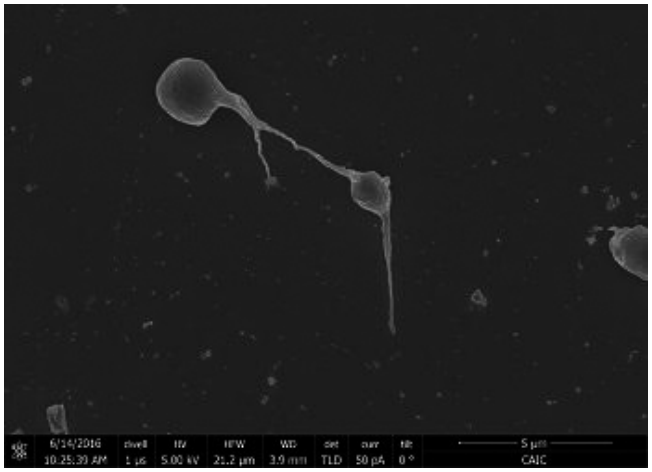

FIGURE 2 Washed platelets from elderly (>1 year) Crlf3-/- mice were imaged by scanning electron microscopy. Pre/pro-platelets were readily seen

ASY 20.4 | RNA Sequencing of Roifman Syndrome Megakaryocytes Reveals a Role for a Small Nuclear RNA in Platelet and Granule Biology

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Background: Roifman syndrome is a rare inherited multisystem disorder characterized by spondyloepiphyseal dysplasia, growth retardation, cognitive delay and antibody deficiency. In some cases, immune thrombocytopenia has been reported. Compound heterozygous variants in *RNU4ATAC*, a nuclear RNA essential for minor intron splicing, were recently identified to cause Roifman. Pathological RNA processing has previously been described in TAR syndrome due to variants in *RBM8A*.
Aims: Investigate platelet defects in 3 patients with Roifman phenotypes from 2 unrelated pedigrees. All cases had low platelet counts with abnormal alpha- and dense-granules but no bleeding. The patients underwent whole genome sequencing in the BRIDGE-BPD study.
Methods: Phenotype similarity regression identified a significant association between two rare alleles in *RNU4ATAC* and a Roifman-like phenotype. CD34+ hematopoietic stem cells of 2 patients and controls were differentiated to megakaryocytes (MKs) for RNA sequencing. Minor intron retention was assessed by DEXseq, adapted for differential intron usage. Immunostaining and Western blot were done on MKs and platelets to study cytoskeleton and granules.
Results: We found novel pathogenic variants in *RNU4ATAC*. Relative to controls, Roifman MKs reach similar ploidy levels, but are smaller and generate less proplatelets with abnormal cytoskeletal organization. Roifman platelets are also larger, show elevated tubulin and actin levels and an increase in granule markers CD63 and vWF. RNA-seq of Roifman MKs revealed minor intron retention in 354 genes of which the top 50 were mainly involved in vesicular transport, cytoskeleton organization or cognitive function. One of the most disrupted genes (P=1.49x10^{-127}) was *DIAPH1*, which is linked to cytoskeleton and macrothrombocytopenia.
Conclusions: Roifman syndrome is the second disorder after TAR syndrome implicating RNA processing in megakaryopoiesis, but the first in which variants in a non-coding RNA are pathogenic.

ASY 33.1 | Differential Signaling through Platelet Collagen Receptors Investigated Using Metabolomics

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[1]*University of Cambridge, Biochemistry, Cambridge, United Kingdom,* [2]*MRC Human Nutrition Research, Fulbourn Road, Cambridge, United Kingdom*

Background: Platelet lipids provide multiple para- and autocrine signaling molecules. Collagens elicit aspirin-sensitive platelet thromboxane (Tx) synthesis that amplifies their activation. Glycoprotein (GP) VI is the main signalling receptor, whilst integrin alpha2beta1 supports platelet adhesion and less prominent signals.
Aims: We sought to discriminate GPVI from alpha2beta1 signaling by investigating lipid metabolism in activated platelets.
Methods: We used open-profiling of the platelet lipidome, by Folch extraction and liquid chromatography-mass spectrometry (LC-MS), to identify classes of lipid mobilised by triple-helical peptides specific for GPVI and alpha2beta1, CRP-XL and GFOGER-XL, respectively. Specific stimuli were resolved by principal component analysis, followed by targeted analysis of eicosanoids by tandem LC/MS.
Results: GPVI stimulated both the cyclooxygenase (COX) and cytochrome P_{450} (CYP450) pathways, generating abundant TxA_2, measured as the stable metabolite, TxB_2. Alpha2beta1 promoted little flux through these pathways, but caused marked production of lipoxygenase (LOX) metabolites, notably LTB_4, 15-HEPE and 15-HETE. These eicosanoids, established mediators of inflammation that may be relevant to atherothrombosis, tended to inhibit thrombus deposition from flowing blood, and to modulate platelet aggregation. Inhibition of the COX pathway by aspirin enhanced the activity of the CYP450 and LOX pathways.
Conclusions: The platelet lipidome is a sensitive indicator of platelet signals from collagen, including those arising through the adhesive receptor alpha2beta1, for which little detailed functional activity has been described.
The LOX pathway is very responsive to alpha2beta1 ligation, whereas the COX and CYP450 pathways are the primary route for GPVI signaling to the lipidome.
The LOX metabolites tend to exert a moderating effect on platelet thrombus formation, but their known action on leukocytes suggest that alpha2beta1 may also contribute to inflammatory processes in the vessel wall.

ASY 33.2 | Characterization of the Gp1ba-Cre Transgenic Mouse: A Novel Approach for Generating Megakaryocyte/Platelet-specific Knockout Mice

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[1]*University of Birmingham, Institute of Cardiovascular Sciences, Birmingham, United Kingdom,* [2]*The Jackson Laboratory, Bar Harbor, United States*

Background: The *Pf4-Cre* transgenic mouse developed by Skoda and co-workers has been used extensively to generate megakaryocyte/platelet (MK/plt)-specific knockout (KO) mouse models. Although highly efficient at deleting *floxed* genes in the MK lineage, several studies have emerged challenging the specificity of this transgene,

and raising the possibility that some of the phenotypes associated with this model may be due to non-specific deletion of *floxed* genes in other cell types.

Aims: To develop an alternative approach to produce MK/plt-specific KO mice. In this study, we investigate the specificity and efficiency of deletion of *floxed* genes by this deleter strain.

Methods: *Gp1ba-Cre* mice were generated by knocking-in a transgene coding for *Cre recombinase* downstream of the endogenous *Gp1ba* locus by homologous recombination. Potential effects of the transgene on platelet parameters and function were assessed by standard assays. Expression pattern of the transgene *in vivo* was investigated by crossing deleter mice with *mT/mG* double-fluorescent Cre reporter mice and quantifying GFP^+ haematopoietic cells by flow cytometry.

Results: *Gp1ba-Cre* heterozygous mice are born at normal Mendelian frequency, have normal platelet count, a 17% increase in platelet volume and marginally reduced GPIb-IX levels; however, platelets from these mice function normally *in vitro* and *in vivo*. Virtually all platelets and nearly half of αIIb^{high} bone marrow (BM) cells from *mT/mG*;*Gp1ba-Cre* reporter mice were GFP^+, demonstrating highly efficient Cre-mediated gene excision in the MK lineage. Notably, no significant GFP expression was detected in erythrocytes, leukocytes, splenocytes or $CD45^+$ BM cells from these mice.

Conclusions: We demonstrate that the *Gp1ba-Cre* transgene is expressed specifically in the MK lineage. This mouse model will be an invaluable tool for generating MK/plt-specific KO mice, for validating conclusions from *Pf4-Cre* generated KO mice and for studying the spatiotemporal expression pattern of GPIbα during development.

ASY 33.3 | Real-time Platelet Production from Human iPS-derived Megakaryocytes in a Micropillar-textured Microfluidic Chip

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Background: Bioreactors that recapitulate some features of the bone marrow microenvironment and cell reprogramming techniques to produce megakaryocytes (MKs) represent major improvements towards ex vivo platelet generation for therapeutic applications to treat or prevent bleeding in severe thrombocytopenic patients.

Aims: In this study, we provide evidence of efficient production of platelets from MK of different sources, based on a new microfluidic chip that upregulates and synchronizes platelet production by exposure of MKs to von Willebrand Factor (VWF) at a high shear rate.

Methods: Mature MKs are directly injected into microchannels comprising arrays of VWF-coated pillars acting as anchors. Time-lapse observation allows measuring single MK elongation velocity, as well as number of releasable platelets per MK, calculated as ratio of released bead volume / smallest volume of released element defined as a platelet.

Results: Captured MKs adopt a beads-on-a-thread conformation before being fragmented into proplatelets and releasing platelets in the flow of the perfusion. MKs derived from hUES cells and iPS cells, as well as from primary HSC, underwent similar sequences of fragmentation into proplatelets and platelets, indicating an efficient and robust process. In each of the 4 experiments, mean elongation velocities of MKs obtained from hUES cells and iPS cells were calculated and varied between 23. 7 and 48. 1 μm/min and 30. 0 and 57. 3 μm/min, respectively. The number of releasable platelets per MK varied between 20 and 800, and 39 and 833, for MK derived from hUES cells and iPS cells, respectively.

Conclusions: In conclusion, these data indicate that high numbers of platelets are releasable from iPS cell-derived MK anchored onto VWF-coated micropillars at high shear rates and provide an innovative way to overcome the limitations of low platelet shedding from iPS cell-derived MKs in static conditions.

ASY 33.4 | Quantification of Platelet Contractile Movements during Thrombus Formation

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Background: During thrombus formation platelet contractile forces can redefine the overall geometry of the thrombus as well as the distribution of aggregatory and procoagulant platelets within the thrombus. Thrombus formation, contraction and individual platelet movements can be monitored and evaluated through different approaches. However, none of the existing methods can generate information about both single platelet movements and thrombus contraction at the same time. Such information would be valuable to better understand the details and dynamics of thrombus development and contraction.

Aims: To characterize platelet movement and thrombus contraction by tracking a large number of platelets during in-vitro thrombus formation experiments.

Methods: Time-lapse and z-stack fluorescence microscopy were used to capture thrombus formation in hirudinised blood in a flow chamber. 5% of the platelets were labelled using CD42a (AF647) and Annexin V (AF488) was present in the blood during experiments. Thrombus formation was studied both in the presence of inhibitors; ASA, cangrelor, abciximab, 6B4 (antibody inhibiting VWF binding), and without inhibition at different shear rates. The thrombus development was quantified as described previously (Claesson et al., 2016, Thromb. Hemost).

Results: Platelet movements within the thrombus were tracked and quantified in x, y and z-axis. The movement patterns of the annexin V and CD42a labelled platelets was distinctively different (Fig 1 and 2, showing the same thrombus) and the movement was affected by the shear rate. The contractile movements were reduced during inhibition.

Conclusions: By tracking individual platelets during thrombus formation, we have the possibility to get new insights into the platelet driven contraction process of the thrombus.

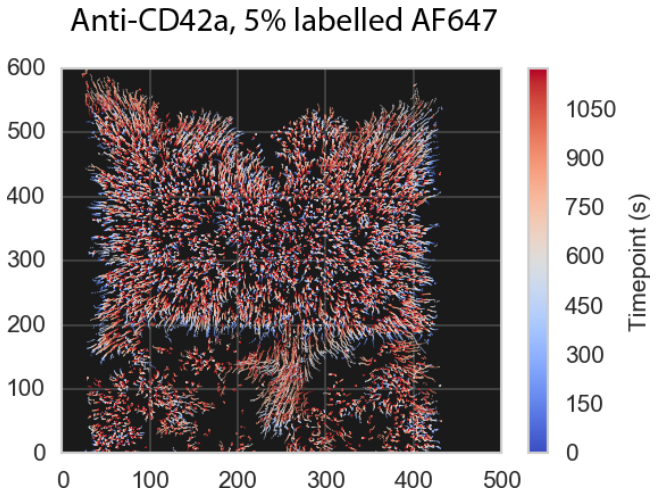

FIGURE 1 Platelet tracks at 400s-1 (without inhibition) for CD42a labelled platelets. The platelet positions were tracked throughout the whole experiment

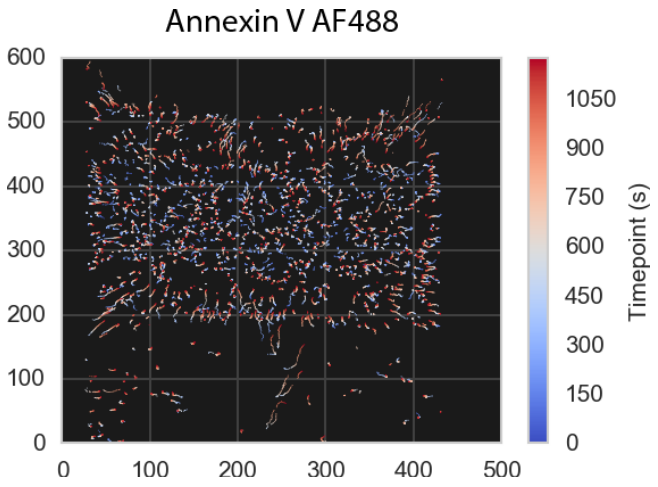

FIGURE 2 Platelet tracks at 400s-1 (without inhibition) for A-V labelled platelets. The platelet positions were tracked throughout the whole experiment

ASY 38.4 | miR-125a-5p Regulates Megakaryocyte Differentiation and Proplatelet Formation

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Background: Numerous reports indicate microRNAs (miRs) regulate or are associated with megakaryocyte (MK) differentiation (MKpoiesis), but none have been correlated with human peripheral blood platelet number *in vivo*.

Aims: To use an unbiased approach to identify miRs regulating MK progenitor differentiation and proplatelet formation (PPF).

Methods: Umbilical cord blood (CB) derived $CD34^+$ stem cells were grown in MK favoring cultures and $CD61^+$ MKs were isolated on day 6, 9 and 13 (Fig 1A). Primary MKs were isolated from human bone marrow (BM) aspirates by laser capture micro-dissection (Fig 1B). All cell types were profiled for miRs (Exiqon) and mRNA (RNA sequencing) expression (Fig 1C).

Results: To identify miRs regulating MKpoiesis and PPF, we considered only miRs expressed in both primary BM MKs and cultured MKs (107 miRs) (Fig 1D), whose expression either increased (33 miRs) or decreased (18 miRs) during MK differentiation (Fig 1E), and significantly associated with platelet number (Fig 1F). This stringent filtering left only 3 miRs. Of these, we focused on miR-125a-5p, because it is known to positively regulate stem cells in mice, but its effects on MKpoiesis is unknown. We found miR-125a-5p was positively correlated with platelet count (p=0.02). Knock down of miR-125a-5p caused a 70% reduction in MK PPF ($p=9.58x10^{-5}$) and 35% reduction in MK CD41 expression. Overexpression of miR-125a-5p increased PPF by ~15%. We also identified 5 putative targets of miR-125a-5p (*LCP1*, *JAZF1*, *PRKCH*, *AHNAK*, *RPL32A*) that decreased expression during MKpoiesis and were negatively associated with platelet number ($p< 0.05$).

Conclusions: miR-125a-5p positively regulates MKpoiesis and PPF. As miRs are increasingly becoming clinical therapeutic targets, miR-125a-5p over expression has potential for improving the in vitro manufacture of platelets or treating thrombocytopenia, whereas its inhibition could be effective in myeloproliferative disorders.

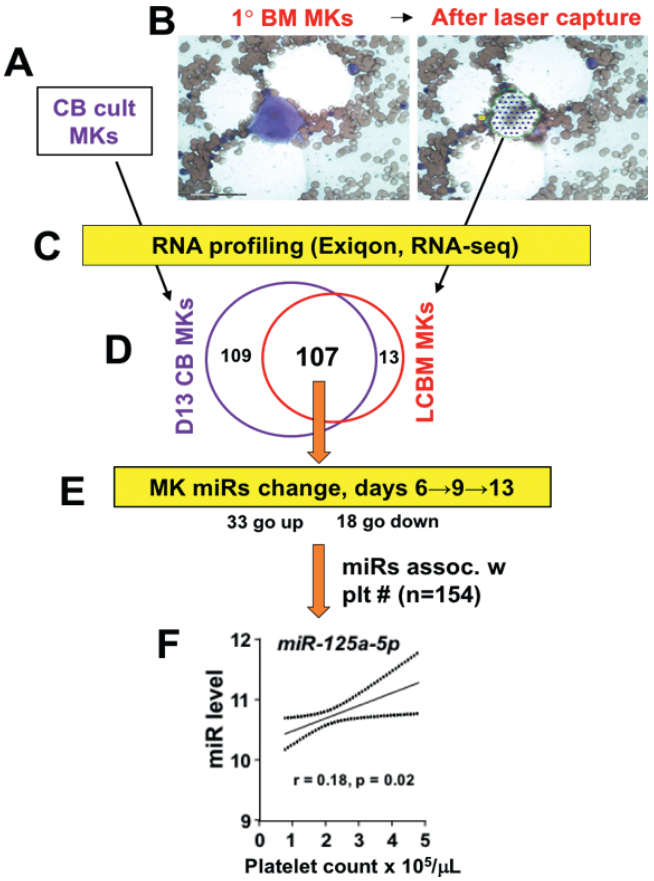

FIGURE 1 Identification of miRs that regulate proplatelet formation

PLATELETS – CLINICAL

ASY 05.1 | Enhancing Autophagy Protects Platelets in Immune Thrombocytopenia Patients

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Background: Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder, in which platelets show increased apoptosis. Autophagy is an essential pathway for platelets to maintain their life and physiological functions. However, the role of autophagy in ITP platelets was previously unclear.

Aims: The objectives of this study were to estimate the level of platelet autophagy in ITP patients, and to investigate the effects of autophagy regulation on platelet protection and life extension.

Methods: Peripheral blood was collected from ITP patients and healthy donors for further cultivation with RAPA, 3-MA and ABO. Previously we have first identified that ABO (6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine) could stimulate autophagy in an mTOR-independent but Annexin A7-dependent manner. LC3 level, platelet viability and apoptosis were measured. Statistical data were expressed as mean ± standard error of the mean (SEM). $P < 0.05$ was considered statistically significant.

Results: Immunofluorescence revealed that accumulation of LC3-positive puncta was reduced in ITP patient platelets and was increased upon RAPA or ABO treatment (Fig. 1A). Western blotting demonstrated that the LC3-II/I ratios in RAPA- or ABO-treated ITP platelets were significantly higher than in ITP platelets. No significant difference was found between the ABO-treated ITP platelets and the RAPA-treated ITP platelets or healthy platelets (Fig. 1B, 1C). Platelet apoptosis in ITP decreased and that platelet viability improved significantly after treatment with either RAPA or ABO, with no difference from that in healthy controls (Fig. 2A, 2B, 2C).

Conclusions: Platelet autophagy in ITP patients was diminished, whose increase could alleviate platelet destruction by inhibiting apoptosis and improving platelet viability. These results suggest a role for autophagy regulation in the pathogenesis of ITP and offer a novel treatment.

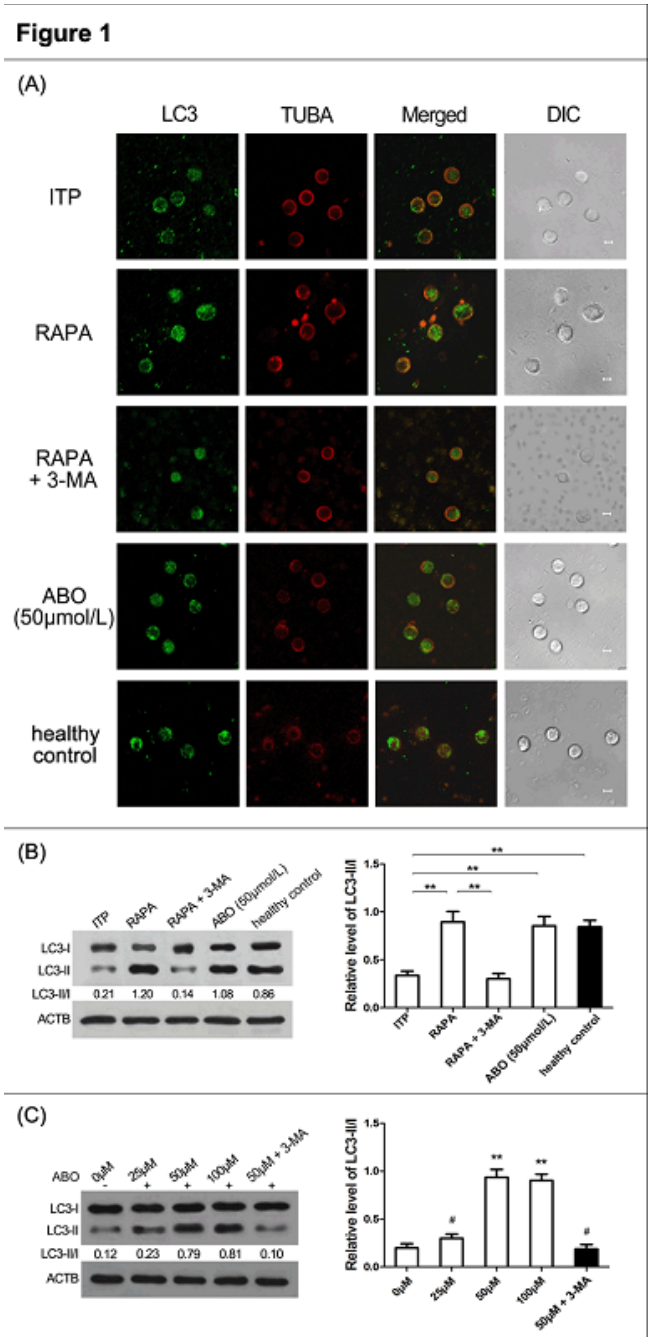

FIGURE 1 Platelet autophagy in ITP patients and healthy controls

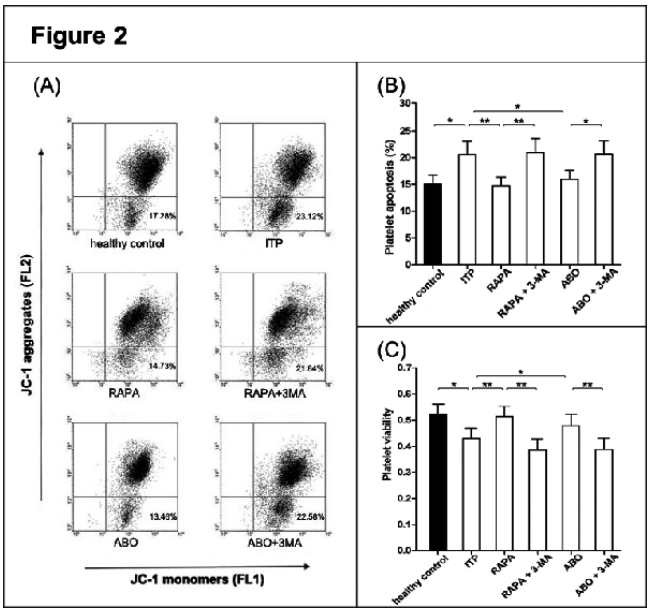

FIGURE 2 Inducing autophagy alleviated destruction of ITP platelets

ASY 05.2 | Antibody Mediated Glycan Modification: A Potential Role in Platelet Destruction in Autoimmune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is a bleeding disorder caused by autoantibodies (AAbs) directed against platelet (PLT) glycoproteins (GP). Recently, Fc-independent PLT clearance via Ashwell-Morell receptors, that recognize glycan changes on PLT surface, was proposed as a new way of Ab-mediated PLT destruction in mice.

Aims: In our study we analyzed the impact of AAbs from ITP patients on the glycan pattern of human PLTs and its effect on their survival *in vivo*.

Methods: Sera from ITP patients and healthy donors were analyzed using monoclonal platelet antigen capture assay (MAIPA) and lectin binding assay (LBA). In LBA, after incubation of sera with PLTs from healthy donors the change of glycan pattern (flow cytometry) and its impact in NOD/SCID mice were analyzed.

Results: 37 sera (ITP patients) and 25 sera (healthy donors) were analyzed and different patterns of glycan modification were observed after incubation with AAbs (LBA). 17/37 sera induced a significant increase in Peanut agglutinin binding compared to heathy donors (median fold increase (FI): 1.21, range 1.08-1.40). 9/37 sera caused higher Erythrina cristagalli lectin binding (median FI: 1.02, range: 1.08-1.15). While, 8/37 sera showed strong decrease in Ricinus communis agglutinin binding (median FI: 0.52, range: 0.50-0.59). Interestingly, not only GP-Ib/IX AAbs but also GPIIb/IIIa AAbs were able to modify glycan pattern. No significant change was induced by sera from healthy donors. The injection of AAbs resulted in accelerated clearance of human PLTs in NOD/SCID mice. The destruction of human PLTs by ITP-AAbs was reduced but not completely inhibited by a specific neuraminidase inhibitor that prevents glycan changes on PLT surface (survival of human PLTs after 5h: 29%, range 22-40% vs. 48%, range 41-53%, respectively).

Conclusions: Our data indicate that AAbs from ITP patients can induce modifications of glycan pattern on PLT surface which seem to contribute to PLT destruction in vivo.

ASY 05.3 | Fc-independent Immune Thrombocytopenia via Mechanomolecular Signaling in Platelets

M. Quach[1]; M. Dragovich[2]; W. Chen[1]; A. Syed[1]; X. Liang[1]; W. Deng[1]; H. Ni[3]; S. De Meyer[4]; J. Ware[5]; H. Deckmyn[4]; F. Zhang[2]; R. Li[1]

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which anti-platelet autoantibodies induce thrombocytopenia. First-line treatments are intravenous immunoglobulin (IVIG) or corticosteroids. Unfortunately, 20-25% of patients are refractory to this type of treatment. ITP mediated by anti-GPIb-IX antibodies (Abs) is not ameliorated by IVIG. Although most Abs targeting the ligand-binding domain (LBD) of GPIba clear platelets in an Fc-independent manner, there are some exceptions. Further, many Abs targeting the mechanosensory domain (MSD) of GPIbα, GPIbβ, or GPIX do not cause platelet clearance. We have showed that VWF binding to GPIbα under physiological shear induces unfolding of the MSD and subsequent GPIb-IX signaling leading to platelet clearance.

Aims: To elucidate the mechanism of anti-LBD Ab-induced platelet clearance.

Methods:

(1). Shear was applied to human and murine platelet-rich plasma mixed with anti-LBD Abs. Platelets were analyzed via flow cytometry for GPIb-IX signaling and crosslinking.

(2). Transgenic mice expressing human GPIba (hTg) were treated with IVIG and injected with anti-LBD Abs. Platelet count was assayed.

(3). Single-molecule force spectroscopy was performed to pull anti-LBD Abs bound to an anchored GPIb-IX complex.

Results: Clearance-inducing anti-LBD Abs, such as 6B4, induce GPIb-IX signaling in human and murine platelets in a shear-dependent manner. 6B4 Fab or anti-LBD Ab AK2 does not induce platelet signaling under the same conditions. Pulling with 6B4 and other Abs, but not AK2, on GPIb-IX causes MSD unfolding. Similarly, 6B4 and other Abs, but not 6B4 Fab or AK2, induce ~20% aggregation or agglutination of platelets in the aggregometer and IVIG-resistant platelet clearance in hTg mice.

Conclusions: Anti-LBD Abs induce GPIb-IX signaling and platelet clearance via unfolding the MSD on the platelet. AK2 is not able to because its unbinding force (force required to cause AK2 to unbind from the LBD) is not sufficiently large to allow unfolding of the MSD.

ASY 05.4 | Induction of Immune Thrombocytopenia in Mice Expressing Human Fcy Receptors: An Improved Experimental Model that Better Reflects the Inflammatory State Associated with ITP

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Background: Fcγ receptors (FcγRs, e.g. FcγRIIa) contribute to the pathophysiology of immune thrombocytopenia (ITP). Mouse models are often used to study the biology of ITP. In these,

thrombocytopenia is achieved by passive administration of anti-platelet antibodies. However, they fail to reflect the inflammatory state associated with human ITP. Differences in the types and expression patterns of FcγRs between humans and mice may contribute to this discrepancy.

Aims: Here, using mice transgenic for human FcγRs, we assessed ITP induced by native or chimeric anti-mouse platelet antibodies.

Methods: Mice transgenic for either the entire human Fcγ-receptor family (huFcγR) or only FcγRIIa (hFc), and wild type (WT) mice, were injected i.v with anti-mouse platelet antibodies: MWReg30, 6A6 or chimeric 6A6 (c6A6), containing rat, mouse or human Fc domains, respectively. Core temperature and platelet counts were measured before and 30 min after injection. Alternatively, MWReg30 and c6A6 were injected i.p and platelets counted daily for 7 days. Plasma IFN-γ, TNF-α, IL-2, IL-6 and IL-10 were measured 30 min after i.v, and on days 0, 3, 5 and 7 after i.p injections.

Results: MWReg30 injected i.v induced severe thrombocytopenia (>90% platelet loss), hypothermia, and shock in huFcγR and hFc but not WT mice. 6A6 caused severe thrombocytopenia in huFcγR and hFc but hypothermia and shock only in huFcγR. In contrast, c6A6 caused mild thrombocytopenia (30% platelet loss) without hypothermia or shock in all strains. Severe sustained thrombocytopenia was achieved in all strains injected i.p. Significant elevation of IFN-γ, TNF-α, IL-6 and IL-10 levels was observed in huFcγR mice injected i.v or i.p with MWReg30, and in both huFcγR and hFc mice with c6A6, but not in WT mice.

Conclusions: The use of anti-platelet antibodies having a human effector region in mice expressing human FcγRs is an improved model that more closely reflects the pathophysiology of human ITP and reveals its inflammatory nature.

ASY 19.1 | The Platelet COX-1 Knockout Mouse as a Model to Study the Effects of Aspirin in the Cardiovascular System

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Background: The anti-thrombotic effect of low dose aspirin is due to its inhibition of platelet cyclooxygenase-1 (COX-1) and blockade of the production of thromboxane A_2. Aspirin also has off-platelet actions that may limit its effectiveness. Since mice have very marked differences in aspirin pharmacokinetics they cannot be used to explore the effects of low dose aspirin in humans.

Aims: To address these questions, we developed platelet COX-1 knockout mice (*platelet-COX-1*$^{-/-}$) to recapitulate the effect of low-dose aspirin in humans.

Methods: *Platelet-COX-1*$^{-/-}$ mice were generated by pairing *Cox-1*$^{fl/fl}$ mice with *Pf4-Cre* mice. The presence of COX-1 in washed platelets was investigated by western blot analysis and confocal immunofluorescence microscopy. Platelet function and eicosanoid production was investigated by aggregometry and ELISA, respectively.

Results: Western blot analysis and confocal microscopy confirmed the absence of COX-1 in platelets. The production of thromboxane B_2, but not 12-HETE induced by arachidonic acid, collagen, A23187 and the PAR-4 activating peptide in platelet rich plasma and whole blood was significantly decreased in *platelet-COX-1*$^{-/-}$ mice. This effect was not further reduced by the addition of aspirin in vitro. *Platelet-COX-1*$^{-/-}$ mice also displayed decreased arachidonic acid-induced platelet aggregation.

Conclusions: We have produced the first *platelet-COX-1*$^{-/-}$ mouse. We envisage that this mouse will allow mechanistic dissection of the platelet and non-platelet effects of aspirin, identification of the range of eicosanoids whose production is affected by aspirin at different sites, and better modelling of the effects of clinically important anti-thrombotic therapy.

ASY 19.2 | Low Platelet Count is an Independent Risk Factor for Infection in Patients with Primary Immune Thrombocytopenia

A.J.G. Jansen[1,2]; M. Qu[3,4]; Q. Liu[3,4]; H.-G. Zhao[5]; J. Peng[3,4]; H. Ni[6,7,8]; M. Hou[3,4]

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Background: Thrombocytopenia is a common and severe complication after infection. Recent *in vitro* evidence suggests a potential role of platelets in the inflammation process.

Aims: In this study we further investigated the role of thrombocytopenia on infections in patients with primary immune thrombocytopenia (ITP).

Methods: Data of a recently published large randomized clinical trial in patients with primary ITP, who were randomized for prednisone or high dose dexamethasone, were used. From 195 patients who were analyzed in this study we could use data on platelet count, infections and clinical characteristics from 158 patients.

Results: Twenty four percent of the patients with primary ITP had an infection in the first month of treatment with prednisone or high dose dexamethasone. Patients who had an infection had significantly lower platelet counts during the first month of treatment (Figure 1) leading to a significantly lower response rate at 1 month ($P < 0.05$) and a significantly longer hospital stay (9.8 vs. 14.0 days, $P < 0.01$). Additionally Cox regression analysis showed that an increase in platelet count of $20x10^9$/L led to a risk reduction of 52% for infections in the next week

(P< 0.01). Although not significant, platelet transfusion led to a large increase in platelet count in ITP patients without infection, but not in patients with infection.

Conclusions: Infections are common in patients with primary ITP leading to significantly worse response rate and longer hospital stays. Additionally low platelet count was independently associated with an increased risk of infection. The role of platelet transfusion as therapeutic tool however remains unclear.

ASY 19.3 | Increased Circulating T Follicular Helper Cells in Patients with Chronic Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is a common hematologic disorder characterized by isolated thrombocytopenia. T follicular helper(TFH) cells are a subset of CD4+ T-helper cells and can activate B cells.

Aims: This study aims to investigate the role of T follicular helper (TFH) cells in the immunopathogenesis of chronic immune thrombocytopenia (CITP),and its chang before and after treatment.

Methods: Fifty-four patients with CITP and 30 age-matched healthy controls were enrolled into this study.The frequencies of circulating TFH cells were characterized using flow cytometry in patients with CITP and in healthy controls. The levels of IL-2, IL-4, IL-10 and IL-21 associated with TFH cells were measured using ELISA. The expression of transcription factors and regulatory factors of Bcl-6, c-Maf, Blimp-1 and PD-1 mRNA in CD4+ T cells were detected by real-time PCR .All these indicators were analyzed before and after treatment.

Results: The proportion of TFH cells were significantly higher (P< 0.05) in CITP compared with controls and decreased percentage of TFH cells were present in CITP responders after treatment (p< 0.05); correlation analysis showed that the TFH cells were positively correlated with the levels of PAIgG and negatively with the platelet counts in peripheral blood. Transcription levels of Bcl-6 and c-Maf mRNA in CD4+ T lymphocyte cells were significantly elevated, the Blimp-1 and PD-1 mRNA in CD4+ lymphocyte cells were lower in CITP patients in comparison with healthy controls. The higher plasma concentration of IL-21, and lower concentration of IL-2, IL-10 were found in CITP patients. After treatment these abnormal indicators were improved in CITP responders.

Conclusions: These data demonstrate that circulating TFH cells and CD4+ TFH lineage-associated cytokines may play a role in CITP. The overactivation of TFH cells may contribute to the immunopathogenesis of CITP, blocking the pathway of TFH cells may be reasonable cellular targets for therapeutic intervention.

ASY 19.4 | Anti-ADAMTS13 Autoantibodies against Cryptic Epitopes in Acquired TTP Patients

E. Roose[1]; G. Vidarsson[2]; O. Verhagen[2]; R. Visser[2]; M. Berkowska[2]; J. Caron[1]; I. Mancini[3]; L. Desender[1]; I. Pareyn[1]; N. Vandeputte[1]; A. Vandenbulcke[1]; M.-A. Azerad[4]; H. Deckmyn[1]; S.F. De Meyer[1]; F. Peyvandi[3]; K. Vanhoorelbeke[1]

[1]KU Leuven Campus Kulak Kortrijk, Laboratory for Thrombosis Research, Kortrijk, Belgium, [2]Sanquin Research, Department of Experimental Immunohematology, Amsterdam, The Netherlands, [3]Università degli Studi di Milano, and Fondazione Luigi Villa, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondaione IRCCS Ca' Granda Ospedale Maggiiore Policlinico, Department of Pathophysiology and Transplantation, Milan, Italy, [4]CHU Brugmann, Jules Bordet, Hematology, Brussels, Belgium

Background: Acquired thrombotic thrombocytopenic purpura (aTTP) is characterized by the presence of anti-ADAMTS13 autoantibodies (autoAbs). ADAMTS13 can adopt different conformations which can be induced by its substrate VWF or an activating Ab that disrupts the spacer-CUB interaction. In other pathologies, like heparin induced thrombocytopenia, autoAbs recognize cryptic epitopes which become exposed after a conformational change in the antigen.

Aims: To clone anti-ADAMTS13 autoAbs from aTTP patients to identify autoAbs with cryptic epitopes.

Methods: Peripheral blood mononuclear cells (PBMCs) from aTTP patients were single cell sorted using FITC and RPE labelled ADAMTS13 and CD19-APC. Anti-ADAMTS13 specific B cells were identified via ELISA. Variable sequences of the Ab heavy and light chains were determined and cloned onto an IgG_1 or kappa constant backbone respectively. Cloned Abs were expressed, purified and tested in ELISA to determine their specificity for ADAMTS13, their epitope and their capacity to recognize MDTCS, T2C2 or full length ADAMTS13.

Results: After sorting PBMCs from two aTTP patients seven anti-ADAMTS13 positive B cell clones were identified: 2 IgG's (TTP73-1 and TTP1a) and 5 IgM's. The IgG's were cloned and expressed. Both IgG's detected coated ADAMTS13, which shows that they are indeed ADAMTS13 specific. Epitope mapping revealed that Ab TTP73-1 is directed against the cysteine/spacer domain, while Ab TTP1a recognizes the TSP1-2/3 domain of ADAMTS13. Remarkably, the Abs were not able to capture ADAMTS13, MDTCS or T2C2, implying that their epitopes are cryptic. Indeed, direct coating of ADAMTS13, MDTCS or T2C2, which induces a conformational change and exposes cryptic epitopes, did result in binding of both Abs TTP73-1 and TTP1a.

Conclusions: We cloned two anti-ADAMTS13 autoAbs from aTTP patients which recognize cryptic epitopes in ADAMTS13. Identifying which factors induce conformational changes in ADAMTS13 in aTTP patients will allow to elucidate autoAb formation in these patients.

ASY 39.1 | Elevated Plasma Soluble GPVI (sGPVI) Can Aid Detection of Platelet-activating Autoantibodies in Heparin-induced Thrombocytopenia (HIT)

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Background: HIT is caused by platelet-activating antibodies binding an antigenic complex of heparin and platelet factor-4 (PF4). This immune complex engages platelet FcγRIIa, causing platelet activation, aggregation and clearance. Identifying activating (pathological) HIT antibodies is challenging because not all patients with detectable HIT antibody develop HIT. Engagement of FcγRIIa induces metalloproteolytic shedding of the platelet-specific collagen receptor, GPVI, to release soluble GPVI (sGPVI).

Aims: To characterise GPVI shedding upon engagement of FcγRIIa by activating HIT antibodies and assess the utility of sGPVI to identify pathological heparin-PF4 antibodies by prospectively evaluating sGPVI in samples from 310 query HIT patients.

Methods: Donor washed platelets were mixed with plasma from healthy donors, or thrombocytopenic patients and therapeutic or saturating heparin doses. Levels of sGPVI were assessed by enzyme-linked immunosorbent assay (ELISA).

Results: HIT patient plasma induced heparin-dependent metalloproteolytic shedding of GPVI and was blocked by saturating heparin dose or 10 mM EDTA or 10 μg/mL FcγRIIa-blocking antibody, IV.3. Of the 310 query HIT patients, 32 had clinically confirmed HIT. In the prospective cohort, sGPVI levels positively correlated with antibody levels (r=0.131; p=0.021) and sGPVI was significantly elevated (p=0.044) in HIT-positive (heparin-PF4 ELISA and serotonin release assay positive) patients with 4Ts score ≥4. Using a sGPVI level ≥59 ng/mL together with 4Ts ≥4 and positive heparin-PF4 ELISA improved HIT diagnostic specificity from 87% (using only 4Ts and heparin-PF4 ELISA) to 96%, and the overall positive predictive value from 0.48 to 0.76.

Conclusions: Engagement of platelet FcγRIIa by HIT patient plasma releases sGPVI. Considering sGPVI levels in addition to 4Ts score and heparin-PF4 antibody levels improved specificity and overall accuracy of HIT diagnosis. Patient sGPVI levels may help identify a platelet-activating HIT antibody.

ASY 39.2 | Pathogenic Antibodies in Heparin-induced Thrombocytopenia (HIT) Activate Monocytes and Induce Profound Membrane Remodeling

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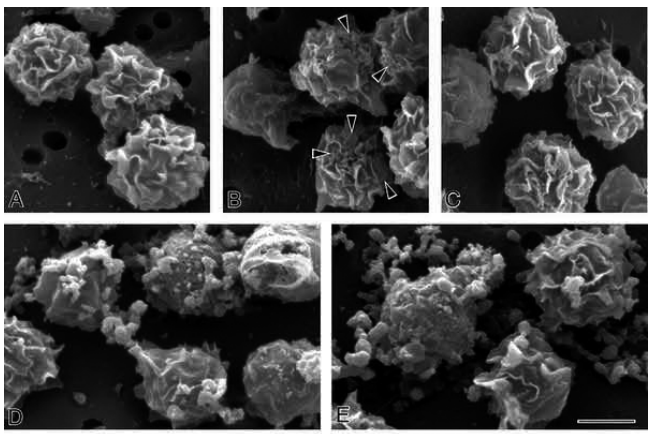
Background: HIT is an antibody mediated thrombocytopenia and thrombosis. HIT antibodies react with a multimolecular complex of PF4 with cell membrane glycosaminoglycans (GAGs) initiating cell activation. We have shown that monocytes are preferentially targeted and contribute to the prothrombotic state, but the effect of HIT antibodies on cell structure has not been described.

Aims: To study changes in the structure of the monocytes caused by HIT antibodies.

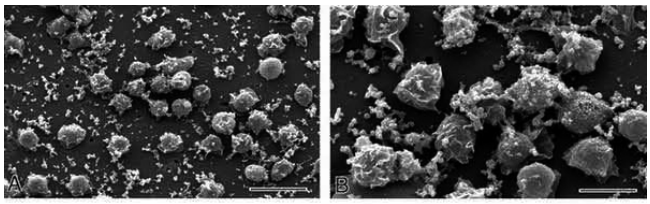
Methods: We studied isolated human and transgenic Fcγ receptor IIA positive (FcγRIIA+) and wild type (wt) (FcγRIIA-) mouse monocytes by scanning electron microscopy, confocal microscopy and flow cytometry to characterize their morphology, function and release of microparticles induced by HIT antibodies.

Results: PF4 binds to monocytes, inducing time-dependent "knobs" that project from the cell surface (average size 148 ± 27nm after 1 hr). Addition of the pathogenic HIT-like monoclonal antibody KKO caused expression of phosphatidylserine and profound remodeling of the cell



Monocyte membrane remodeling in the presence of PF4 and HIT antibodies.
A. Control monocytes - No PF4: few knobs on the cell surface.
B. Monocytes with PF4 - Addition of 100 μg/ml recombinant human PF4 initiates appearance of small knobs (black arrows; average size 148 ± 28 nm).
C. Monocytes with PF4 and RTO show similar morphology as control monocytes.
D, E. Monocytes with PF4 and KKO (50 μg/ml, 60min) dramatically enhanced clustering of PF4/GAGs complexes, forming much larger surface blebs (701 ± 208 nm).
Magnification bar = 5 μm

FIGURE 1 Monocyte membrane remodeling in the presence of PF4 and HIT antibodies



Microparticles released from monocytes.
A. Low magnification. Magnification bar = 20 μm
B. Higher magnification. Magnification bar = 10 μm

FIGURE 2 Microparticles released from monocytes

membrane characterized by loss of membrane ruffles and clustering of PF4/GAG complexes into large surface "blebs" (Fig. 1). These blebs increased in number and size over time (701 ± 208nm, after 1 hr) and were shed as microparticles (average diameter 356 ± 307nm) (Fig. 2). Flow cytometry confirmed these microparticles contained cell membrane lipids and receptors. In contrast, RTO, a monoclonal antibody that blocks PF4 oligomerization and prevents thrombocytopenia/thrombosis in an animal model, inhibited PF4/KKO-induced modification of monocyte surfaces. Comparing monocytes isolated from FcgRIIA+ vs wt mice indicated that bleb formation results from clustering of knobs caused by bivalent HIT antibodies through crosslinking of FcγRIIA's.

Conclusions: Binding of pathogenic HIT antibodies to PF4 antigenic complexes assembled on the monocyte plasma membranes promotes large scale remodeling, activation through FcγRIIA and shedding of procoagulant microparticles, all of which may contribute to thrombosis.

ASY 39.3 | Dynamic Redistribution of Platelet Factor 4 on Vascular and Intravascular Cell Surfaces: Implications for Heparin-induced Thrombocytopenia (HIT)

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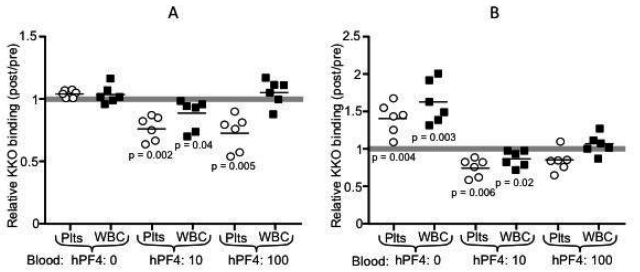
Background: HIT is an iatrogenic disorder involving antibodies (Abs) that recognize PF4/heparin complexes, leading to thrombocytopenia and thrombosis. We posit that PF4 released from platelets redistributes along the surface of leukocyte and endothelial glycosaminoglycans where it becomes a target for pathogenic Abs leading to a systemic hypercoagulable state.

Aims: To assess the redistribution of PF4 released from activated platelets among various vascular and intravascular cells.



Effect of endothelial cells on binding of PF4 to platelets and total WBCs.
Whole PF4^{null} murine blood was flowed through a channel coated with HUVECs either (A) previously unexposed to PF4 or (B) saturated with 100 μg/ml of recombinant hPF4. In both, relative surface-bound PF4 as estimated by KKO binding after traversing the channel was compared to the level prior to entering the channel. The cells in whole blood had been exposed to 0, 10 or 100 μg/ml of recombinant PF4 as indicated. The mean is shown for each study as a horizontal line. N = 6 separate studies per arm.

FIGURE 1 Effect of endothelial cells on binding of PF4 to platelets and total WBCs

Methods: Surface-bound PF4 was analyzed by flow cytometry using labeled HIT-like monoclonal Ab (MoAb) KKO, anti-PF4 MoAb RTO, and polyclonal anti-PF4 Ab. Intravital confocal imaging of the liver and a microfluidic model were used to evaluate PF4-binding to endothelial cells.

Results: PF4 released from platelets activated in whole blood formed HIT antigenic complexes predominantly on monocytes. After inducing HIT in transgenic mice overexpressing human PF4, there was a >80-fold increase in HIT Ab binding to monocytes compared with a < 4-fold increase on platelets ($p < 0.008$). This increase in HIT Ab targeting was associated with ~4 hrs of monocytopenia. Binding of PF4 to platelets correlated inversely with total WBCs added. Depletion of monocytes increased binding of PF4 to platelets 2-3 fold. Intravital confocal imaging showed PF4-mediated binding of KKO to hepatic sinusoid endothelium. When whole blood was perfused through endothelial-lined microfluidic channels, PF4 was displaced preferentially from platelets to the endothelium relative to loss of PF4 from WBCs (Fig. 1). Hematoporphyrin mediated endothelial injury enhanced this redistribution of platelet PF4.

Conclusions: Our studies support a model in which PF4 released from activated platelets binds to diverse intravascular and vascular cell surfaces that become targets of HIT antibody and potential sites of prothrombotic activity. Methods that shift PF4 binding away from monocytes and endothelium may reduce the risk of thrombosis in patients with HIT.

ASY 39.4 | Neutrophils and Released Neutrophil Extracellular Traps (NETs) Contribute to the Prothrombotic State in HIT

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Background: HIT is a prothrombotic thrombocytopenic disorder that occurs in heparin-exposed patients due to antibodies (Abs) to PF4/

heparin complexes. Although the high risk of thrombosis in HIT has been attributed to platelet (Plt) activation, recent data suggests that other cells, including neutrophils (PMNs), may contribute to clot formation. We studied the role of PMNs and NETs, extruded PMN chromatin that promotes clot formation, in this disorder.

Aims: We used in vitro and in vivo methods to study if HIT Ab exposure leads to enhanced PMN adhesion to endothelial cells (EC) and increased PMN thrombi infiltration. We also explored if inhibition of NET formation can attenuate these effects.

Methods: PMN exposed to HIT Abs were flowed through human endothelial umbilical vein cell (HUVECs)-lined microfluidic channels and EC adhesion was quantified. Peptidyl arginine deiminase (PAD) 4, which citrullinates histones and is an essential NET mediator, was disrupted in human PF4/FcgRIIa mice that develop HIT after infusion of a HIT Ab. Clot formation following cremaster venule laser injury was studied in$PAD4^{+/+}$ and $PAD4^{-/-}$ HIT mice. Plt and PMN accumulation within thrombi was assessed.

Results: HIT Ab exposure led to increased PMN adhesion in HUVEC-lined microfluidic channels (Figs. 1A and B). Enhanced EC adhesion (Fig. 1C) and PMN thrombus infiltration in HIT mice was observed following HIT induction (Fig. 2A). Plt and PMN accumulation was decreased in $PAD4^{-/-}$ HIT mice relative to $PAD4^{+/+}$ HIT mice (Fig. 2B-D).

Conclusions: HIT Ab-exposed PMN are recruited to sites of vascular injury where they contribute to plt adhesion and thrombus growth. These findings are less pronounced in $PAD4^{-/-}$ mice demonstrating that NETs contribute to the prothrombotic state in HIT. We propose that inhibition of PMN recruitment and/or NET release should be explored as strategies in the treatment of HIT and may be relevant to other immune-based prothrombotic disorders.

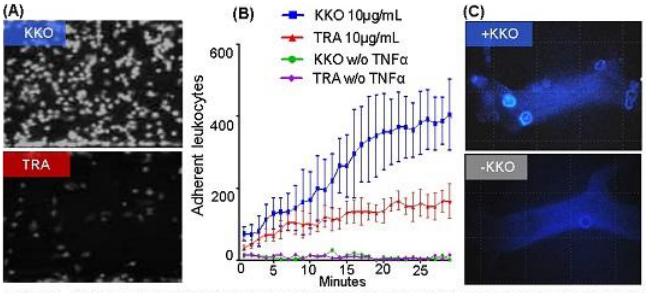

Figure 1. KKO promotes neutrophil adhesion to inflamed ECs. Microfluidic channels coated with 50µg/ml fibronectin. HUVECs infused through channels, grown to confluence, ± incubated with TNFα 1 ng/ml x 6 hrs. Whole blood labeled with calcein AM(34) flowed through channels after exposure to KKO or its isotype control, TRA. Leukocytes counted using imaging software FIJI, with particle-size exclusion. **(A)** Representative fields from studies showing adherent neutrophils. **(B)** Temporal profile of adherent neutrophils under conditions noted in graph. Mean ± 1 SEM is shown (N = 3 per arm). P = <0.0001 for KKO vs. TRA on TNFα-treated ECs using a two-tailed Student T test. **(C)** Representative image of neutrophil-EC adhesion in cremaster venule of HIT mouse before and 30 minutes after 1µg/g KKO injection.

FIGURE 1 KKO promotes neutrophil adhesion to inflamed endothelial cells

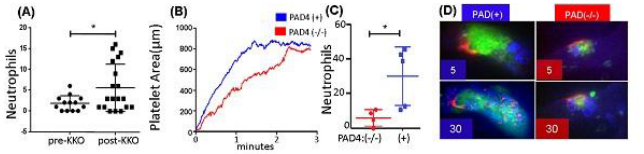

Figure 2. Cremaster injury studies in PAD4-/- HIT mice. Cremaster venule injuries were induced with a nitrogen laser. Platelets were detected using $Alexa^{488}$ anti-CD41 Ab and neutrophils using $Alexa^{647}$ anti-ly6G Fabs. Confocal videos were done. **(A)** Neutrophils incorporated in thrombi 5 minutes following laser injury were counted in injuries made before and after HIT induction. 13 venous injuries made prior to KKO injection were compared to 19 injures made after KKO injection. * P = 0.03 comparing the two arm by two-sided Student T test. **(B)** Platelet accumulation measured in 4 injuries made in 2 $PAD4^{-/-}$ mice compared to 6 injuries made in in 3 $PAD4^{+/+}$ mice. **(C).** After HIT induction, a significantly smaller number of neutrophils infiltrated venule thrombi 30 minutes following laser injury in $PAD4^{-/-}$ mice compared to $PAD4^{+/+}$ controls. ** P = 0.03 comparing the two arm by two-sided Student T test. **(D)** Representative images of injured cremaster venule in KKO-treated $PAD4^{+/+}$ mouse compared to $PAD4^{-/-}$ sibling 5 and 30 minutes following laser injury, demonstrating the typical extent of neutrophil accumulation. Red = KKO, green = platelets, blue = neutrophils.

FIGURE 2 Cremaster injury studies in PAD4-/- HIT mice

TRANSFUSION & BIOTHERAPEUTICS

ASY 11.1 | Synthesis of the First GMP Grade Biotin 3-sulfo N-Hydroxysuccimide Is now Available to Label Blood Cells Intended for Human Transfusion Studies, but not Exclusively

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Background: Platelet or red blood cell (RBC) survival and recovery is currently measured with radioactive reference tracers such as ^{111}In or ^{51}Cr. However due to regulatory obstacles such radiolabeling studies are only carried out in designated institutes. We propose an alternative tracer (Biotin-Sulfo-NHS) which could be used in human. A good comparison between the survival of RBC labeled with either a non GMP-biotin or ^{51}Cr was demonstrated in a clinical trial (Mock, 2014). The benefits are:

1) non-radioactive compound,
2) safe,
3) lower cost,
4) limited device for tracking labeled cells by flow cytometry (FC).

Aims: To develop a non-radioactive and safe cell tracker, Biotin-Sulfo-NHS, manufactured in accordance with Q7 GMP Guidance for API.

Methods: Synthesis of GMP-Biotin-Sulfo-NHS was performed by mixing *N*-hydoxysulfosuccinimid in anhydrous *DMF* and diclohexylcarbodiimide during 24h under argon. All chemical steps were performed according to the EudraLex guidelines: *The Rules Governing Medicinal Products in the European Union Volume 4; GMP; Medicinal Products for Human and Veterinary Use, Part II: Basic Requirements for API used as Starting Materials.*The labeling efficacy of RBC or platelets with this GMP-Biotin was compared to a non GMP-biotin, by FC.

Results: Mean Fluorescence Intensities (MFI) were obtained with washed RBC, labeled with 2 densities of either GMP- or non GMP-biotin, overlaid. Biotin (µg/mL): 0 (MFI 0.5 vs 0.5), 2 (MFI 3 vs 3), 20 (MFI 41 vs 32). Same results were obtained with washed platelets: 0 (MFI 0.4 vs 0.4), 4 (MFI 12 vs 11), 18 (MFI 48 vs 52). No microparticles were detected excluding cell damage.

Conclusions: The new GMP-Biotin was efficient to label RBC and platelets *ex vivo*. It will increase the safety and contribute to overcome the need for non-radioactive methods within the framework of studies in human. Its field of application is extended: transfusion, oncology -hematology, chronic disease (anemia, diabetes).

ASY 11.2 | French Lyophilized Plasma versus Fresh Frozen Plasma for the Initial Management of Trauma Induced Coagulopathy: A Randomized Open-label Trial

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Background: Hemorrhage is the major cause of preventable death in trauma patients. Guidelines recommend to begin haemostatic resuscitation immediately, including plasma transfusion.

Aims: We aimed to investigate if French Lyophilized Plasma (FLyP) was more effective than FFP for the initial management of the trauma-induced coagulopathy (TIC).

Methods: In an open-label, phase 3, randomised trial, we enrolled trauma patients aged 18 years or older needing an emergency pack of 4 plasma units within 6 hours of injury. We randomly assigned patients in a 1:1ratio to receive 4FLyP or 4FFP units. The primary endpoint was the fibrinogen concentration at 45 minutes after randomisation. Secondary outcomes included changes in hemostatic parameters, intervals transfusion, hemostatic product requirement and in-hospital mortality at 30 days. NCT0275015.

Results: 48 patients were randomised (24/group); one patient (FLyP group) discontinued intervention before any blood sampling and was excluded for primary efficacy analysis. FLyP reduced the time from randomisation to transfusion of first plasma unit ($p< 0.0001$). Compared to FFP, FLyP achieved a higher fibrinogen concentration 45 minutes after randomisation (baseline-adjusted mean difference, 0.29 g/L; 95% CI, 0.08-0.49). Similarly, a greater improvement in Prothrombin Time ratio, Factors V and II with FLyP were found at 45 minutes. The between-group differences in coagulations parameters remained significant at 6 hours but not after 12 and 24 hours. FLyP reduced the need of fibrinogen concentrates [J1] but not blood product requirement. 30-day in-hospital mortality rate was 22% in FLyP and 29% in FFP (ns).

Conclusions: FLyP was more effective than FFP for the initial management of trauma patients as increase in fibrinogen concentration and TIC improvement were faster and greater. FLyP represents an attractive option for trauma management, especially when facing logistical issues including combat casualty care and terrorism.

ASY 11.3 | The Neutralization of CD40/CD40L Complex Inhibits TRALI Development in a Mouse Model Induced through Lipopolysaccharide and Anti-MHC I mAb Injection

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Background: Even though used systematically with leukocyte reduction, platelet transfusions still cause adverse reactions in the recipient. They include TRALI (Transfusion-Related Acute Lung Injury), respiratory distress that occurs within 6 hours of the transfusion. The pathophysiology of this transfusion complication brings complex cellular communication into play which depends on a number of signalling channels. The role, particularly inflammatory, played by blood platelets in TRALI pathophysiology is evident but still under debate. Blood platelets play a major role in inflammation, particularly via the immunomodulator complex CD40/CD40L (sCD40L).

Aims: In this work, we studied the specific function of the CD40/CD40L (sCD40L) complex in regulating TRALI in an inflammatory context.

Methods: We developed a mouse model of immune TRALI, triggered by a double injection of LPS and an anti-MHC I antibody. A neutralizing anti-CD40L antibody was injected 30 minutes before inducing TRALI. The characteristics of TRALI we studied were a decrease in body temperature, pulmonary lesions, infiltration into the alveolar space and death. Blood counts of the target cells, and CD41 and Ly6G markers on lung sections enabled an evaluation of pulmonary infiltration. Cellular communication was measured from a cytometric study. Lastly, the inflammatory state of the mice and the cellular phenotype of the neutrophils were assessed.

Results: Inhibiting this immunomodulator complex significantly reduces the development of pulmonary oedema. The neutralizing antibody targets mainly the three-way cellular communication normally established during TRALI between the endothelial cells, the neutrophils and the platelets, and thus blocks the cell migration essential for the pathology to develop.

Conclusions: This target protein appears to play a major role in preventing the induction of TRALI. Improving the conditions in which the platelet concentrates are prepared and stored would thus help to control the risks of non-immune TRALI.

ASY 11.4 | Controlling Hydrophobicity to Minimize Thrombogenic Risk on Medical Devices in an Arterial Environment

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Background: Medical devices placed in the arterial circulation are at high risk for thrombosis associated with shear induced platelet activation and protein adsorption followed by platelet adhesion. Superhydrophobic surfaces and hydrophilic surface coatings have both been proposed for use on medical devices to prevent thrombosis from occurring by passivating the surface but none have been implemented commercially due to drawbacks.

Aims: To isolate the mechanism(s) in superhydrophobic surfaces that mitigates thrombus risk, while reproducing the characteristics on a hydrophilic surface to mitigate high affinity hydrophobic plasma protein binding.

Methods: We analyzed the potential for "slip" between the surface and blood through a computational physics model to determine the potential role in reducing shear stress experienced by blood, while also reducing the potential for cells to adhere to the surface. We further develop coatings in a polydimethylsiloxane (PDMS) microfluidic channel consisting of a sharp stenosis that exhibits characteristics of a high shear gradient, a region of high shear stress, and a region of flow stagnation, all of which are proposed to promote thrombosis. We evaluate slip in the channel and quantify thrombus growth on the surface relative to bare PDMS.

Results: Surface slip in the channel does reduce shear stress. Through computations and experiments, we further show that the coated surface significantly reduces the potential for platelet adhesion and activation. Surface feature size and "slipperiness" of the surface both played a significant role in the platelet response.

Conclusions: Hydrophobic surfaces are prone to protein and then cell adhesion, while hydrophilic are not. Counterintuitively, superhydrophobic surfaces are resistant to adhesion and thrombus formation. We show potential reasons why these surfaces can mitigate thrombus formation and propose new potential coatings for arterial-based medical devices.

ASY 24.1 | Sequential Mesenchymal Stromal Cell Treatment of Thrombocytopenia after Allogeneic Stem Cell Transplant: Results from a Multicenter Phase II Trial

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Background: Post-transplant thrombocytopenia is a complex complication with remarkable morbidity and mortality for which there is no effective treatment in refractory cases. Mesenchymal stromal cells (MSC) are a potentially attractive therapeutic tool because of their immunomodulatory properties and regulation of the bone marrow (BM) niche.

Aims:

a) To analyze the safety and feasibility of the sequential MSC infusion.
b) To analyze the effectiveness in terms of platelet recovery and length of the response.

Methods: Eleven patients with platelets count (P)< $50x10^9$/L and complete BM chimerism were treated within a phase II clinical trial. MSC were isolated and expanded from 50 mL of BM from third-party healthy donors. Partial and complete response (PR and CR) at day 90, was defined by achieve P=$50\text{-}100x10^9$/L or >$100x10^9$/L, respectively; adverse effects (AE) and survival was analyzed after infusion 4 doses of $1x10^6$ MSC/kg on days 1, 4, 11 and 18.

Results: Baseline patients' characteristics are shown in Table 1. MSC infusion was performed after a median of 86 days from allo-transplant (range 35-633). There were no AE related to infusion. Within the first 90 days, 9 out of 11 patients (82%) responded to cellular therapy and 7 of them achieved CR. On day 90, six patients maintained CR and one PR, two patients had no response and two were non-evaluable. At the last follow-up, with a median of 202 days (range 76-820), 7 patients (CR=3, PR=3) maintained the response, 1 patient achieved CR with an alternative treatment and 4 patients deceased due to the progression of disease (n=1) or sepsis (n=3). Patients that achieve response to MSC therapy seemed to have an advantage in terms of long-term survival.

TABLE 1 Baseline patients characteristics

AGE	Median 49 years (Range, 20-66 years)
SEX	Male = 9; Female = 2
HEMATOLOGICAL DISEASE	Acute Myeloid Leukemia = 7; Non-Hodgkin Lymphoma = 3; Acute Lymphoblastic Leukemia = 1
DONOR	Unrelated = 3; Sibling = 3; Cord Blood = 1
CONDITIONING REGIMEN	Reduced intensity = 6; Myeloablative = 5
PRIOR TREATMENT	Median 1 line (Range, 1-3)
MEDIAN PLATELET COUNT BEFORE INFUSION MSC	22x103/mm3 (Range, 12-47x103/mm3)
MEDIAN PLATELET COUNT AT 90 DAY POST INFUSION MSC	106.000 Range (5-170x103/mm3)
CONCOMITANT GVHD	8 out of 11 patients (73%)

Conclusions: Treatment of peripheral thrombocytopenia with MSC is feasible, has no adverse effects and is potentially useful in most patients. Achieving either CR or PR to MSC therapy seems to favor long-term survival for those patients.

Funding: Clinical trial was funded by the EC11-389 grant from Ministerio de Sanidad, Spain.

ASY 24.2 | Optimization of in vitro Megakaryocyte and Platelet Production by Inducible Overexpression of Supporting Factors

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Background: Induced pluripotent stem cells (iPSC) are of great interest for generation of patient-specific megakaryocytes (MK) and platelets for cellular therapies.

Aims: Although differentiation of platelets from iPS cells is possible, the recovered numbers are low. To optimize the process we focus on the overexpression of supporting factors like transcription factors (TF) e.g. Gata-1 and Nfe2. To avoid off target effects in pluripotent cells and during differentiation TF expression must be time-controlled. We therefore established a tet-inducible system in murine iPSC based on retroviral vectors. To overcome problems caused by vector silencing during differentiation, the reverse tetracycline-controlled transactivator M2 (rtTA M2) is expressed from the ROSA26 safe harbor locus.

Methods: MKs and platelets were generated via embryoid body formation. After dissociation and co-cultivation on OP-9 feeder cells with thrombopoietin, cytomorphological analysis and cell surface phenotype identified culture-derived MK/ proplatelets.

Results: Overexpression of Gata-1 resulted in expandable megakaryocytes which were almost 20% double positive for CD41/ CD42d and maintained their phenotype for >6 weeks until termination. Similarly, Nfe2 overexpression supported in vitro MK development, however, the expansion capacity was lower. More than 70% of the platelets from Gata-1 overexpressing MK were positive for CD41, hereof almost 25% were double-positive for CD41/CD42d after 6 weeks. To modify human iPSC, Tet-inducible all-in-one retroviral vectors were developed containing different sized chromatin opening elements for prevention of vector silencing. Repeated induction/repression by doxycycline was most faithful when using a 670bp element of the CBX3 5‘ region/gene, which also showed the highest fold-induction and strongest expression in human iPSC.

Conclusions: Inducible Gata-1 and NFe2 expression by retroviral modification of iPSC improves in vitro MK and platelets production.

ASY 24.3 | Endothelial and Cancer Cell Gene Silencing by Platelet-vehiculated siRNAs

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Background: Platelets interact actively with normal and pathologic cells in the circulation. In particular, platelets and platelet-derived-microparticles (PMP) release some of their miRNAs to endothelial cells. Short interfering RNAs (siRNAs) are a novel class of drugs that downregulate the expression of a target gene by degrading its relative mRNA. A major limitation of siRNAs therapy is their degradation in blood and insufficient achievement of target cells. We have developed a novel method to transfect therapeutically-designed siRNAs into platelets.

Aims: To assess whether coincubation with siRNA-transfected platelets can silence an endothelial-cell or cancer-cell specific gene.

Methods: Platelets were transfected with a fluorescent TYE563-labeled-siRNA and transfection efficiency was assessed by flow-cytometry and confocal microscopy. Fluorescent siRNA-transfected platelets were coincubated with human umbilical vein endothelial cells (HUVEC) or HeLa cells (cervical tumor) to assess transcellular siRNA transfer. Moreover siRNA-HPRT transfected platelets were coincubated with HUVEC and HeLa to assess gene silencing efficacy by measuring HPRT mRNA by Real-Time PCR and HPRT protein by Western Blotting.

Results: Around 90% of platelets were transfected with fluorescent siRNA. TYE563-labeled-siRNA was detected in HUVECs and in HeLa cells after co-incubation with siRNA-transfected platelets. Coincubation with platelets transfected with siRNA-HPRT determined a significant downregulation in target cells of HPRT mRNA (-57%±11% in HUVEC and -55%±4% in HeLa) and protein (-43%±18% in HUVEC and -55% in HeLa).

Conclusions: siRNA-transfected platelets deliver therapeutically-designed siRNA to HUVEC and HeLa silencing the target gene. The ability of platelets to deliver active siRNA to endothelial and tumor cells can be used to downregulate pathogenic gene expression in vivo. Using platelets as carriers is a potentially new original approach to RNAi therapy.

ASY 24.4 | Targeting Peripheral Blood Mononuclear Cells to Activated Platelets Preserves Cardiac Function after Ischemia-reperfusion Injury

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Background: One of the major hurdles and potential reason of failure of intravenous regenerative cell therapy is a low homing efficacy to the infarcted myocardium post MI.

Aims: Developing a highly specific anchor to guide cells to the ischemic myocardium and thus minimize cardiac remodeling/injury and restore cardiac function after ischemia and successful reperfusion.

Methods: A bispecific tandem single-chain antibody (scFv) that binds with high affinity and specificity to activated platelets via the GPIIb/IIIa receptor and to peripheral blood mononuclear cells (PBMC) via the Sca-1 receptor was engineered and characterized. The tandem-scFv was tested in a mouse model of ischemia-reperfusion (IR) injury by LAD ligation for 60 min. Fluorescence (IVIS) imaging, cell infiltration studies, echocardiographic and histological analyses were performed.

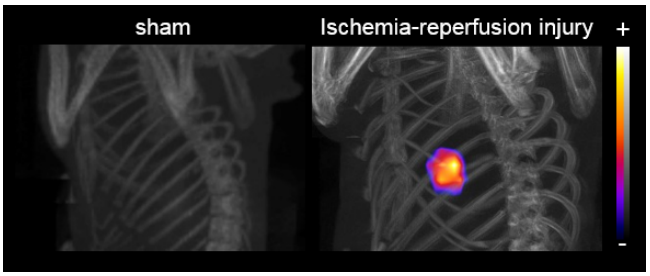

FIGURE 1 PET/CT images show accumulation of activated platelets post IR injury

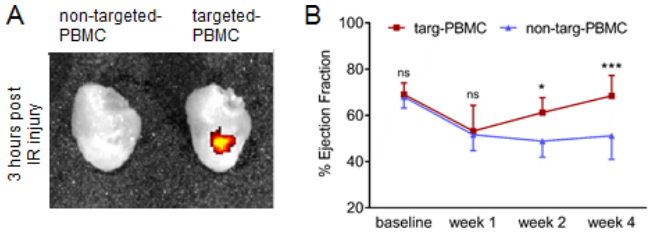

FIGURE 2 A) IVIS image shows enhanced delivery of targeted-PBMCs post IR injury. B) Treatment with Targ-PBMC preserves cardiac function post IR injury

Results: Dynamic flow assays applying physiological shear stress showed strong binding of tandem-scFv pre-incubated cells to activated platelets. After IR injury, a PET/CT scan using a platelet specific radiotracer showed abundant accumulation of activated platelets in the post-ischemic myocardium and thus activated platelets represent an ideal target to redirect cells.

Systemic delivery of tandem-scFv targeted-PBMCs led to successful cell delivery to the infarcted myocardium followed by a significant decrease in infiltrating inflammatory cells. Homing of targeted-PBMCs ultimately decreased necrosis (7.2±3.3 vs. 11.9±2.1%, $p< 0.01$), increased capillary density (929±148 vs. 544±103 capillaries, $p< 0.0001$), and most importantly nearly completely restored cardiac function 4 weeks post IR injury (68.3±5.3 baseline vs. 68.4±8.8 targ-PBMC vs. 51.1±10.2 %EF control, $p< 0.001$).

Conclusions: Targeting of Sca-1$^+$-PBMCs to activated platelets allows effective intravenous delivery of cells to the infarcted myocardium thereby preventing IR injury and loss of cardiac function. Overall, activated-platelet targeting of PBMCs represents a novel and promising approach for cell therapy after MI.

VASCULAR BIOLOGY & ANGIOGENESIS

ASY 09.1 | Thrombin-reduced miR-27b Attenuates Platelet Angiogenic Activities via Enhancing Platelet de Novo Synthesis of Anti-angiogenic Thrombospondin-1

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Background: Platelets can synthesize proteins upon activation. Platelets contain microRNAs (miRNA) and a functional miRNA effector machinery. It is, however, unclear if platelet miRNAs can regulate protein synthesis of platelets.

Aims: To investigate if and how platelet miRNAs regulate de novo syntheses of angiogenic factors.

Methods: Platelet miRNA profiling was performed using an oligonucleotide microarray allowing detection of both mature and precursor miRNAs. Transfection of Meg-01 cells and platelets were carried out using specifically developed techniques for these transfection-resistant cells. Impact of miRNA intervention on platelet angiogenic activities was assessed by an endothelial tube formation assay.

Results: Microarray-based miRNA profiling showed that thrombin stimulation down- or up-regulated a number of platelet miRNAs, whilst thrombin consistently decreased Ago2-associated miRNAs. Among those altered miRNAs, miR-27b was down-regulated in both the total and Ago2-immunoprecipitated miRNA profiles of platelets. Using western blotting assays, we showed that thrombin stimulation induced platelet de novo synthesis of thrombospondin-1 (TSP-1), and that the level of TSP-1 content reached 3-fold higher than that before thrombin stimulation. With either platelet precursor megakaryocyte cell line MEG-01 cells or platelets, we showed that transfection of miR-27b mimic, but not the negative control of miRNA mimic, markedly reduced TSP-1 protein levels, implying that miR-27b inhibits, or reduced miR-27b can enhance TSP-1 synthesis. Notably, reduced TSP-1 synthesis by miR-27b overexpression potentiated platelet-enhanced endothelial tube formation.

Conclusions: Thrombin stimulation reduces platelet miR-27b levels, and evokes platelet de novo synthesis of TSP-1. Elevation of miR-27b by transfection inhibits platelet TSP-1 de novo synthesis, and enhances platelet angiogenic activities, indicating that platelet activation-reduced miR-27 is a novel regulatory mechanism of platelet angiogenic activity.

ASY 09.2 | Human Endothelial Cells Infected with Staphylococcus aureus Leads to an Up-regulation of miR-330, Resulting in Loss of Barrier Integrity in an ex vivo Model of Sepsis

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Background: Sepsis is one of the major causes of mortality in critically ill patients and is commonly caused by *Staphylococcus aureus*. Typical characteristics of sepsis are oedema and swelling due to excessive

vascular leakage, caused by endothelium degradation. However the nature of the signals leading to vascular leakage is currently unknown and is often difficult to control pharmacologically. MicroRNA are short non-coding RNA that control many cellular processes. Their dysregulation is often linked to disease.

Aims: This study aims to investigate if endothelial miRNA are dysregulated following infection and if this contributes to sustained and excessive responses in sepsis.

Methods: Human endothelial cells, sheared at physiological rates, were infected with *S. aureus* and resulting changes in miRNA expression analysed by TaqMan® Arrays (RQ=2-ΔΔCt). Whole genome sequencing and bioinformatics uncovered potential miRNA-mRNA interactions linked to infection with effects on endothelial function determined by mirmimetic transfections.

Results: Following *S. aureus* infection, 93 endothelial miRNA were significantly modified, of which 35 were up- and 58 were down-regulated ($p< 0.05$). miR330 was significantly up-regulated and using next generation sequencing and bioinformatics we identified 102 potential targets in the cells ($p< 0.0005$). Of particular interest were key genes responsible for ensuring barrier integrity of the cells. Therefore changes to permeability following infection were investigated. Results suggest that *S. aureus* significantly increases permeability ($p< 0.05$) by reducing expression of a key junctional protein, VE-Cadherin. Consistent with this, transfection of a miR330 mimetic to uninfected cells resulted in an increase in permeability.

Conclusions: We propose that following *S. aureus* infection of human endothelial cells, rapid dysregulation of miRNA occurs contributing to degradation of the endothelial permeable barrier through down-regulation of essential junction barrier proteins such as VE-Cadherin.

ASY 09.3 | Identification of Eight Oestrogen-sensitive miRNAs that Regulate Coagulation Factors, One of which (miR-365a-3p) Inhibits Tissue Factor Expression

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Background: High oestrogen (E_2) levels during pregnancy are associated with an increased risk of venous thrombosis, however, the role of E_2-responsive miRNAs (miRs) binding to the 3'UTR regions of coagulation genes regulating function remains poorly understood. NanoString nCounter® miRNA array analyses identified a number of E_2-responsive miRs in HuH-7 cells that may be involved in regulating haemostasis.

Aims: This study sought to validate the E_2-responsiveness of the candidate miRs and investigate their direct effects on predicted coagulation gene targets.

Methods: Nine significantly E_2-responsive miRs ($p< 0.05$) detected by NanoString nCounter® miRNA array were selected and their putative coagulation gene targets were identified using *in silico* miRNA target prediction tools. E_2 effects on the expression of the candidate miRs and mRNA levels of predicted gene targets were determined by RT-qPCR in HuH-7 cells. The direct interaction between the miRs and the putative miRNA-binding sites in Tissue Factor (*F3*) and Factor VIII (*F8*) were measured via dual luciferase assays. Statistical significance was assessed by Student t-test.

Results: Out of the 9 potential miRs, we confirmed by RT-qPCR E_2 significantly downregulated ($p< 0.05$) 8 miRs, let-7f-5p, miR-26b-5p, miR-98-5p, miR-128-3p, miR-365a-3p, miR-423-5p, miR-455-3p and miR-548aa. We also found E_2 significantly increased ($p< 0.05$) *F3* and *F8* mRNA levels. One miRNA, miR-365a-3p showed a functional miR-365a-3p binding site in the *F3*-3'UTR, whereby deletion of the predicted miR-365a-3p binding site in the *F3*-3'UTR abolished the inhibitory activity, confirming miR-365a-3p direct regulation of *F3* mRNA expression.

Conclusions: We have confirmed 8 miRs that are downregulated by E_2, and that one, miR-365a-3p, inhibits *F3* mRNA expression which could lead to an increase in E_2-driven tissue factor expression. NanoString nCounter® miRNA array analysis is a useful screen for E_2-responsive miRs but positive findings need confirmation by RT-qPCR to determine their significance.

ASY 09.4 | Everolimus Eluting Coronary Stenting Elicits Less Cellular Activation via Altered miRNA Levels in Plasma and Endothelial Cells

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Background: We have recently described higher levels of soluble E-selectin and VCAM-1 after bare-metal stenting (BMS, n=28) in contrast to drug-eluting stenting (DES, n=21) in stable angina patients. One fifth of BMS subjects displayed in-stent restenosis (ISR) in the first 6 months, while no individuals with DES suffered from such complication.

Aims: Here we compared plasma miRNA levels of these patients with or without ISR, and the background of some of these miRNA alterations was further analyzed via the evaluation of RNA content of endothelial cells *in vitro*.

Methods: Total RNA was extracted from plasma samples obtained after 1 month of intervention, and a global expression pattern of miRNAs was analyzed by using TaqMan Open Array (ABI) in 3 samples from each study group. UPL-probe based RT-qPCR assay (Roche) was used for validation of selected miRNAs showing at least 2-fold alteration. We investigated the effect of vascular inflammation and that of everolimus on endothelial miRNA and mRNA levels after

stenting using RT-qPCR. For this purpose, cultured human umbilical vein (HUVEC) and coronary artery (HCAEC) endothelial cells (Sigma-Aldrich) were challenged with recombinant TNF-α (100 ng/mL) for 4-24 hours in the presence or absence of externally added everolimus (0.5 μM).

Results: There were 36 significantly decreased (e.g. miR-126, miR-181b) and 21 upregulated

(e.g. miR-185) miRNAs in BMS with ISR (P< 0.01) vs. those BMS without complication and all DES patients. TNF-α enhanced miR-146a, miR-155 and miR-185 expression in endothelial cells indicating the development of inflammatory response and endothelial dysfunction. Decreased miR-424 and miR-181b were found with elevated E-selectin and VCAM-1 mRNA levels by 4 hours. In contrast, everolimus via raising these miRNAs caused significantly (P< 0.01) depressed mRNAs of these adhesion proteins.

Conclusions: Everolimus shows a substantial effect on endothelial cell activation in DES via altering the level of circulating and cellular miRNAs.

ASY 17.1 | Vascular Endothelial Cells Derived from Human PAI-1 Deficient iPS Cells Reveal Physiological Functions of PAI-1 in Angiogenesis

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Background: Plasminogen Activator inhibitor-1 (PAI-1), the principle inhibitor of plasminogen activators (PAs), play essential role not only in the regulation of fibrinolysis but other pathophysiological-events including angiogenesis. We have identified two distinct PAI-1 deficient patients having apparent phenotypes of severe bleeding and impaired wound healing.

Aims: In order to investigate the intrinsic function of human PAI-1 in endothelial cells, we have established iPS cells from the patients.

Methods: iPS cells from the patients were generated, and differentiated to endothelial cells. The endothelial cells were isolated by magnetic sorting with anti-VEGFR-2 antibody, and their functions and the related gene expressions were analyzed.

Results:

(1) Endothelial cells derived from PAI-1 iPS cells (PAI-1 iPS-ECs) detached from gelatin-coated dishes easier than control.

(2) PAI-1 iPS-ECs migrated faster in migration assay.

(3) The expression of Dll4 gene, known to be highly expressed in so-called tip cells existing at the angiogenic-edge, was enhanced, and the number of branching in tube formation assay was less in PAI-1 iPS-ECs.

(4) As these results suggested that PAI-1 iPS-ECs acted like mesenchymal cells, we checked the cell response to TGF-beta induced endothelial mesenchymal transition. TGF-beta induced alpha-smooth muscle actin expression more effectively in PAI-1 iPS-ECs.

(5) In in-vitro endothelial sprouting assay, tube-like vascular formation was less mature and tip-like cells were more dominant in PAI-1 iPS-ESs.

Conclusions: Immature vascular sprouting, due to dominance of tip-like cells, seems to be responsible for the impairment in both angiogenesis and wound healing in human PAI-1 deficiency.

ASY 17.2 | Perivascular Progenitor Cells Regulate the Balance between Regeneration vs. Fibrosis via PDGFRa Signalling

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Background: PDGFRα is restricted to mesenchymal progenitors that reside in the perivascular tissue of multiple organs. Although the stem cell capacity of vascular and perivascular cells has been extensively studied in numerous models, the cellular and molecular mechanisms responsible for fibrosis are poorly understood.

Aims: Herein we aimed to study the molecular mechanism regulating fibrogenesis of perivascular cells and developed a new antifbrotic treatment to reduce cardiac and skeletal muscle fibrosis.

Methods: We utilized the *Collagen1a1-GFP* transgene and conditional Cre mouse lines to identify cells producing Collagen-I matrix in wild type mice exposed to acute injury or those mutated at the dystrophin gene locus (*mdx*) as a model of Duchenne Muscular Dystrophy (DMD), to study the mechanisms of cardiac and skeletal muscle injury/repair and fibrosis.

Results: Fate mapping experiments indicate that these perivascular progenitors contribute to regeneration whereas in injury they become collagen producing fibroblasts. While in acute injury/repair of muscle and heart, PDGFRα signalling is transiently upregulated during the regenerative phase, in the DMD model and in human DMD it is chronically over-activated. Conditional expression of the constitutively-active PDGFRα D842V mutation in perivascular cells, during injury/repair, hindered the repair phase and instead promoted fibrosis. In DMD, treatment of *mdx* mice with crenolanib, an investigational drug, highly selective PDGFRα tyrosine kinase inhibitor, reduced fibrosis, improved cardiac function, muscle strength and was associated with decreased activity of Src, a downstream effector of PDGFRα signalling.

Conclusions: Transient PDGFRα-activation of perivascular progenitors is required for repair of the injured heart and muscle, but persistent and excessive activation of this pathway directly drives fibrosis and hinders repair. The PDGFRα pathway is a potential new target for treatment of fibrosis in progressive skeletal muscle and cardiac diseases.

ASY 17.3 | Co-injection of Mesenchymal Stem Cells with Endothelial Progenitor Cells Accelerates Muscle Recovery in Hind Limb Ischemia by an Endoglin-dependent Mechanism

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Background: Endothelial colony-forming cells (ECFCs) are progenitor cells committed to endothelial lineages that have robust vasculogenic properties. Mesenchymal stem cells (MSCs) have been described to support ECFCs angiogenic process in different kind of matrices.

Aims: MSCs potential interaction with ECFCs in hind limb ischemia (HLI) remains largely unknown. Therefore, we assessed whether co-administration of ECFCs and MSCs can support vasculogenic properties in HLI of nude mice.

Methods: We previously described endoglin as a key adhesion molecule; we thus evaluated its implication in ECFCs/MSCs interaction. We examined the effect of ECFCs+MSCs injection after HLI in athymic nude mice. Immunohistochemistry for human and mouse CD31 and *in situ* hybridization (ISH) for ALU sequence were performed.

Results: Foot perfusion was increased after ECFCs injection in day 7 and was even better 14 days after injection. Co-administration of MSCs significantly increased vessel density and foot perfusion at day 7, although this difference was no longer significant at day 14. Capillary density was enhanced in ECFCs+MSCs mice by analysis of mouse CD31, human CD31 incorporation and ISH detecting human ALU sequence. We then examined injection of ECFCs silenced for endoglin + MSCs and found a decreased vessel density and foot perfusion at 7 and 14 days ($p < 0.001$). Silencing endoglin in ECFC did not block MSC differentiation potential in perivascular cells or other mesenchymal lineages. However, silencing endoglin in ECFC dramatically decreased their adhesive properties on MSCs.

Conclusions: We demonstrate that MSCs in combination with ECFCs accelerate muscle recovery by an endoglin-dependent mechanism. Our data suggest the systematic use of MSC as a means to improve ECFCs engraftment in hind limb ischemia.

ASY 17.4 | Thromboembolic Events in Patients with BCR-ABL1-negative Myeloproliferative Neoplasms Are Not Related to in vitro Endothelial Cells Adhesiveness Nor JAK2-V617F Expression

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Background: BCR-ABL1-negative myeloproliferative neoplasms (MPN) are a group of hematological malignancies characterized by the proliferation of mature myeloid cells, a tendency towards acute leukemia transformation and a high risk of venous and arterial thromboembolic events (TEE). They harbor several driving mutations, among which *JAK2*-V617F is the most frequent and is associated with TEE.

Aims: To better understand the role of endothelial cells in the pathogenesis of TEE in patients with *JAK2*-V617F-positive MPN, we analyzed the in vitro adhesive properties of these cells, isolated from these patients with and without a prior history of TEE, as well as from healthy individuals.

Methods: Endothelial colony-forming cells (ECFCs) were isolated from peripheral blood mononuclear cells and seeded on collagen with conditioned media. Red blood cells (RBCs) were obtained after centrifugation of whole blood. The presence of *JAK2*-V617F mutations on ECFCs was assessed by RT-PCR. Adhesion was evaluated after incubation of ECFCs with RBCs with or without TNF-α. Circulating endothelial cells (CEC) were quantified by flow cytometry.

Results: ECFCs were successfully cultured from 7/19 patients (3 with polycythemia vera; 1 with essential thrombocythemia and 3 with primary myelofibrosis), 3 of which have had a TEE. The number of CECs was not associated with the success in isolating ECFCs. However, we observed a difference in the median number of CECs in patients with (0.03 CECs/µl; n=7) and without (0.08 CECs/µl; n=8) a prior TEE ($p=0.09$). We also observed a higher median adhesion of RBCs from healthy individuals with ECFCs isolated from patients with MPN without TEE (31%; n=3) than those with TEE (7.2%; n=4) without TNF-α stimulus ($p=0.0023$). We could not detect a *JAK2*-V617F mutation in the ECFCs from any of the patients.

Conclusions: Our findings do not support an increased adhesive capacity of ECFCs nor the presence of JAK2-mutations in these cells as drivers of thromboembolic events in patients with JAK2-mutated MPN.

ASY 30.1 | Targeting the Angiopoietin-Tie2 Axis to Regulate Microvascular Thrombosis in Sepsis

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Background: Treatment of disseminated intravascular coagulation (DIC) in the context of sepsis is a therapeutic challenge because patients can present concurrently with thrombosis and bleeding. Inflammation-induced changes in endothelium contribute to systemic activation of coagulation. We hypothesized that DIC is initiated largely by these endothelial changes and that targeting the endothelium could ameliorate thrombosis without causing bleeding.

Aims: To determine whether modulation of the Angiopoietin(Ang)-Tie2 axis can reduce microvascular thrombosis in endotoxemia.

Methods: We monitored laser-induced thrombus formation in cremaster arterioles of TIE2+/- mice and following IP injection of 10 mg/kg LPS in C57B6 mice, infused with an adenovirus expressing Tie2 receptor agonist Ang1 (Ang1Ad) or control adenovirus (CtlAd).

Results: LPS exposure (1-3 hrs) significantly increased platelet and fibrin formation at injury sites to 190% ($p< 0.05$) and 195% ($p< 0.001$) of control, respectively. Platelet function was unaffected in these experiments; however, phosphorylation of endothelial Tie2 was substantially reduced. Evaluation of thrombus formation in TIE2+/- mice (without LPS infusion) showed enhanced fibrin formation at injury sites to 197% ($p< 0.01$) of TIE2+/+ controls. Given that Tie2 deficiency and prothrombotic responses are linked, we assessed whether Tie2 activation could reduce the LPS-induced phenotype. Fibrin formation was significantly attenuated ($p< 0.05$) in endotoxemic mice injected with Ang1Ad compared to CtlAd. In contrast, elevated Ang1 levels did not prolong bleeding times in tail clip assays. Studies using cultured endothelium showed that Ang1 pre-treatment significantly blocked LPS-induced phosphatidylserine and tissue factor expression and inhibited thrombin and FXa generation.

Conclusions: These data suggest that the Ang-Tie2 axis controls clot formation in endotoxemia. Targeting this axis represents a novel approach for reducing thrombotic complications without increasing bleeding risk in sepsis.

ASY 30.2 | AMPK Promotes VEGF-induced Angiogenesis via GFAT1 Inhibition

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Background: Activation of AMP-activated protein kinase (AMPK) in endothelial cells regulates energy homeostasis, stress protection and angiogenesis, but the underlying mechanisms are incompletely understood. We identified glutamine:fructose-6-phosphate amidotransferase 1 (GFAT1) as an AMPK substrate. GFAT1 is the rate-limiting enzyme in the hexosamine biosynthesis pathway (HBP) and as such controls the modification of proteins by O-linked β-N-acetylglucosamine (O-GlcNAc).

Aims: The aim of this study was to check if AMPK controls O-GlcNAc levels and cellular function via GFAT1 phosphorylation.

Methods: Using phosphoproteomic and biochemical analyses, we identified GFAT1 as AMPK target and studied the functional significance of its regulation by AMPK in primary human endothelial cells with biochemical, pharmacological, genetic and in vitro angiogenesis techniques.

Results: Activation of AMPK in cells by 5-aminoimidazole-4-carboxamide riboside (AICAR) led to GFAT1 phosphorylation at serine 243 and inhibition of its activity. In parallel, reduced O-GlcNAc levels were observed. Vascular endothelial growth factor (VEGF), a major angiogenic stimulus, induced GFAT1 phosphorylation in an AMPK-dependent manner. Inhibition of GFAT1 potentiated VEGF-induced sprouting indicating that GFAT1 acts as negative regulator of angiogenesis. In cells expressing non-phosphorylatable S243A-GFAT1, VEGF-induced sprouting was reduced compared to cells expressing wild-type GFAT1. These data suggest that VEGF relieves the inhibitory action of GFAT1/HBP on angiogenesis via AMPK-mediated GFAT1 phosphorylation. Activation of GFAT1/HBP by high glucose impaired VEGF-induced sprouting, while GFAT1 inhibition improved sprouting even at high glucose.

Conclusions: Our findings provide novel mechanistic insights into the role of HBP in angiogenesis. They suggest that targeting AMPK in endothelium might help to ameliorate hyperglycaemia-induced vascular dysfunction associated with metabolic disorders.

ASY 30.3 | Endothelial Primary Cilia Coordinate Vascular Regression During Development

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Background: The primary cilium plays a major role during embryonic development as a sensor for chemical and mechanical morphogenetic signals. However, its role in endothelial cells during blood vessel formation is essentially unknown. During development, newly-formed blood vessels remodel into mature networks to ensure efficient perfusion of the tissue. This process is based on the divergent migration of endothelial cells to remove superfluous connections, and is driven by blood flow.

Aims: We evaluated the possible role of the primary cilium as a mechanosensor for blood flow during vessel regression.

Methods: We depleted endothelial cells of their primary cilium in vivo using endothelial-specific inducible gene knockout of IFT88 in mice. In vitro, we silenced IFT88 in endothelial cells exposed to different shear stress conditions.

Results: In vivo, knockdown of IFT88 promoted vascular remodeling in the retina, without affecting endothelial cell death or proliferation. Interestingly, we found that the proportion of endothelial cells carrying a primary cilium decreased with the level of shear stress. In vitro, silencing of ITF88 modified the response of endothelial cells to flow. Under low levels of shear stress, cilium-depleted cells polarized (front-rear polarity) better in the direction of flow compared to control cells, while at high levels of shear stress cells polarized better against the direction of flow. In addition, endothelial cells devoid of primary cilia migrated faster than control cells. Finally, BMP signaling is down-regulated in vitro following IFT88 knockdown, suggesting that the effect of the primary cilium on cell polarity could be mediated by BMPs.

Conclusions: In vitro, we found that endothelial cell polarization and migration are enhanced by the loss of the primary cilium. Together with the early vascular regression we observed in the absence of primary cilia in vivo, this could suggest that the primary cilium negatively regulates divergent migration of endothelial cells during vascular remodeling.

ASY 30.4 | Nterm-Phosphatase of Soluble Epoxide Hydrolase (sEH) in the Regulation of Vascular Endothelial Inflammation

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Background: Soluble epoxide hydrolase (sEH) is a homodimeric enzyme consisting of C-terminal epoxide hydrolase (Cterm-EH) and N-terminal lipid phosphate phosphatase (Nterm-phos) domains that allosterically interact with each other. Cterm-EH regulates the activity of bioactive epoxy-fatty acids through hydrolysis to corresponding diol-fatty acids, whereas information about the natural substrate of Nterm-phos is limited, leaving its physiological role unknown. The small molecule SMTP-7 inhibits both of the two activities of sEH and suppresses endothelial inflammation, while contribution of each inhibition remains unknown.

Aims: To investigate the role of Nterm-phos in SMTP-mediated suppression of endothelial inflammation and to identify endogenous substrate of Nterm-phos.

Methods: Gene expression was assessed by DNA microarray and qPCR. LPS was used to induce CAM expression in microvessel endothelial cells. Nterm-phos inhibitors were generated based on SAR of *N*-substituted amino acids.

Results: SMTP-7 inhibited LPS-induced cell adhesion molecule expression (CAM) in endothelial cells. There was a striking similarity in the gene expression pattern between SMTP-7-treated mice and sEH-KO mice (Fig. 1), exemplified by marked elevation *Saa* and *Lcn2* expression. Fmoc-Tyr, which selectively inhibits Nterm-phos, but not a Cterm-EH-specific inhibitor suppressed CAM expression and induced *Saa* or *Lcn2*. For the screening for endogenous substrate of Ntem-phos, total lipids isolated from mouse liver were treated with purified sEH (wild-type, EH-deficient D335S, or Phos-deficient D9A). Alcohols pre-existed or emerged after sEH reaction were analyzed by LC-MS after respectively derivatized with deuterated or unlabeled dansyl chloride, resulting in identification of candidate substrates.

Conclusions: sEH Nterm-phos is responsible for the regulation of endothelial inflammation. Nterm-phos substrate may control CAM expression and endothelial inflammation.

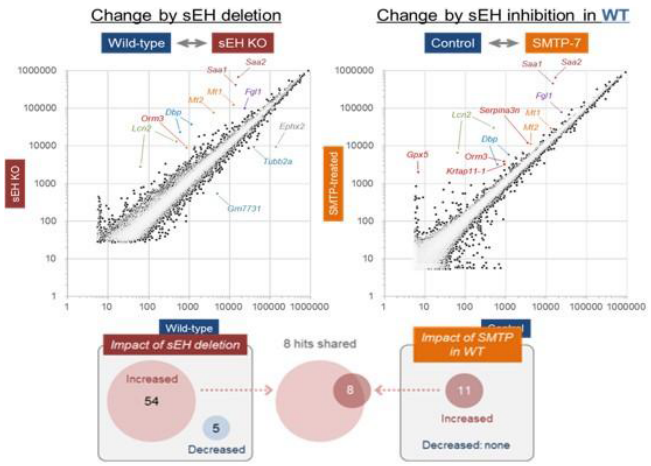

FIGURE 1 SMTP causes gene expression change similar to that observed in sEH deletion

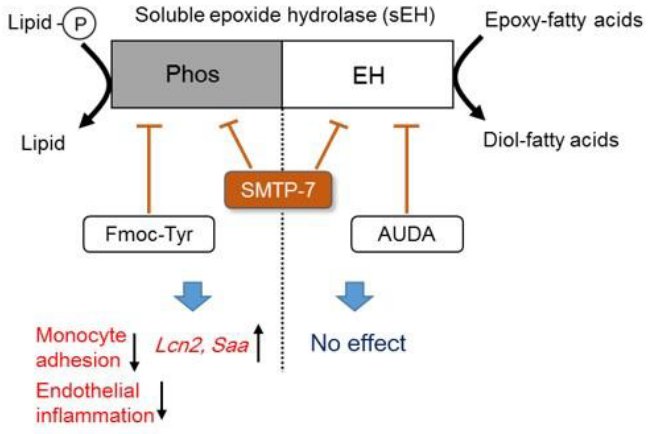

FIGURE 2 Schematic diagram of the mechanism of SMTP-7-mediated anti-inflammatory effect

ORAL COMMUNICATION ATHEROTHROMBOSIS & STROKE

OC 27.1 | Hypertension Upregulates Tissue Factor Expression in Bone Marrow Megakaryocytes and in Circulating Platelets

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Background: Hypertension is a predisposing factor to thrombotic complications of cardiovascular diseases. Indeed, increased levels of circulating tissue factor (TF) have been reported in hypertensive patients. Angiotensin II (Ang) has been shown to induce TF expression in monocytes. Also platelets (PLT) express TF but whether Ang affects PLT-TF expression is still unknown.

Aims: To investigate whether:

1) Ang modulates *in vitro* PLT-TF expression;

2) in an animal model of hypertension the number of TF^{pos} PLT and megakaryocytes (MK) is affected;

3) pharmacological control of hypertension with captopril restores PLT- and MK- TF expression.

Methods: TF^{pos} PLT from Wistar Kyoto rats were analyzed by flow cytometry (FC) after stimulation *ex vivo* with Val5Ang (1-100nM). *In vivo*, spontaneously-hypertensive stroke-prone rats (SHRSP) received standard diet (SD,) or high-sodium permissive diet (HSD) plus vehicle or plus captopril (50 mg/kg/die). PLT- and MK- TF expression was analyzed by whole blood FC. Thrombin generation was measured by CAT assay.

Results: *Ex vivo* stimulation of PLT with Ang resulted in a concentration dependent increase in the surface expression of TF, which was significantly inhibited by valsartan preincubation. *In vivo*, 4 week-HSD induced hypertension in SHRSP. The number of TF^{pos} PLT and MK increased compared to SD rats (PLT:64±6.7% vs 33.8±5%; p< 0.0001; MK: 47.8±6.8% vs 32.2±4%; p=0.007) and was reduced by captopril (PLT: 32.8±10.6%, p< 0.0001; MK: 36.7±5.2%; p=0.04). Similarly, PLT-TF activity was increased in hypertensive rats. Treatment with captopril reverted these effects.

Conclusions: Ang induces TF expression on the platelet surface, an effect mediated by AT1 receptor. *In vivo* hypertension upregulates MK TF expression leading to an increased number of PLT expressing a functionally active TF..In turn, the PLT prothrombotic potential is increased. Pharmacological treatment with captopril reverts TF up-regulation in MK and PLT.

OC 27.2 | Elevated Mean Platelet Volume is Associated with Lower Risk of Death in a General Population, but is a Predictive Marker of Death in Patients with History of Cardiovascular Disease

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Background: Larger mean platelet volume (MPV) has been associated with adverse health outcomes. Yet, the majority of studies have addressed the role of MPV in high-risk populations or patients with cardiovascular disease (CVD).

Aims: To test the possible association of MPV with all-cause mortality in a large prospective population-based cohort study.

Methods: 17,402 adult subjects randomly recruited from a general population within the Moli-sani study (2005-2010), were analysed and subsequently subdivided in two subgroups (with or without CVD at baseline). MPV was measured within 3 hours from blood collection, that was performed in the same laboratory. The same operator used the same cell counter (Coulter HMX, Beckman Coulter, IL, Milan, Italy) for all subjects. Hazard ratios were calculated by multivariable Cox-proportional hazard models.

Results: Over a median follow up of 8 years (137,547 person-years), 925 all-cause deaths occurred. In a multivariable model controlled for platelet and leukocyte counts, platelet distribution width and C-reactive protein, the highest MPV quintile (mean MPV=10.0 fL) was associated with 21% reduced risk of death (HR=0.79; 95% CI 0.64-0.98) as compared to the lowest one (mean MPV=7.4 fL). The inverse association appeared even stronger in the subgroup without CVD at baseline (HR=0.64; 95% CI 0.50-0.81). In contrast, within 920 subjects reporting a previous CVD event, increased MPV was associated with higher risk of death (HR=1.69; 95% CI 1.05-2.72 for highest *vs* lowest quintile; p for interaction=0.048). Similar results were obtained when a sample (n= 4,777) of the Moli-sani population was enrolled in a different laboratory, where MPV was measured by a different counter and a different operator.

Conclusions: Elevated MPV is associated with lower risk of death in subjects free from CVD, but appears to be a predictive marker of death in CVD patients. History of CVD is an effect modifier of the inverse association between MPV and all-cause death.

OC 27.3 | Acute Phase Serotonin-mediated Neutrophil Trafficking is Independent of Endothelial Adhesion Molecules

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Background: Acute phase neutrophil endothelial interactions are partly regulated by platelet-derived serotonin (5-HT), as mice deficient for tryptophan hydroxylase 1 (*Tph1*$^{-/-}$), the rate limiting enzyme for serotonin synthesis show attenuated neutrophil recruitment in inflammation.

Aims: Evaluation of serotonin mediated effects on endothelial cells (EC) and neutrophils *in vitro* and *in vivo* following endotoxic shock and myocardial ischemia reperfusion (I/R) injury.

Methods: HUVEC or isolated neutrophils were stimulated with 100µM 5-HT to assess cell adhesion under flow *in vitro*. Endotoxic shock in WT and *Tph1*$^{-/-}$ *mice* was induced by i.p. administration of LPS. 30 min LAD ligation was followed by 24 h of reperfusion. Endothelial adhesion molecule expression profile and neutrophil transmigration into the peritoneum and affected heart tissue was analyzed using flow cytometry and histology.

Results: 5-HT stimulation of HUVEC did not affect neutrophil adhesion, while pre-incubation of neutrophils with 5-HT increased neutrophil adhesion by 50% under flow. Neutrophil kinetics in blood of mice after endotoxic shock were similar in WT and *Tph1*$^{-/-}$ mice (2,4 vs. 2,7 x10^5 cells/mL; n.s.) whereas infiltrated peritoneal neutrophils were reduced by 40% in *Tph1*$^{-/-}$ mice. Flow cytometry revealed similar levels of ICAM, VCAM, E- and P-selectin expression on EC.

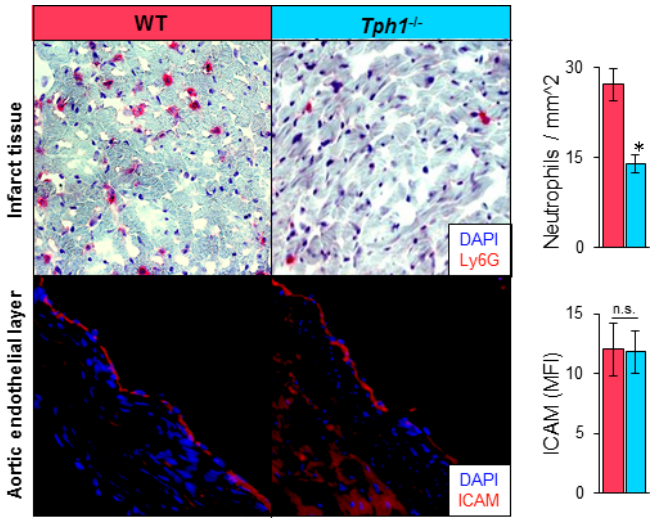

FIGURE 1 Top: Neutrophil infiltration in infarct tissue. Bottom: ICAM expression on aortic roots. 24 h I/R, students t-test *= p<0.05, n≤6/group

Tph1$^{-/-}$ mice show less neutrophil infiltration into infarct tissue after 24 h of myocardial I/R compared to WT (14 vs. 28 cells/mm^2 tissue; Fig. 1 top). Blood neutrophil levels were similar. Analysis of aortic root cross sections showed similar ICAM expression in both genotypes (Fig. 1 bottom).

Conclusions: Serotonin triggers neutrophil recruitment *in vitro* and transmigration *in vivo* during acute inflammation as it occurs upon bacterial infection or during reperfusion after myocardial infarction. In contrary to what was proposed in earlier studies, this effect seems to be mediated by direct stimulation of blood neutrophils by 5-HT and is independent of EC-derived adhesion molecules.

OC 27.4 | Circulating Microparticles in Cardiovascular Disease: Predictive Value in CABG Patency

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Background: Microvesicles (MV) are biomarkers of vascular injury and inflammation in several cardiovascular diseases (CVD) including atherothrombosis where elevated levels of MV are correlated with disease severity. Graft patency is one of the major determinant of long-term outcome following coronary artery bypass grafting (CABG). The issue of identifying predictors of graft patency has been addressed by several studies but none has assessed the potential involvement of MV.

Aims: To elucidate whether a specific signature of MV is associated with CABG occlusion.

Methods: MV analysis was carried out in platelet free plasma (PFP) collected from 179 patients the day before CABG (T0). After 18 months, a CT scan evaluation of graft patency was performed. The number of MV, their cell origin and the expression of platelet activation markers (Pselectin, CD40L, TF) were evaluated in PFP by flow cytometry. Thrombin generation was measured by CAT assay.

Results: Patent and occluded grafts were observed in 75% and in 25% of patients, respectively. Analysis of MV signature at T0 indicated that patients that would have had occluded bypass at follow up had higher number of MV derived from activated-platelet while no significant differences were observed in monocyte-, granulocyte- and endothelium-derived MV. Occluded patients had 5- and 3-times more TF$^+$ MV (p=0.0008) and AnnV$^+$ MV (p=0.05), respectively, compared to patent ones. Of interest, the MV-associated thrombin generation capacity significantly correlated with the number of TF$^+$/AnnV$^+$ MV (p=0.03). A reclassification analysis indicated that the inclusion of the number of TF$^+$/AnnV$^+$ MV to CVD risk factor model resulted in a significant improvement in predicting CABG occlusion (IDI=0.190, p=0.001).

Conclusions: These data show that patients that would have had occluded bypass-graft had a significant higher number of MVs compared to patients with patent graft. Moreover they provide the evidence that the signature of MV before CABG surgery has a predictive value of graft patency.

OC 27.5 | Proteomic Analysis of Premature Myocardial Infarction Subjects Indicates Impairment in ‚Hedge Hog' Signaling Protein DYNC2H1

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Manipal University/Thrombosis Research Institute, Proteomics and Coagulation Unit, Bangalore, India

Background: Premature Coronary Artery Disease (PCAD) is highly prevalent in Asian Indians and lacks association of most risk factors. The plasma proteomic profiling, along with network statistics holds enormous potential in the identification of novel biomarkers and also gain a better understanding of the diseases.

Aims: This study is aimed at assessing the differential proteomic profile associated with PCAD and identifying factors unique to the Asian Indians.

Methods: The global proteomic analysis was performed by using 2DE coupled with MALDI-TOF for protein identification. The differentially expressed proteins identified were used in the construction of protein-protein interaction network using STRING v10. Furthermore, all the proteins in the network were classified into CAD associated and non-CAD proteins. The average shortest path length between known CAD and non-CAD proteins was identified using Pesca v.3.0.8 (Cytoscape). The top 3 (5%) proteins were selected for ELISA based biological validation on (n= 40 CAD patients & n= 40 Controls). SVM analysis was used to prioritize the top protein and was validated on (n= 440 samples).

Results: We have identified 26 significantly differentially expressed proteins, of which 12 proteins (FN1, APOA1, ALB, FGG, FGB, C3, C4B, F2, HLA-DQB1, HP, FGA, ACTB) were known to be associated with CAD. Whereas 14 proteins (ACTN3, AKAP9, CD5L, DYNC2H1, ERVW-1, FAM190B, FER, GC, KDM2A, PLK3, SH3D21, SPATA8, TTR, ZNF624) were novel proteins with respect to CAD. Based on average shortest path length the top 3 proteins (DYNC2H1, PLK3, FER) were identified. The SVM analysis suggested that DYNC2H1 with the classification accuracy of 63.64% and AUC of 0.719 was a top scoring protein. The increased levels of DYNC2H1 showed 3.81 fold risk in CAD patients (95%CI; 2.27-6.38, p< 0.001).

Conclusions: These findings suggest that network-based shortest path analysis is useful in the omics-based novel biomarker identification. Our data also suggests that DYNC2H1 could be an important predictor of PCAD.

OC 41.1 | Role of Endothelial NF-κB Signalling in Atherosclerosis

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Background: Endothelial cell activation via the transcription factor NF-κB constitutes one of the first steps in atherogenesis, leading to the recruitment of leukocytes to the arterial wall. The central activator of NF-kB is IκB kinase 2 (IKK2), which phosphorylates its inhibitor, resulting in release of NF-κB and induction of genes involved in the development of atherosclerotic plaques and inflammatory responses.

Aims: We aim to study the role of endothelial IKK2 in the development of atherosclerosis to elucidate the underlying mechanisms linking endothelial inflammation and atherosclerosis.

Methods: We established a conditional transgene mouse model mimicking chronic endothelial inflammation by crossing mice containing an inducible, aortic endothelial-specific Cre recombinase with a strain bearing constitutive active IKK2 (caIKK2) downstream of a loxP-flanked stop cassette. CaIKK2-mice were crossed on an ApoE deficient background and fed a cholesterol-rich diet for 10 weeks to investigate the role of caIKK2 in the development of atherosclerosis.

Results: Aortic mRNA levels of the activation markers E-selectin, ICAM-1, and VCAM-1 were significantly upregulated after induction of caIKK2. Lymphocyte and granulocyte numbers were found to be significantly reduced in the circulation, with a parallel increase in aortic draining lymph nodes. To monitor potential changes in aortic gene expression due to inflamed endothelial cells, we performed RNA-sequencing of wildtype and caIKK2 aortas. Evaluation of the results with *Ingenuity Pathway Analysis* software revealed that the most upregulated pathways were associated with B- and T-cell signalling. Upon feeding the mice a cholesterol-rich diet for 10 weeks, increased atherosclerotic plaque areas could be observed in aortas from caIKK2 mice.

Conclusions: In summary, endothelial expression of caIKK2 led to endothelial cell activation resulting in an accelerated development of atherosclerosis, which was associated with increased infiltration of B and T cells.

OC 41.2 | Autophagy Is Required for Endothelial Cell Alignment and Atheroprotective Signaling Under Physiological Blood Flow

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Background: Atherosclerotic lesions usually develop in curvature or branch point of the vasculature due to low shear stress while high shear stress areas are devoid of plaques. Endothelial inflammation, apoptosis and senescence are involved but the mechanisms remain elusive. Autophagy is a protective mechanism allowing recycling of defective organelles and proteins to maintain cellular homeostasis.

Aims: Our aim was to understand the role of autophagy in atheroprotective effect of high shear stress.

Methods: We used parallel plate chamber in vitro to generate different shear stress conditions on endothelial cell deficient (shRNA Atg5) or not in autophagy. In vivo, we silenced autophagy in endothelial cell specifically using *Atg5$^{flox/flox}$; VE-cadherin-cre and Atg7$^{flox/flox}$; VE-cadherin-cre* mice.

Results: We first demonstrated in human and murine arteries that atheroprotective high shear stress activates endothelial autophagic flux. On the opposite, endothelial cells exposed to atheroprone low shear stress were characterized by inefficient autophagy. *In vitro*, low shear stress activated mTOR, inhibited AMPKα and blocked the autophagic flux contributing to the decrease in autophagy. Both in cultured endothelial cells and in transgenic mice, deficiency in endothelial autophagy was associated with a defect in endothelial cells alignment in flow direction, a hallmark of endothelial cell health. Cultured endothelial cells deficient in autophagy presented a senescent profile under high shear stress and displayed an enhanced inflammatory response when co-stimulated with TNFα. In transgenic mice, high shear stress areas of aortas harbored increased senescence and apoptosis. Finally, in transgenic hypercholesterolemic mice, deficiency in autophagy lead to increased plaque burden in areas usually athero-resistant.

Conclusions: Altogether, these results show that adequate endothelial autophagic flux under high shear stress limits atherosclerotic plaque formation by preventing inflammation, senescence and apoptosis.

OC 41.3 | Immune Regulation by Oral Tolerance Induces Alternative Activation of Macrophages and Reduces Markers of Plaque Vulnerability in Apobtm2Sgy/Ldlrtm1Her/J Mice

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Background: Atherosclerosis, a well-accepted autoimmune disease needs a novel therapeutic approach in restoring the tolerance and methods for stabilizing the vulnerable plaque. Our earlier studies have shown that an oral tolerance to recombinant molecule expressing three peptides derived from ApoB100, human HSP60 (hHSP60) and outer membrane protein of Chlamydia pneumonia (Cpn) (AHC) protective role in controlling atherosclerosis in mice.

Aims: To explore the effect of immune tolerance to established by AHC molecule in controlling advanced atherosclerosis and inducing plaque stabilization in ApoBtm2Sgy/Ldlrtm1Her/J mice.

Methods: Groups of ApoBtm2Sgy/Ldlrtm1Her/J mice were given a diet rich in cholesterol (HFD) for 10 weeks to establish atherosclerosis. Diseased mice, orally dosed for 5 times with AHC molecule or ova-albumin as a control on alternate days and HFD was continued for another 12 weeks before sacrificing.

Results: Tolerance was associated with significantly higher regulatory T cells both in aortic sinus and spleen with higher mRNA expression of CTLA4 (3 fold), Foxp3 (1.4 folds) and TGF-b (1.62) in the aorta. Tregs cells were found to induce alternate activation of macrophages to M2 phenotype (arginase1, IL-10, TGF-β) with a reduction in plaque inflammation (IL-23, iNOS, TNF-α). AHC treatment showed evidence of plaque stabilization as observed by the reduction in plaque necrosis in aortic sinus (35.8%) and in the brachiocephalic artery (26%), with reduced expression of Tissue factor and MMP9. Macrophage apoptosis was reduced and collagen content enhanced by treatment.

Conclusions: Our results suggest that tolerance to atherogenic peptides in a multi-antigenic construct increases regulatory T cells which activate M2 macrophages, prevent T cell proliferation and reduce plaque vulnerability and inflammatory markers thus providing evidence for plaque stabilization in mice with advanced atherosclerosis.

OC 41.4 | IL-33 Stimulates the Release of Procoagulant Microvesicles from Human Monocytes and Differentially Increases Tissue Factor in Human Monocyte Subsets

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Background: Monocytes and monocyte-derived MVs are the main source of circulating TF. An increase in monocyte TF expression and circulating procoagulant MVs contribute to a prothrombotic state in patients with cardiovascular disease. IL-33 is a pro-inflammatory cytokine involved in atherosclerosis and several other chronic inflammatory diseases, but its role in regulating thrombosis is still unclear.

Aims: In this study, we aimed to investigate the effects of IL-33 on the procoagulant properties of human monocytes and monocyte-derived MVs.

Methods: Protein levels were analyzed by WB, flow cytometry, or ELISA and gene expression by real-time PCR. The MVs were detected by flow cytometry and the procoagulant properties of monocytes and MVs were determined by a FX-activity assay.

Results: IL-33 induced a time- and concentration-dependent increase of TF mRNA and protein levels in isolated human monocytes via binding to the ST2-receptor and activation of the NF-kB-pathway. The IL-33 treated monocytes also released procoagulant CD14+TF+ MVs and IL-33 was found to increase the TF activity of both the isolated monocytes and of the monocyte-derived MVs.

The intermediate monocytes (IM) displayed the highest ST2-receptor expression, followed by non-classical monocytes (NCM), and classical monocytes (CM). IL-33 induced a significant increase of TF only in the IM ($p< 0.01$), with only a tendency detected in NCM ($p=0.06$) and no increase observed in CM.

We extended our data by measuring plasma levels of IL-33 and CD14+TF+ MVs in 20 patients with carotid artery stenosis undergoing carotid endarterectomy. Plasma IL33 positively correlated with CD14+TF+ MVs ($r=0.480$, $p=0.032$) but not with CD14+ MVs ($r=0.397$, $p=0.083$).

Conclusions: We hereby provide novel evidence that the proinflammatory cytokine IL-33 induces differential TF expression and activity in monocyte subsets and the release of procoagulant MVs both *ex* and *in vivo*. In this manner, IL-33 may contribute to the formation of a prothrombotic state in cardiovascular disorders.

OC 41.5 | Mir-146a from the Hematopoietic Compartment is Not Involved in Atherosclerosis Development Despite Provoking an Increased Inflammation and NET Formation

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Background: Atherosclerosis is a chronic inflammatory disorder in which monocytes and macrophages recruited in atherosclerotic plaques activate pro-inflammatory pathways. Mir146a negatively regulates the IRAK1/TRAF6/NF-kB pathway. Moreover, systemic overexpression of miR146a decreases inflammation and atherosclerosis. We recently described that the rs2431697 (TT) variant of *MIR146A*, associated with lower levels of miR-146a, predicts adverse cardiovascular events in patients with atrial fibrillation by promoting an increased inflammatory state.

Aims: To evaluate the role of miR-146a from the hematopoietic compartment in the development of atherosclerosis in a mouse model.

Methods: $LDLr^{-/-}$ mice irradiated with lethal γ-rays doses (N=30) were transplanted with bone marrow (BM) ($0.5e10^6$ cells/mouse) of wild-type (WT) or $miR146a^{-/-}$ mice. Animals were feeded by fat-rich diet with cholesterol. Plasma was obtained for chemistry, hematology, IL-6 (ELISA), DNA (sytox green), and elastase (ELISA) quantification. Expression in thoracic artery of miR-146a and its targets (*Irak1*, *Traf6*, *and Tlr4)* were measured by qRT-PCR. Plaque size was quantified in aortic arch by Oil-Red staining and in heart valves by immunohistochemistry.

Results: Hematological count and lipid profile were not different between groups, neither were atherosclerotic lesion sizes. However, IL-6 levels were higher in mice transplanted with$miR-146a^{-/-}$ BM (209.8±97.8 *vs*. 6.5±2.7 pg/mL; $p< 0.05$), as well as DNA (348 *vs* 177 ng/ml; $p< 0.05$) and elastase (229 vs 113 ng/ml; $p< 0.01$). *Irak1*, *Traf6* and *Tlr4* expression were also higher ($p< 0.05$) in mice transplanted with $miR-146a^{-/-}$BM.

Conclusions: miR-146a deficiency in the hematopoietic compartment is not sufficient to aggravate atherosclerosis despite increasing inflammation. Interestingly, we showed for the first time a potential role of miR-146a in NET formation. Future studies are necessary to unravel the mechanisms of this regulation and the pathophysiologic impact.

ISCIII y FEDER P14/00253; 19873/GERM/15; SEV-2015-0505

COAGULANT & ANTICOAGULANT MECHANISMS

OC 02.1 | Maintaining Extraembryonic Expression Allows Generation of Adult Mice with Tissue Factor Pathway Inhibitor Gene Disruption

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Background: The Kunitz1 domain of TFPI binds the active site of fVIIa and is required for inhibition of Tissue Factor/Factor VIIa activity. TFPI-Kunitz1 domain-deficient mice die *in utero*; the critical vascular site of pathology remains unknown. TFPI is highly expressed on trophoblast cells of the placenta. The interface of maternal blood and extraembryonic trophoblast cells is a potential target of pathological coagulation resulting in fetal growth restriction and death.

Aims: To examine if maintaining extraembryonic expression of TFPI allows *in utero* survival and generation of mice lacking TFPI Kunitz1 domain.

Methods: Mice with floxed K1 TFPI domain ($TFPI^{K1\ Lox/Lox}$) were crossed with an epiblast-specific Cre strain ($Meox2Cre^{tg/+}$) to generate $Meox2Cre^{tg/+}$ $TFPI^{K1\ Lox/Lox}$ experimental animals (Table 1). These animals delete the floxed TFPI K1 domain in the embryo while maintaining expression in trophoblast and primitive endoderm cells of the placenta. Embryos, placentae, pups and adult animals were observed for phenotypes and evaluated by real time PCR, western blot analysis and histology.

Results: Cre-Lox strategy resulted in expected Mendelian frequency of TFPI K1 domain-deficient embryos and pups
(Table 1). Real time genomic PCR and RT-PCR on embryos confirmed 95 to 99% deletion of TFPI K1 domain and a similar reduction in transcript expression. Western blotting confirmed the absence of full length protein in adult animals (Figure 1A). Most TFPI deficient animals (termed $TFPI^{K1\_Lo}$) exhibited a normal life span. Lowest expression (≤ 1%) correlated with non-specified acute illness between 3 to 5 months of age and presence of large ischemic regions in the brain (Figure 1B).

Conclusions: Severe deficiency of TFPI is compatible with normal mouse development, if extraembryonic expression is maintained. Brain ischemia is the most prominent phenotype of severely deficient animals. These results highlight a critical role of Tissue Factor/Factor VIIa inhibition by TFPI in extraembryonic circulation and in the brain.

TABLE 1 Results of genetic crosses showing expected Mendelian frequencies of live TFPIK1_Lo pups

Genetic Cross	Stage of Analysis	Genotypes Observed				#$TFPI^{K1\_Lo}$ Expected
		$TFPI^{K1\ Lox/+}$	$TFPI^{K1\ Lox/-}$	$TFPI^{K1+/-}$	$TFPI^{K1\_Lo}$	
Male $Meox2Cre^{tg/+}$ $TFPI^{K1+/-}$ X Female $TFPI^{K1\ Lox/Lox}$	Pups at 4 weeks	40	23	44	32	34.75

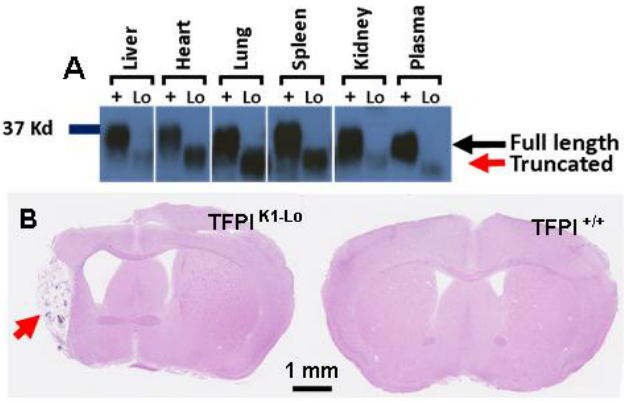

FIGURE 1 Brain ischemia (B) and absence of full length protein (A) in TFPI K1_Lo mice

OC 02.2 | Prostacyclin Prevents Deep Vein Thrombosis in COX-2 Knockout Mice

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Background: Venous thrombosis is a common condition that often leads to pulmonary thromboembolism and death. Cyclooxygenase (COX)-1 and -2, catalyzing prostanoids from arachidonic acid, play a critical role in the occurrence of thrombotic events.

Aims: To investigate the molecular mechanisms underlying venous thrombosis (VT) focusing on the impact of COX-2 deletion.

Methods: Thrombi were induced by inferior vena cava ligation (IVCL); thromboelastomethry, mass weight and histology and venous ultrasonography approaches were used to assess the effect of COX-2 ablation in mice ($COX\text{-}2^{-/-}$). Tissue factor (TF), Annexin A2 (ANXA2) and S100A10 were analysed in venous thrombi and in peritoneal macrophages (PM) isolated from WT and $COX\text{-}2^{-/-}$ mice.

Results: COX-2 deletion predisposes to VT as suggested by greater clot firmness and clot elasticity, by higher plasma levels of functional fibrinogen, factor VIII and PAI-1 activity, and proved by bigger thrombi detected after IVCL compared to WT mice. $COX\text{-}2^{-/-}$ thrombi have greater fibrin content, higher number of $F4/80^{+}$, TF^{+} and $ANXA2^{+}$ cells, and lower $S100A10^{+}$ cells. Remarkably, monocyte depletion reduced thrombus size in mutant mice, suggesting a key role of $COX\text{-}2^{-/-}$ monocytes in this experimental setting. Interestingly, COX-2 deletion increased ANXA2, reduced S100A10, promoted assembly of ANXA2/p50NF-kB complex and its nuclear accumulation, and induced TF in PM, whereas ANXA2 silencing decreased dramatically TF. Finally, Carbaprostacyclin treatment prevented VT formation in mutant mice, and reduced the ANXA2 binding to p50NF-kB subunit and its nuclear trafficking, and decreased TF in COX-2-/- PM.

Conclusions: The increased activation of haemostatic system observed in $COX\text{-}2^{-/-}$ mice may partly explain their predisposition to thrombosis. In addition, COX-2 deletion promotes macrophage TF activity by nuclear accumulation of ANXA2 sustaining venous thrombus growth, which suggests a new role for ANXA2 in venous thrombosis.

OC 02.3 | Deficiency of Platelet eNOS Produces a Pro-thrombotic Phenotype

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Background: Mice deficient of eNOS ($eNOS^{-/-}$) are characterized by endothelial dysfunction, hypertension and cardiac dysfunction. However, their thrombotic phenotype is controversial and it has not been fully explored.

Aims: Aim of our study was to clarify the role of NO, and in particular of platelet-derived NO, on arterial and venous thrombosis in mice.

Methods: Wild type (WT) mice made thrombocytopenic were transfused with highly-purified $eNOS^{-/-}$ platelets to generate chimeric mice selectively lacking platelet eNOS. Pulmonary thromboembolism (PT) was induced by i.v. collagen+epinephrine (coll+epi); arterial thrombosis by photochemical damage to the femoral artery; inferior vena cava thrombosis by the partial flow restriction method; platelet production of NO was analysed by DAF-FM DA; platelet activation by flow cytometry.

Results: Highly purified platelets from WT, but not from $eNOS^{-/-}$, mice release NO upon collagen-induced activation. Platelets from $eNOS^{-/-}$ mice show enhanced adhesion to collagen under high shear rate flow condition. I.v. coll+epi-induced in vivo platelet activation is significantly greater (38±4 vs 25±6% of P-Sel positive platelets, $p< 0.05$) and PT mortality is significantly increased in $eNOS^{-/-}$ than in WT mice. Femoral artery thrombosis occurs in a shorter time (7.7±0.3 min vs 11.9±0.7 min, $p< 0.05$) and with larger thrombi (0.59±0.02 mg vs 0.27±0.01 mg, $p< 0.05$) in $eNOS^{-/-}$ mice. Chimeric mice lacking eNOS only in platelets show a significantly shorter time to occlusion (8.2±0.4 min, $p< 0.05$ vs control). Inferior vena cava thrombus weight is enhanced in $eNOS^{-/-}$ mice (16.4±12.3mg vs 11±2.5mg, $p=0.02$).

Conclusions: Our data show that mouse platelets produce NO, that NO-released by platelets regulates platelet deposition on collagen, that endogenous NO exerts an antithrombotic activity in vivo both in the arterial and venous circulation and that platelet-derived NO contributes to this effect.

OC 02.4 | Amyloid Precursor Protein is a Regulator of Venous Thromboembolism in Mice

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Background: The amyloid precursor protein (APP), primarily known as the precursor of amyloid peptides that accumulate in the brain of Alzheimer's disease patients, is also an abundant platelet protein whose physiological function remains largely unknown.

Aims: In this work, we investigate the role of APP in hemostasis and thrombosis.

Methods: Platelet activation, blood coagulation, arterial and venous thrombosis were compared in APP knockout (APPKO) versus wild type (WT) mice.

Results: *Ex vivo* aggregation, secretion, and integrin aIIb3 inside-out activation induced by a wide panel of agonists were normal in APP-deficient platelets, indicating that this protein is dispensable for basic platelet functions. Nevertheless, the number of circulating platelets was reduced by about 20% in APPKO mice and their size was slightly increased. The absence of APP was associated to enhanced proplatelet formation by bone marrow megakaryocytes. Tail bleeding time was comparable in control and APPKO mice and, *in vivo*, the absence of APP did not alter thrombus formation in the femoral artery. By contrast, in a model of vein thrombosis induced by flow restriction in the inferior vena cava, APPKO mice developed much larger thrombi than control animals. Consistent with this, analysis of pulmonary thromboembolism revealed that larger vessels were occluded in APPKO mice. APPKO mice displayed an accelerated APTT but not PT time compared to WT mice, when measured in the presence of platelets. Moreover, the activity of factor XIa, but not factor XIIa, was higher in APPKO mice. APPKO mice presented a significantly higher number of circulating platelet-leukocyte aggregates and neutrophils displayed a constitutive stronger tendency to protrude extracellular traps.

Conclusions: These results indicate that platelet APP limits venous thromboembolism, through a negative regulation of both fibrin formation and neutrophil function.

OC 02.5 | Absence of Platelet Thrombin Receptor Par4 Prevents Thrombotic and Stroke-like Phenotypes of Thrombomodulin Deficient mice

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Background: Thrombomodulin (Thbd) is a multifunctional membrane glycoprotein with pleiotropic functions. It forms a high affinity complex with thrombin and suppresses excessive activation of blood clotting. Mice with Thbd deletion in the endothelium succumb to severe and fatal thrombosis at an early age.

Aims: To determine if absence of thrombin receptor, Par4, ameliorates thrombotic phenotype of endothelial-specific and global Thbd deficient mice.

Methods: We crossed Par4 null and Thbd floxed mice with endothelial- and epiblast-specific Cre strains (Tie2Cre and Meox2Cre respectively) to generate $Par4^{-/-}$ $Tie2Cre^{tg}$ $Thbd^{Lox/Lox}$ (comparison group $Par4^{+/+}$ $Tie2Cre^{tg}$ $Thbd^{Lox/Lox}$) and $Par4^{-/-}$ $Meox2Cre^{tg}$ $Thbd^{-/-}$ (comparison group $Par4^{+/+}$ $Meox2Cre^{tg}$ $Thbd^{-/-}$) mice in C57Bl6 genetic background. Animals were observed and characterized by PCR, immunohistochemistry and histology.

Results: $Tie2Cre^{tg}$ $Thbd^{Lox/Lox}$ mice recapitulated the previously described spontaneous and severe thrombosis seen in the extremities.

Some animals exhibited circling behavior without signs of limb injury, indicative of stroke. Epiblast restricted Thbd-deficiency resulted in live Meox2Cre^{tg} Thbd$^{-/-}$ embryos in normal Mendelian frequency at term, but these exhibited high neonatal lethality (~1/3rd surviving) (Table 1). About half of the pups that survived the first 3 weeks succumbed to severe tail and limb thrombosis or exhibited circling behavior by 8 to 10 weeks of age. The thrombotic and stroke-like phenotypes of Thbd-deficient animals were not observed in the absence of Par4 in both models, resulting in dramatically improved survival (Figure 1). In contrast to amelioration of thrombotic disease in adult animals, the absence of Par4 did not significantly improve survival of Thbd-deficient neonates (Table 1).

TABLE 1 Genetic distribution showing live endothelial and global Thbd-deficient pups at 3 weeks of age

Genetic Cross	Genotypes of pups				Expected Thbd$^{-/-}$	95% confidence interval for observed Thbd$^{-/-}$
	Thbd$^{Lox/+}$	Thbd$^{Lox/-}$	Meox2Cre^{tg} Thbd$^{+/-}$	Meox2Cre^{tg} Thbd$^{-/-}$		
Male Meox2Cre^{tg} Thbd$^{+/-}$ X Female Thbd$^{Lox/Lox}$	53	39	45	12	25%	4.2 - 13.7%
Male Par4$^{-/-}$ Meox2Cre^{tg} Thbd$^{+/-}$ X Female Par4$^{-/-}$ Thbd$^{Lox/Lox}$	23	24	28	10	25%	5.2 -18.5%
Male TieCre^{tg} Thbd$^{+/-}$ X Female Thbd$^{Lox/Lox}$	13	18	18	7	25%	5.2 - 24.1%
Male Par4$^{-/-}$ Tie2Cre^{tg} Thbd$^{+/-}$ X Female Par4$^{-/-}$ Thbd$^{Lox/Lox}$	6	8	4	7	25%	12.1- 49.4%

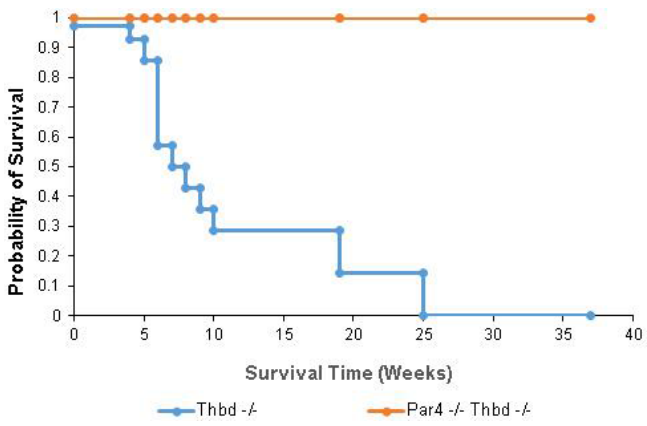

FIGURE 1 Kaplan-Meier plot comparing survival of Thbd-null weanlings in the presence or absence of Par4 expression

Conclusions: Global deficiency of Thbd results in thrombotic and stroke-like phenotypes similar to endothelial-specific gene deletion. Thrombin receptor Par4 plays a key role in mediating these phenotypes in both models.

OC 14.1 | Revisiting Antithrombotic Therapeutics; Sculptin a Novel Class of Specific, Competitive, Reversible, Scissile and Tight Binding Inhibitor of Thrombin

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Background: Thrombin is a multifunctional enzyme with a key role in the coagulation cascade. Its functional modulation can culminate into normal blood coagulation or disorders like thrombosis. Thus, the identification of novel potent and specific inhibitors of thrombin are of immense importance.

Aims: To characterize sculptin, a novel, reversible, potent and specific inhibitor of thrombin and its anti-thrombotic effect.

Methods: Sculptin was identified in transcriptomics profile of the salivary glands from tick *Amblyoma cajanensis*. Recombinant sculptin was investigated using bioinformatics, phylogenetic, mass spectrometry, inhibition and binding kinetics, global coagulation assays.

Results: Here we report, sculptin a novel class of direct thrombin inhibitor identified in transcriptomics profile of the salivary gland from tick. It consists of 168 residues with four exactly similar repeats of 34 amino acids and evolutionary diverged from classical hirudin. Sculptin is a specific and reversible inhibitor of thrombin with K_i of 18.5 ± 2.2 pM. It is slowly consumed by thrombin and loses its inhibition activity. Likewise, sculptin is hydrolyzed by factor Xa and each polypeptide fragment was able to inhibit thrombin independently. A single domain of sculptin alone retained ~45% of inhibitory activity, which we proposed to bind thrombin in a bivalent fashion. The formation of a small turn/ helical-like structure by active site binding residues of sculptin might have made it a more potent thrombin inhibitor than hirudin analogs. In addition, it prolongs coagulation via both extrinsic and intrinsic pathways.

Conclusions: Sculptin is a novel class of specific, competitive, reversible, scissile and tight binding inhibitor of thrombin. A single domain of sculptin is more potent thrombin inhibitor than hirudin analogs i.e. Bivalirudin. Taken together, our data allow us to conclude that sculptin and its independent domain(s) have strong potential to become a novel antithrombotic therapeutics.

OC 14.2 | Study of the Antithrombin Folding through the N-glycosylation

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Background: As a member of the serpin superfamily, antithrombin (AT) has propensity to polymerize during the folding process due to certain factors, although the mechanism is still unknown. AT is an N-glycoprotein and N-glycosylation is a post-translational modification that plays a key role in the folding of proteins.

Aims: To use the N-glycosylation as a tool to study the folding of AT.

Methods: Recombinant expression of AT variants created by mutagenesis. EndoH treatment, electrophoresis and western blot analysis of variants.

Results: Eighteen variants with one new N-glycosylation sequence in each one of the beta-strands were constructed-Table and Fig. Only 8 mutants became glycosylated and were secreted, but only 3 of them retained their anticoagulant activity. Two mutants were secreted at very low levels. All glycosylated mutants polymerized and the extra glycan was sensitive to EndoH, except in 2 cases. Two non-glycosylated mutants were also sensitive to EndoH. Polymerization and low secretion levels of P172N/D174T and L146T seemed to be caused by the mutation as no extra glycosylation was produced. Most of the mutations at A-sheet induced polymerization andat B-sheet clearly affected the secretion levels. Two out of 3 mutants at C-sheet were glycosylated, secreted and were functional.

Conclusions: Mutations creating new N-glycosylation sitesat strands s1A, final region of s2A, s5A, final region of s6A, s1B, s4B, and s3C were not glycosylated, suggesting that folding of these strands precedes N-glycosylation. Moreover, these mutations mostly impaired the inhibitory function of AT. For all mutations glycosylated, the extra glycan did not follow the proper maturation through the Golgi, affecting in some cases the accessibility of other glycans. Our data suggest that A and B-sheets are crucial for protein folding since most N-glycosylation mutations at these regions provoke polymerization or low secretion. These results broaden our knowledge on AT folding and may help to rationally design drugs to impair polymerization.

Mutants	Structure location	Extra glycan	EndoH sensitivity	Polymerization	Secretion level	Anti FIIa activity
P172N/D174T	Strand 1A (s1A)			XX	Low	
L146T	Strand 2A (s2A)			XX	Low	
S142N/N144T	Strand 2A (s2A)	YES	YES	XXX		YES
I219T	Strand 3A (s3A)	YES	YES	XXX		
A371N/L373T	Strand 5A (s5A)			X	Low	
E326N/G328T	Strand 6A (s6A)	YES	YES	XXX		YES
G328N	Strand 6A (s6A)					YES
R261N/V263T	Strand 1B (s1B)				Low	YES
Q268N/L270T	Strand 2B (s2B)	YES			Very Low	
V282N/I284T	Strand 3B (s3B)	YES	YES		Low	
V410N//I412T	Strand 4B (s4B)		YES		Low	
P407T	Strand 4B (s4B)				Very Low	
I420T	Begining of strand 5B (s5B)		YES	XXX		
M423N/R425T	Strand 5B (s5B)	YES	YES	XXXX	Low	
F77N	Strand 6B (s6B)	YES	YES	XXXX	Low	
M315N/V317T	Strand 2C (s2C)	YES	YES	XX	Very high	YES
Y253N/E255T	Strand 3C (s3C)					
K236N/L238T	Strand 4C (s4C)	YES	YES	X		YES

FIGURE 1 Mutations generated by site directed mutagenesis. The degree of polymerization has been indicated with an increasing number of "X"

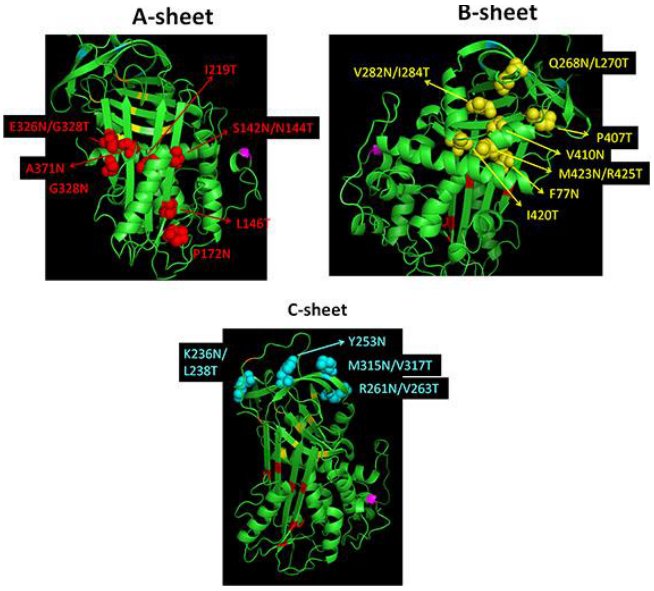

FIGURE 2 Structural representation of the mutations generated in the beta-strands of AT. Images were rendered with Pymol using PDB: 1t1fA as template

OC 14.3 | Palmitoylation of the Cytoplasmic Domain of Tissue Factor Regulates its Encryption, Mediated through Alteration in the Orientation of the Transmembrane Domain

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Background: The release of tissue factor (TF) maintains the balance of haemostasis. One enigma in the regulation of TF cofactor activity has been the ability of this protein to exist on the surface of the cells in encrypted form without triggering coagulation. As such, the de-encryption of TF leads to the availability of this protein as a coagulation

cofactor. Palmitoylation of TF was one of the earliest post-translational modifications that was reported and occurs on Cys245 within human TF.

Aims: In this study we examined the role of Cys245 and transmembrane domain of TF on the encryption of its activity.

Methods: The pCMV-Ac-TF-tGFP was mutated to express TF with Cys245→Ser and Cys245→Phe substitutions to prevent or mimic palmitoylation respectively. Additionally, the transmembrane domain was shortened by deleting Ser241-Leu242, or made inflexible by substituting Gly225 with Val. Endothelial cells devoid of exogenous TF were transfected to express the proteins and the cellular/cell-surface TF antigen, factor Xa generation, and factor VIIa binding potentials were assessed in resting cells and PAR2-activated cells. In addition, to prevent TF de-palmitoylation the expression of palmitoyl deacylase enzymes in the cells was suppressed.

Results: Substitution of Cys245 with Ser resulted in increased TF activity in resting cells while Phe-substitution of this residue resulted in rapid suppression of TF activity. Either shortening of the transmembrane domain, or replacing the flexible Gly225 also prevented the activation of the otherwise wild-type TF in cells. Finally, suppression of palmitoyl deacylase suppressed the TF activity following PAR2 activation.

Conclusions: Palmitoylation of TF incorporates the proximal boundary region of the cytoplasmic-transmembrane domain into the membrane altering the orientation of the transmembrane domain and altering the membrane span that may be accommodated. This in turn may prevent the incorporation of TF within lipid raft regions necessary for the increased activity of TF.

OC 14.4 | Characterization of the p.Arg393Cys Variant of Antithrombin. Extracellular Formation of Antithrombin-albumin Disulphide Linked Dimers through a Conformational Dependent Mechanism and Role in Thrombosis

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Background: Antithrombin (AT) is an anticoagulant serpin whose deficiency significantly increases the risk of thrombosis. The conformational sensitivity of this serpin explains why certain *SERPINC1* missense mutations are able to affect its structural stability with pathogenic consequences.

Aims: To characterize new pathogenic and prognosis effects of *SERPINC1* mutations associated with conformational changes of AT.

Methods: *SERPINC1* and plasma AT was studied by molecular and biochemical methods including proteomic and functional analysis of purified proteins in 2 members of a family with AT deficiency: a 20 year-old male with deep-vein thrombosis and pulmonary embolism and his asymptomatic mother.

Results: The *SERPINC1* p.Arg393Cys mutation affecting the P1 residue of the reactive center loop fully explained the type II RCL deficiency observed. This variant (AT Milano), allowed the interaction with albumin through disulphide bonds. However, analysis of plasma AT of carriers by native gels revealed 2 different variants with similar levels. Heat (42˚C) induced in plasma the transformation of the variant with faster mobility (mutant monomer) into the variant with slow mobility (dimer of mutant AT with albumin), as demonstrated by biochemical and proteomic analysis. The dimer, which was purified by heparin affinity and gel filtration, has no anticoagulant activity but higher heparin affinity than the wild-type molecule. Thus, formation of dimers impaired the anticoagulant capacity of carriers as they reduce the activity of wild-type molecules, particularly under low heparin concentrations.

Conclusions: The p.Arg393Cys mutation caused an AT variant with null anticoagulant activity that has conformational sensitivity, as demonstrated in P1 variants. Transformation to the latent conformation, allowed the formation in plasma of dimers with albumin through a disuphide bond, with dominant negative effect, that might exacerbate the risk of thrombosis under stress conditions.

Funding: PI15/00079;CB15/00055;SETH;19873/GERM/15;GATRA

OC 14.5 | Involvement of Heparanase Procoagulant Domain in Bleeding and Wound Healing

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Background: Heparanase, known to be involved in angiogenesis and metastasis, was shown to form a complex with tissue factor (TF) and to enhance the generation of factor Xa. Our study demonstrated that peptides derived from TF pathway inhibitor (TFPI)-2 impeded the procoagulant effect of heparanase and attenuated inflammation, tumor growth and vascularization.

Aims: The present study aimed to identify the procoagulant domain in the heparanase molecule and to evaluate its effects in a model of wound healing that involves inflammation and angiogenesis.

Methods: Twenty-four potential peptides derived from heparanase, were generated and their effect was studied in an assay of factor Xa generation. Peptides 14 and 16 that demonstrated the best procoagulant effect were studied in a bleeding mouse model and in a wound healing mouse model.

Results: Peptides 14 and 16 increased factor Xa levels by 2-3 fold and at high levels caused consumption coagulopathy. The TFPI-2 derived peptides explored in our previous study were found to inhibit the procoagulant effect induced by the peptides 14 and 16. In the bleeding model, time to clot formation was shortened by 50% when peptides 14 or 16 were topically applied or injected subcutaneously ($p<0.001$). In the wound healing model the wound became more vascular and its size was reduced to 1/5 compared to controls, upon one week of exposure to peptides 14 or 16 applied topically or injected subcutaneously ($p<0.001$).

Conclusions: The putative heparanase procoagulant domain was identified. Peptides derived from this domain significantly shortened bleeding time and enhanced wound healing.

OC 15.1 | Proteolytic Activity of Single-chain Prekallikrein

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Background: During the process of plasma contact activation factor XII (FXII) and prekallikrein (PK) undergo reciprocal activation, forming factor XIIa (FXIIa) and kallikrein (KLK). Recently, we showed that FXII in its single-chain precursor form exhibits proteolytic activity toward PK that is enhanced by polyanions such as polyphosphate. This activity could serve as a trigger for contact activation when FXII and PK are exposed to a surface.
Aims: To determine if single-chain PK has proteolytic activity toward its substrates FXII and high molecular weight kininogen (HK).
Methods: We prepared recombinant wild type PK (PK-WT), and PK variants with alanine replacing arginine at the activation cleavage site (PK-R371A) or serine in the catalytic active site (PK-S559A). PK-R371A cannot be converted to KLK by FXIIa, and is constrained in a single chain form. We also prepared FXII-WT, and a variant with alanine replacing the active site serine (FXII-S554A). We tested the ability of PK species to cleave FXII species or HK in the absence or presence of polyanions (polyphosphate or DNA).
Results: No PK species cleaved FXII-S544A in the absence of a polyanion, however, both PK-WT and PK-R371A cleaved FXII-S544A to a form representing FXIIa in the presence of polyphosphate. The PK-WT-mediated reaction was substantially faster than reactions with PK-R371A. PK-R371A, but not PK-S559A, increased the rate of FXII-WT "autoactivation" in the presence of DNA. Both PK-WT and PK-R371A cleave HK in reactions that are inhibited by the serpin C1-Inhibitor.
Conclusions: Similar to FXII and the fibrinolytic enzymes tPA and urokinase, PK exhibits proteolytic activity in its single-chain form and, therefore, is not a true zymogen. While the capacity of single chain PK-R371A to cleave FXII and HK is considerably weaker than that of KLK, the activity could be relevant for initiation of contact activation (when KLK levels are low), and to basal bradykinin generation through cleavage of HK.

OC 15.2 | The Proline Rich Domain of Factor XII Mediates Contact Activation

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Background: Factor XII is activated to its enzyme by plasma kallikrein or by its unique property to autoactivate when bound to negatively charged surfaces. The mechanism for autoactivation is not completely understood. New interest in this pathway has emerged by the recognition that several biologic substances support FXII autoactivation and due to the observation that FXII deficient ($FXII^{-/-}$) mice are protected from thrombosis in several murine models.
Aims: Determine the molecular domains responsible for FXII contact activation. We used a combination of recombinant FXII mutants, FXII domain-specific antibodies and murine arterial and venous thrombosis models.
Methods: We cloned and recombinantly expressed 19 FXII deletion mutants and studied their susceptibility to kaolin and polyphosphate-induced contact activation *in vitro*. We then reconstituted $FXII^{-/-}$ mice with FXII deletion mutants and challenged them in models of contact activation-mediated thrombosis.
Results: Each FXII deletion mutant was used to reconstitute FXII deficient human plasma. With the exception of the FXII mutant that lacked the proline-rich region, the remaining mutants normalized the prolonged aPTT and corrected thrombin generation to normal. Reconstituting $FXII^{-/-}$ mice with full length FXII restored the time to carotid artery occlusion induced by $FeCl_3$ and resulted in fatal pulmonary emboli (PE) in the collagen-epinephrine model. In contrast, $FXII^{-/-}$ mice reconstituted with the FXII variant lacking the proline-rich region exhibited significantly prolonged time to carotid artery occlusion and survived the fatal collagen-epinephrine PE challenge. We generated an antibody against the proline-rich domain and confirmed that it ameliorates thrombosis *in vitro* and *in vivo*.
Conclusions: The FXII proline-rich region mediates surface activation of zymogen FXII and its inhibition represents a novel therapeutic approach to interfere with thrombotic disorders.

OC 15.3 | Physiological Activation of Factor XII Occurs on the Platelet Surface in a Zinc-dependent Manner

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Background: Factor XII (FXII) inhibition could allow antithrombotic therapy with minimal risk of hemorrhage. FXIIa activity is typically measured in plasma assays following addition of negatively charged activators. However, the physiological mechanism of FXII activation during thrombosis is poorly understood.
Aims: To explore the relative contributions of endothelium and platelets to FXII activation during thrombus formation.
Methods: We used antibody X210-C01, a specific inhibitor of mouse and human FXIIa, in the mouse cremaster laser injury model in order to test the effect of FXIIa inhibition *in vivo*. Fluorogenic and chromogenic assays of thrombin and FXIIa generation were utilized in conjunction with platelet flow cytometry.

Results: Treatment with X210-C01 abolished both platelet accumulation and fibrin formation following laser injury of mouse arterioles. We next explored the differential contributions of platelets and endothelium to FXII-mediated thrombin generation. X210-C01 inhibited platelet-dependent thrombin generation in a dose-dependent fashion, while blocking tissue factor (TF) and factor VIIa (FVIIa) with specific antibodies had no effect. By contrast, thrombin generation by TNF-stimulated endothelium was unaffected by X210-C01 but required FVIIa and TF. Evaluation of the interaction between FXII and platelets by flow cytometry showed that FXII-FITC binds to stimulated platelets in a specific and saturable manner in the presence of Zn^{2+}. This effect was reversed by addition of cold FXII in molar excess and partially reversed by lactadherin, a specific inhibitor of phosphatidylserine. Both platelet-dependent thrombin and FXIIa generation were inhibited by the presence of CaEDTA, a specific zinc chelator, while CaEDTA had no impact on standardized clotting times.

Conclusions: Our findings demonstrate a Zn^{2+}-dependent, FXII-mediated pathway for thrombin generation on platelets that is distinct from fluid-phase coagulation as measured by clinical assays.

OC 15.4 | Neutralizing Blood-borne Polyphosphate In vivo Provides Safe Thromboprotection

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Background: Polyphosphate is an inorganic polymer that initiates fibrin formation by activating the factor XII-driven intrinsic pathway of coagulation. *E. coli* exopolyphosphatase (PPX) is a cytoplasmic polyphosphate that catalyzes the hydrolysis of intracellular polyphosphate.

Aims: We develop PPX-based recombinant specific inhibitors of polyphosphate and show that targeting blood-borne polyphosphates confers potent thromboprotection in a factor XII-dependent manner in human blood and murine thrombosis models.

Methods: We cloned and recombinantly expressed PPX mutants. We examined fibrin formation in plasma, procoagulant activity of activated platelets and interference with thrombus formation in flowing blood by targeting polyphosphate with PPX variants. Additionally, we tested the *in vivo* anticoagulant activities of PPX variants in murine thrombosis models.

Results: Using 14 PPX deletion mutants that systematically lack single and combination of PPX domains we found that full size PPX specifically degrades polyphosphate while a PPX variant lacking domains 1 and 2 (PPX_Δ12) binds to the polymer without degrading it. Both PPX and PPX_Δ12 interfered with polyphosphate-driven thrombin formation in human plasma. PPX and PPX_Δ12 interfered with polyphosphate-related procoagulant platelet activity in a factor XII-dependent manner, reduced fibrin accumulation and impeded thrombus formation in blood under flow. PPX and PPX_Δ12 *in vivo* infusions protected wild-type mice from arterial thrombosis and protect animals from activated platelet-induced venous pulmonary embolisms. Despite their potent thromboprotective activities targeting polyphosphates did not increase bleeding from injury sites and did not increase blood loss from wounds.

Conclusions: Our data identify the first selective polyphosphate inhibitors, indicate that polyphosphate drives thrombosis in vivo via factor XII activation and reveal that targeting polyphosphate represents a new approach for interference with thrombotic events without increased bleeding risk.

OC 15.5 | Essential role of High-molecular-Weight Kininogen in Endotoxemia

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Background: Endotoxemia causing systemic inflammation results from persistence of lipopolysaccharide (LPS) in circulation. Which host proteins in plasma exploited by LPS to form endotoxemia remains unknown. Studies have suggested that plasma contact activation system (CAS) is associated with endotoxemia, however, the role of each CAS component in endotoxemia has never been characterized.

Aims: To determine the role of the CAS component in the formation of endotoxemia.

Methods: We generated kininogen 1-knockout (*Kng1*$^{-/-}$) mice by disrupting exons 2 and 3 of *Kng1* gene. The mice lacking each CAS component and their littermate controls were intraperitoneally injected with LPS, followed by observation of survival and measurement of cytokines. Histological changes in organs were observed by H&E staining. Plasma LPS levels were measured by LAL assay. Surface plasmon resonance and LPS-conjugate pull-down assay were used to examine the binding capacity and site of HK with LPS. The dynamic binding of HK and its metabolites to LPS were analyzed in a BODIPY FL-LPS fluorescence assay.

Results: *Kng1*$^{-/-}$ mice were resistant to LPS-induced mortality with significant amelioration of inflammation and organ injury; however, mice lacking prekallikrein and Factor XII exhibited comparable survival rate with WT mice. Circulating LPS levels were significantly reduced in *Kng1*$^{-/-}$ mice. Replenishment of *Kng1*$^{-/-}$ mice with human HK recovered LPS levels and rendered them susceptible to LPS-induced mortality. HK bound to LPS via O-polysaccharide/core oligosaccharide portion. LPS induced HK cleavage to two chain form (HKa) containing heavy chain (HC) and light chain (LC). HKa and LC but not HC disaggregated LPS. LC bound to LPS with a Kd of 1.52 nM. The binding site in LC to LPS was localized at a DHG15 amino acids region of Domain 5 (D5).

A monoclonal antibody against D5 significantly reduced LPS-induced mortality and circulating LPS levels in wild-type mice.

Conclusions: HK, as a major LPS carrier in circulation, is essential for endotoxemic shock.

OC 16.1 | Expression Patterns of F8 Promoter-binding-TFs and F8 Promoter Methylation in Different Endothelial Cells

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Background: F8 deficiency causes hemophilia A. Main source of F8 production and secretion is the liver sinusoidal endothelial cells (HHSEC), whereas other endothelial cells also express and secrete F8 but to a less extent; this variability in expression could be caused by epigenetic regulation and/or by differential expression of transcription factors (TFs) binding to the promoter region.

Aims: To study epigenetic regulation and TFs binding sites in 1 kb upstream of F8-TSS.

Methods: Toward explaining the molecular reasons for differences in expression of F8 between different endothelial cells, we used TRANSFAC-database to identify potential-TF binding to the F8 promoter and determine their expression levels in adult and fetal HHSEC and in human umbilical vein endothelial cells (HUVEC) using Illumina human ht12-v4 arrays. Additionally, we analyzed methylation of the F8 promoter region using Illumina-EPIC methylation arrays.

Results: : F8 expression is highest in adult HHSEC, followed by fetal HHSEC than by HUVEC. We found 10 CpGs in the 1 kb upstream of F8-TSS, whose methylation patterns distinguish HUVECs from both fetal and adult HHSEC but with no significant separation power between adult and fetal HHSEC. Twenty TFs matrices were found to bind to the 1 kb region upstream of F8-TSS. These matrices represent unique 155 TFs having 247 probes. After background filtering, 78 out of 155 TFs remained in our dataset having in total 103 probes. We than identified differential expressed TFs that are specifically over expressed in F8 expressing cells. We found that 2 TFs were common between adult and fetal HHSEC against HUVEC. We also found 4 and 7 TFs specific for fetal HHSEC and adult HHSEC respectively in comparison to HUVECs.

Conclusions: In summary, we detected TFs predicted to bind the F8 promoter that are specifically expressed in adult HHSEC. These TFs could be linked to expression and thus secretion potential of F8 by HHSEC.

OC 16.2 | Different N-glycosylation of Factor VIII: Similarities and Differences of Plasma Derived and Recombinant Factor VIII Products

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Background: Neutralizing antibodies against FVIII are a major complication in current therapy for hemophilia A patients. Despite progress in explaining the unwanted immune responses, the cause for development of these antibodies remains unclear. Clinical evidence suggests that there are differences in terms of immunogenicity between plasma derived (pdFVIII) and recombinant FVIII (rFVIII) products, and among different rFVIII. These differences may be attributed to differences in protein glycosylation, which can affect clearance and antigen presentation of FVIII.

Aims: We performed a head-to-head N-glycan analysis of FVIII molecules found in pdFVIII and rFVIII products to identify potential differences and similarities between pdFVIII and rFVIII products.

Methods: N-glycosylation of one pdFVIII and six rFVIII products were analyzed. Each product was prepared by trypsin digestion followed-up with N-glycan release after PNGase F cleavage. The released N-glycans were purified and permethylated, and then analyzed by MALDI-TOF MS. The data were processed using Data explorer® software.

Results: Some N-Glycan epitopes are conserved in both rFVIII and pdFVIII (e.g. high mannose). For the first time, we report the presence of N-glycolylneuraminic acid (Neu5Gc) found on highly purified pdFVIII. rFVIII expressed in HEK cell lines contains glycan epitopes which are absent in pdFVIII (e.g. fucosylated LacdiNAc (LDNF). N-glycan capping with sialic acid differs among the products, and rFVIII expressed in HEK cell lines contain the lowest degree of terminal sialic acid (Table 1).

Conclusions: Plasma derived FVIII and rFVIII show many similarities in N-glycan profile and composition. Surprisingly, rFVIII proteins expressed in HEK cells show the greatest differences to pdFVIII, containing epitopes not detected on pdFVIII. Moreover, we identified Neu5Gc on pdFVIII; thus, this sugar cannot be considered a non-self-epitope found only on rFVIII proteins.

TABLE 1 Terminal N-glycan structure on rFVIII

Terminal glycan structures	rFVIII products						
	pdFVIII	A	B	C	D	E	F
n=1-6 High mannose	+	+	+	+	+	+	+
Blood group ABO	+	–	–	–	–	–	–
N-Acetylneuraminic acid (NANA)	+	+	+	+	+	+	+
N-Glycolylneuraminic acid (Neu5Gc)	+	+	+	+	–	–	+
Fucosylated LDN (LDNF)	–	–	–	–	+	+	–
N-glycan capping with sialic acid	71%	63%	73%	64%	42%	19%	69%

OC 16.3 | Factor VIII with 237 Amino Acids B-domain Has Extended Half-life in Mice

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Background: FVIII consists of a light chain (A3-C1-C2) non-covalently linked to a heterogeneous heavy chain (A1-A2-B). Miao *et al.* (Blood 2004) reported increased expression of FVIII with a 226 amino acid (aa) B-domain compared to B-domain deleted and full-length FVIII. This FVIII variant is therefore used in gene therapy studies (Ward, Blood 2011; McIntosh, Blood 2013).

Aims: To characterise FVIII comprising the 226 N-terminal aa fused to the 11 C-terminal aa of the endogenous B-domain (FVIII-237).

Methods: PK of FVIII-237 and FVIII with other lengths of the B-domain was evaluated in *F8*-KO mice. *In vivo* effect of FVIII-237 and FVIII with a 21 aa B-domain (FVIII-21, NovoEight®) were evaluated in a tail vein transection model in *F8*-KO mice. *In vitro* activity was measured in a chromogenic assay. Binding studies were performed using ELISA and surface plasmon resonance (SPR). U87-MG cell binding was quantified using ELISA.

Results: FVIII-237 had specific activity comparable to that of FVIII-21. FVIII-237 was equally effective in reducing bleeding in *F8*-KO mice as FVIII-21. FVIII-237 had a half-life of 10-12h compared to 7-8h for FVIII-21. FVIII with other lengths of the B-domain did not have further increased half-life. Binding studies did not identify any difference in affinity for VWF or a VWF fragment D'D3A1. Interestingly, cell binding was markedly reduced for FVIII-237 compared to FVIII-21. However, binding studies did not show any reduced affinity to low density lipoprotein receptor related protein 1 (LRP) or cluster II thereof, but the maximal binding capacity was reduced. Likewise, the affinity of FVIII-237 for phospholipids (25%PS/75%PC) was not reduced.

Conclusions: FVIII with a 237 aa B-domain had 1.5-fold increased half-life in *F8*-KO mice. This increased half-life appeared to be independent on affinity for VWF, LRP or phospholipids with 25% PS. However, cell surface binding was markedly reduced suggesting reduced interaction with other cellular components.

OC 16.4 | Altered Cleavage of Human Factor VIII at the B-domain and Acidic Region 3 Interface Enhances *in vitro* and *in vivo* Function

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Background: Furin plays a major role in the proteolytic cleavage of B-domain deleted human factor VIII (hFVIII-BDD), leading to a secretion predominantly in the heterodimer form. Our previous studies demonstrated that when the majority of the furin recognition site (1645-RHQR-1648) is deleted, BDD is cleaved exclusively between S1657 and D1658 in the a3 region.

Aims: Based on our previous results indicating the superiority of hFVIII furin deletion variants and canine FVIII (cFVIII), we hypothesized that modifications of the a3 cleavage site sequence to that of cFVIII (P1657/E1658), with or without furin deletions, may further result in enhanced efficacy.

Methods: hFVIII variants were purified or introduced into AAV8-TTRm-hFVIII to assess hemostatic function.

Results:

SDS-PAGE gel revealed that Δ3 and SP/DE had a 3-fold increase in single chain (SC) polypeptide compared to BDD, while Δ3-SP/DE was secreted almost entirely in the SC form similar to cFVIII (89%). While one-stage aPTT showed no significant difference, two-stage aPTT illustrated that Δ3, SP/DE, and Δ3-SP/DE had a 2-fold increase in activity compared to BDD. When hemophilia A (HA) mice were injected with recombinant protein (10μg/kg) before a tail clip assay, BDD reduced blood loss significantly compared to HA mice injected with PBS (426μL). At the same dose, Δ3, SP/DE, and Δ3-SP/DE were able to reduce blood loss further, comparable to wild-type (WT) mice (58μL). In the gene therapy setting (1e11 vg/mouse), Δ3-SP/DE exhibited a 2-fold increase in expression (97ng/mL) while SP/DE (23ng/mL) resulted in lower FVIII levels compared to BDD (39ng/mL). When these AAV-treated mice underwent a hemostatic tail-clip challenge, BDD decreased blood loss significantly (232μL) compared to HA mice (518μL). Interestingly, both SP/DE (39μL) and Δ3-SP/DE (49μL) further reduced blood loss, comparable to WT mice (31μL).

TABLE 1 Summary of In Vitro Data

Variant		Single Chain (%)	1-stage aPTT ($x10^3$ U/mg) (±SEM)	2-stage aPTT ($x10^3$ U/mg) (±SEM)	Tail Clip Blood Loss (μl) (±SEM)
BDD	hFVIII-BDD	23	10.5 (±0.7)	320.2 (±22.1)	201 (±22)
Δ3	hFVIII-del1645-1647	60	10.6 (±1.5)	673.7 (±17.8)	68 (±16)
SP/DE	hFVIII-S1657P/D1658E	69	12.7 (±0.9)	564.1 (±17.4)	92 (±29)
Δ3-SP/DE	hFVIII-del1645-1647-S1657P/D1658E	99	8.8 (±0.7)	692.0 (±14.0)	98 (±22)

Conclusions: These data suggest that these variants with varying degrees of intracellular processing have enhanced hemostatic function both *in vitro* and *in vivo*.

OC 16.5 | Probing Changes in Activated Factor VIII Upon Complex Formation with Activated Factor IX on Phospholipid Membranes

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Background: The crystal structures of factor VIII (FVIII) and electron microscopy structures of membrane-bound FVIII have provided new insight into the structure and function of this cofactor. The changes in activated FVIII(a) that occur upon assembly with activated factor IX (FIXa) on phospholipid membranes, however, remain unclear. Hydrogen-Deuterium eXchange Mass Spectrometry (HDX-MS) now allows for probing alterations in surface accessibility and/or secondary structure of amino acid regions in FVIIIa upon complex formation with FIXa on phospholipid vesicles.

Aims: To assess the molecular changes in FVIIIa upon assembly of the FVIIIa-FIXa complex on phospholipid membranes.

Methods: Thrombin-activated FVIII was incubated with phospholipid vesicles with or without FIXa in a buffer containing deuterated water. HDX-MS analysis was employed to identify regions in FVIIIa with an altered deuterium uptake upon addition of FIXa.

Results: HDX-MS analysis of FVIIIa in absence of FIXa revealed that residues along the A1-A2 and A2-A3 interface showed a marked increase in deuterium incorporation. This suggests that the A2 domain dissociates from FVIIIa. This finding is compatible with the observation that FVIIIa activity is dampened by spontaneous dissociation of the A2 domain. In presence of FIXa, this increased deuterium incorporation along the A domain interfaces was not observed. Instead, mainly region L631-Y636 revealed a reduced deuterium uptake implying that it may directly interact with FIXa. Site-directed mutagenesis confirmed that this region supports the enzymatic activity of FIXa. Intriguingly, the FVIII crystal structure by Shen et al. shows that region L631-Y636 is covered by the acidic a1 and a2 regions, which are flanked by thrombin cleavage sites.

Conclusions: We propose that activation of FVIII liberates region L631-Y636, which contributes to the stimulation of the enzymatic activity of FIXa.

OC 26.1 | A Novel Mouse Model of Carotid Artery Thrombosis for the Evaluation of Thrombolytic Therapies

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Background: Acute ischaemic stroke is a major cause of disability and death. Currently, only 10% of stroke patients are eligible for rtPA treatment, the only approved thrombolytic agent. The lack of other efficacious therapies is partly due to the paucity of suitable autologous clot-induced stroke models that allow the better understanding of the pathogenesis of stroke and provide insights to the development of effective therapies.

Aims: To develop a mouse model of stroke triggered by *in situ* large artery thrombosis that allows real-time monitoring of recanalization by thrombolysis, in combination with post-recovery functional deficit analysis.

Methods: We developed a mouse *in situ***C**arotid **A**rtery **T**hrombosis (iCAT) stroke model. Occlusive thrombi were created by electrolytic injury to the carotid artery, with transient contralateral carotid occlusion. Cerebral blood flow was monitored using laser speckle contrast imaging at occlusion and 24h post occlusion. The effect of thrombolytic agents on arterial thrombosis was monitored by carotid arterial blood flow for 60min post occlusion, and the impact on brain injury assessed by cerebral perfusion and infarction, and neurological deficits at 24hrs post occlusion.

Results: In our iCAT model, blood flow impairment correlated with brain infarction. These defects were closely associated with neurological deficits and mobility impairment, as analysed with modified Bederson's scoring and open field analysis methodology developed in-house (Samson *et al*., 2015). Intravenous administration of thrombolytic agents (rtPA or integrilin) post occlusion resulted in partial clot lysis and restoration of carotid blood flow in 30% of cases, mild reduction in cerebral infarction and a marked improvement in functional outcome.

Conclusions: This iCAT stroke model represents a new and clinically relevant tool to investigate thrombosis mediated stroke, and can provide multiparameter insights into the cerebral perfusion and functional effects of new thrombolytic agents for stroke.

OC 26.2 | Development of an Inducible Mouse Model for Factor X Deficiency

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Background: A complete absence of coagulation factor X (FX) seems incompatible with live, as no patients are known with a complete absence of FX activity. Indeed, inactivation of the *F10* gene in mice

results in about 50% embryonic death, while the remaining mice display neonatal death due to bleeding complications.

Aims: Our goal was to develop and characterize a viable model of inducible FX deficiency.

Methods: C57Bl/6 mice were created containing a *F10* gene with loxP sequences and the *Cre* gene under control of the murine Mx1-promoter (F10-loxP/Cre+). Deficiency is induced upon polyinosynic/polycytidicylic acid (pI:pC) treatment. F10-loxP/Cre- mice were used as control.

Results: Treatment with pI:pC left FX activity in control F10-loxP/Cre- mice unaffected, whereas it led to a severe FX deficiency in F10-loxP/Cre+ mice (< 5% in RVV-X activity assay). Despite this loss of FX activity and antigen, mice were viable and no spontaneous deaths were observed over a 10-month period. Nevertheless, internal hematomas were regularly observed in mice in post-mortem autopsies. Blood count was similar to that of control mice.

Hemostatic activity was analyzed following standardized transection of the caudal vein, which allows distinguishing between primary and secondary hemostasis. Overall, F10-loxP/Cre+ mice were characterized by a strongly increased blood loss over a 60-min period, compatible with the severe FX deficiency. However, whereas control and FVIII-deficient mice ceased bleeding within the first 3 minutes (primary hemostasis), a significant increase in blood loss was observed for F10-loxP/Cre+ mice during this period.

Conclusions: To conclude, we have generated a new viable model of inducible FX-deficiency. Initial analysis confirms the bleeding tendency upon inactivation of the FX gene. Our model will be useful to further study the role of FX in hemostasis, but also in other (patho)-physiological processes.

OC 26.3 | Gene-based FVIIa Prophylaxis Modulates the Spontaneous Bleeding Phenotype of Hemophilia A Rats

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Background: Gene-based continuous expression of FVIIa successfully treated canine hemophilia[1], however a dose-relationship in preventing spontaneous bleeds was not established in this large animal model. The hemophilia A (HA) rat model also exhibits spontaneous bleeds, and may be a suitable animal model to address this unresolved question.

Aims: To investigate how different circulating levels of ratFVIIa modulate the spontaneous bleeds in HA rats.

Methods: We introduced an intracellular protease cleavage site between the heavy and light chains of ratFVII, resulting in its secretion as ratFVIIa. Stable cell lines expressing ratFVIIa were used for recombinant protein purification. Liver-directed, adeno-associated viral vector (AAV8) mediated ratFVIIa gene transfer was used to obtain sustained expression of ratFVIIa in HA rats, thereby modeling FVIIa prophylaxis. Spontaneous bleeds in naïve and AAV-treated rats were recorded over 16 weeks. Transgene expression above baseline was monitored by ELISA. Spontaneous bleeds were treated on-demand with purified ratFVIIa.

Results: Recombinant ratFVIIa was hemostatic following on-demand administration in HA animals. Naïve HA rats (n=13) exhibited 22 bleeds (1.7 per rat). AAV-treated HA rats (n=34) exhibited 38 bleeds (1.1 per rat) in a ratFVIIa concentration ([ratFVIIa]) dependent manner, as assessed by proportion of bleeding rats at different mean [ratFVIIa] expression: 12/13 (92%) naïve HA rats bled compared to 12/15 (80%) and 9/19 (47%) rats with [ratFVIIa] < 500 or >500 ng/ml, respectively. No bleeds were observed at mean [ratFVIIa] >1250 ng/ml (n=7).

Conclusions: HA rats can be successfully treated with ratFVIIa on-demand. Importantly, using a gene-based FVIIa prophylaxis in HA rats, we determined for the first time that ratFVIIa circulating concentration >500 ng/ml above baseline can reduce spontaneous bleeds, while levels >1250ng/ml can essentially prevent them.

[1]Margaritis P, et al, Blood, 2009;113;3683-3689

This work was funded by Novo Nordisk A/S

OC 26.4 | Distinct Pathogenesis of Cancer-associated Venous Thrombosis Identifies Tissue Factor-phospholipid Interactions as Selective Antithrombotic Target in vivo

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Background: Pancreatic cancer patients are at high risk of developing venous thromboembolism, which is a leading cause of mortality in this population. How exactly malignant tumors promote deep venous thrombosis (DVT) remains unclear. Here, we addressed the thrombogenic action of microparticles (MP) shed by pancreatic tumor cells in a murine model of DVT.

Aims: It is unkown, if the mechanisms that trigger cancer-associated DVT are similar on a cellular and molecular level to those found in non-malignant thrombosis. Identification of differences in the pathophysiology of cancer-associated DVT could have important therapeutic implications, requiring specific pharmacological approaches distinct from those used to prevent or treat non-malignant thrombosis.

Methods: Flow reduction in the inferior vena cava was induced and thrombi were harvested after 48 hours in *IL4-R/Iba, low-hTF, and SELP*$^{-/-}$mice. MPs were isolated from FG, L3.6pl, and KCP pancreatic adenocarcinoma cell lines or healthy donors. Thrombus formation was visualized by intravital 2-photon microscopy. Mouse primary vein endothelial cells were used for cell culture.

Results: MP derived from pancreatic cancer cell lines caused excessive DVT compared to blood cell derived MP from healthy donors. Unlike blood MP, the prothrombotic activity of pancreatic cancer cell-derived MPs (pcMP) did not depend on platelets or myeloid leukocytes. Instead,

pcMP induced DVT depends on tissue factor delivered by tumor MP and released from host endothelial cells once they are in contact with pcMPs. During cancer-associated thrombosis, the association of TF with the phospholipids is essential and inhibition of this interaction attenuates cancer-associated DVT without affecting normal hemostasis.
Conclusions: Together, distinct prothrombotic pathways drive the pathogenesis of cancer-associated DVT compared to non-malignant DVT. Modulation of phospholipids could serve as a selective novel target for prevention of VTE in cancer patients.

OC 26.5 | Recombinant Destabilase-isopeptidase Stimulates Destruction of Preformed Cross-linked (Old) Thrombi in Rats

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Background: The problem of struggle with old (cross-linked) thrombi is one of the urgent problems of cardiovascular surgery. Proteolytic enzymes can lyse only fresh-formed thrombi. Cross-linkings, slowly formed by blood plasma trans-glutaminases (e.g. factor XIIIa), protect thrombi from proteinase-associated dissolution. The mechanism of cross-links degradation was discovered with the discovery of ε-(γ-Glu)-Lys-isopeptidase, destabilase of the medicinal leech.
Aims: We compared arterial isopeptidase thrombolytic activity of destabilase with proteinase thrombolytic activity of plasmin (streptokinase-activated).
Methods: The study was conducted with 27 male Sprague Dawley rats (weight 450-550 g, age 5 months). All animals were surged to provoke 10% FeCl3-induced arterial thrombosis in carotid artery. Groups in the study: 24h after the surgery animals of Group 1 were intravenously injected saline, Group 2 - streptokinase, Group 3 - destabilase, Group 4 - streptokinase & destabilase. 48h after surgery thrombi were removed, weighted and dissolved in 2% acetic acid.
Results: Streptokinase potentiated thrombolysis by 36% vs. saline. Destabilase administration decreased weight of arterial thrombi vs. saline- and streptokinase-treated rats by 74,6% and 59,3% respectively. Joint use of destabilase and streptokinase decreased weight of thrombi vs. saline and streptokinase by 80,8% and 69,1% respectively. Moreover, the quantity fibrin soluble in 2% acetic acid is higher in the destabilase-exposed thrombi than in any other.
Conclusions: Proteolytic mechanism of thrombolysis discovered by Sherry S.et al. (1954) is only one part, but not thrombolysis itself. The second part is isopeptidolysis, dissolving of cross-links, which appears on the late stage of thrombi formation. Degradation of isopeptide bonds by destabilase is a crucial parameter for stabilized fibrin lysis. It results in slow spontaneous transition of the old thrombi from solid to soluble state. Thus, Destabilase is able to perform proteolysis-independent thrombolysis.

OC 40.1 | Cleaved Kininogen as a Biomarker for Bradykinin Production

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Background: High molecular weight kininogen (HK) is the cofactor of the plasma contact system. HK deficiency prolongs clotting times in vitro, but not in vivo, suggesting that the physiological role of HK is unrelated to clotting. When plasma kallikrein (PK) cleaves HK, cleaved HK (cHK) and the inflammatory peptide bradykinin (BK) are generated (Fig 1). Patients with hereditary angioedema (HAE) experience recurrent tissue swelling attacks, in which BK is heavily implicated. Direct analysis of BK in plasma is challenging, but immunoblotting studies previously established that cleaved HK is a surrogate marker for BK release.
Aims: To develop an enzyme-linked immunosorbent assay (ELISA) method for cHK to facilitate clinical diagnostics and research.
Methods: Nanobodies against cHK were selected by phage-display. Samples were diluted in buffer containing protease inhibitors to prevent pre-analytical cHK generation and a polyanionic compound was used to improve assay sensitivity by increasing the avidity of the binding interaction. Proof-of-principle clinical validation studies were performed in HAE patient cohorts and analysed using 1-way ANOVA.

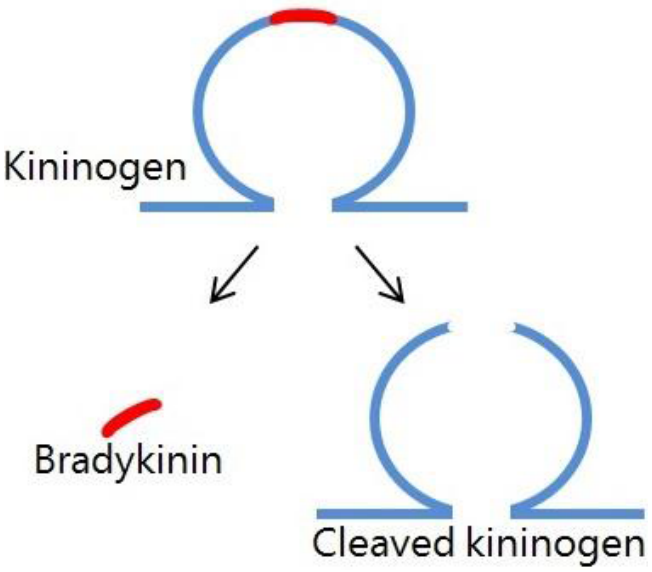

FIGURE 1 Schematic drawing of kininogen cleavage. Kallikrein cleavage of kininogen generates bradykinin and cleaved kininogen

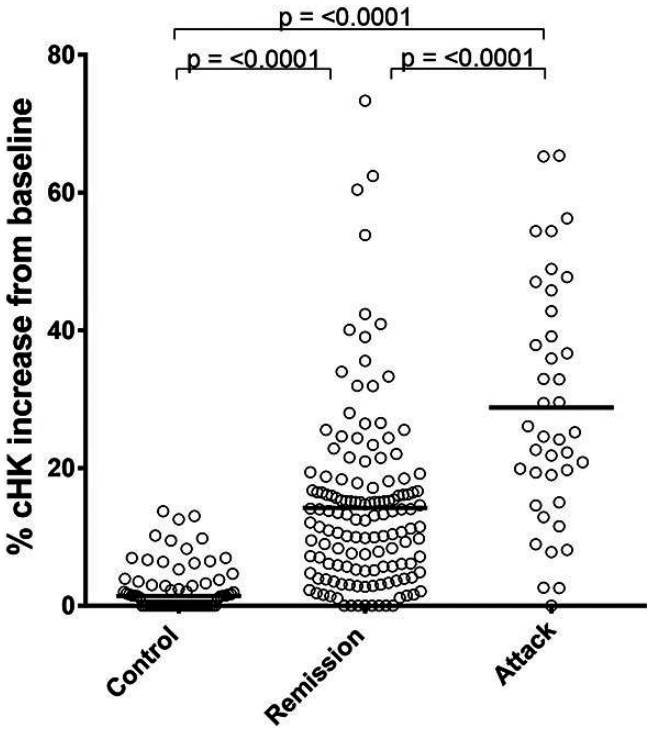

FIGURE 2 cHK levels are increased in HAE. ELISA detection of cHK, bars represent medians, circles samples measured

Results: We developed a nanobody specific for cHK. When we trigger PK activity in plasma, cHK becomes rapidly detectable and remains stable. By comparison, when plasmin activity is triggered in plasma, cHK is only transiently detected. We found that additional cleavage of cHK by plasmin eliminates nanobody recognition. Next, we went on to investigate the levels of cHK in plasma of HAE patient samples (n=178) and controls (n=68). Normal pooled plasma contains low levels of cHK (2-6%). These are significantly elevated in HAE patients that are in clinical remission (n=138) and further elevated during swelling attacks (n=40) (Fig 2).

Conclusions: We developed a detection method for cHK and clinically validated it. We propose that cHK constitutes a promising surrogate biomarker for BK release that should prove useful for investigation of BK-mediated pathology.

OC 40.2 | The Pharmacokinetic Profiles of Intravenously and Subcutaneously Administered Recombinant Factor IX Fc-XTEN in Cynomolgus Monkeys

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Background: Previously we have reported the engineering of rFIXFc-XTEN, a recombinant Factor IX monomeric Fc fusion protein, containing the R338L (Padua) variant for improved activity, combined with a 72 amino acid XTEN insertion in the cleavable activation domain (ASH2015/2016). rFIXFc-XTEN shows in vivo bleeding efficacy and improved pharmacological parameters for subcutaneous dosing in hemophilia B mice.

Aims: Determine the intravenous (IV) and subcutaneous (SC) pharmacokinetic and pharmacodynamic profiles of rFIXFc-XTEN in non-human primates.

Methods: Recombinant FIXFc-XTEN was produced in HEK293 cells and affinity purified. Three cynomolgus monkeys per cohort were dosed with rFIXFc-XTEN (100 IU/kg) by either intravenous or subcutaneous injection. Plasma FIX antigen and FIX activity levels were monitored by rFIXFc-XTEN specific ELISA and FIXFc-XTEN capture chromogenic activity assay, respectively. PK parameters were estimated using Phoenix WinnonLin software. In addition, pre- and post-dosing hematologic and coagulation parameters in blood were assayed.

Results: In cynomolgus monkeys, the IV dosed rFIXFc-XTEN showed a half -life of approximately 43 hours and an AUC/D of 0.301 (h*kg/mL). Subcutaneous dosing of rFIXFc-XTEN showed a prolonged half life of 69 hours and an AUC/D of 0.136 (h*kg/mL). The bioavailability was 45% and clearance 7.5 mL/hr/kg). No adverse effects were observed in this non human primate PK study as determined by various blood parameters.

Conclusions: These non-human primate PK data correlate well with previous findings in HemB mice and support the potential of once weekly or less frequent subcutaneous dosing of rFIXFc-XTEN in humans.

OC 40.3 | Activated FXI Regulates the Catalytic Activity of ADAMTS13 by Removing the CUB Domains

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Background: ADAMTS13 cleaves and inactivates von Willebrand factor (VWF), which binds collagen, facilitating platelet adhesion under vascular injury. But is still uncertain how ADAMTS13 activity is regulated. Thrombin and plasmin have been shown to cleave ADAMTS13. Based on the fact that elevated levels of FXI is an independent risk factor for deep vein thrombosis and ischemic stroke, we hypothesize that FXIa inactivates ADAMTS13 leading to platelet aggregation and thrombus formation.

Aims: To determine the functional role of inactivation of ADAMTS13 by FXIa.

Methods: Recombinant ADAMTS13 (250 nM) was incubated with FXIa (50 nM) for increasing times (0-3 hours) at 37°C before being analyzed by western blot using an anti-ADAMTS13 antibody against the two CUB domains (C-terminal) or against the metalloproteinase (MET) domain (N-terminal). ADAMTS13 activity was measured by a fluorogenic substrate (FRETS).

Results: Our results show that FXIa caused the disappearance of the ADAMTS13 band (~200 kDa) and the appearance of a band at ~150 kDa when the samples were analyzed with the anti-MET antibody

and a ~50 kDa band when the samples were analyzed with the anti-CUB antibody. The presence of aprotinin, which inhibits FXIa activity, blocked the degradation of ADAMTS13. Kallikrein or FXIIa were unable to cleave ADAMTS13. Using a cell surface immunoassay we observed that after incubation with FXIa, the detection of the CUB domain from ADAMTS13 was lost from endothelial cells surface. The incubation of ADAMTS13 with FXIa caused an increase in ADAMTS13 activity as measured by using FRETS.

Conclusions: ADAMTS13 circulates in a closed conformation, which is maintained by a CUB-spacer domain binding interaction. ADAMTS13 becomes conformationally activated through interaction of its CUBs domains with VWF. Here we show that FXIa-mediated deletion of ADAMTS13-CUB domains enhances its capacity to cleave FRETS and blocks the interaction with VWF. Our results suggest that FXIa may limit ADAMTS13-mediated VWF inactivation.

OC 40.4 | A Small-Molecule and Direct Inhibitor of Factor Xia Provides Strong Antithrombotic Activity with Low Bleeding in Non-Human Primate Model of Arterial Thrombosis

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Background: Factor XIa (FXIa) is a novel target for safer antithrombotic therapy. BMS-724296 (BMS) is a selective, reversible and direct inhibitor of human FXIa with Ki of 0.8 nM.

Aims: BMS has been studied in rabbits, but not in higher species. To strengthen FXIa as an antithrombotic target, we examined BMS in cynomolgus monkey models.

Methods: Arterial thrombosis was produced in the carotid artery by electrical stimulation in anesthetized monkeys. Hemostasis was assessed using a provoked kidney bleeding time (KBT) model. In vivo thrombosis, KBT as well as ex vivo clotting times were monitored in the same animal. BMS (0.025+0.05, 0.05+0.1, 0.102+0.2 and 0.4+0.8 (mg/kg+mg/kg/h)) (n=5-6 per group) or vehicle (n=8) were administered intravenously as a bolus dose plus infusion from 30 min prior to initiation of thrombosis until the end of the experiment. Thrombus weight reduction, KBT and clotting times were determined. Clotting times, including activated partial thromboplastin time (aPTT) and prothrombin time (PT), were measured.

Results: BMS at 0.025+0.05, 0.05+0.1, 0.102+0.2 and 0.4+0.8 mg/kg+mg/kg/h reduced thrombus weight by 0±0, 35±7*, 72±4* and 85±4%*, respectively (*P< 0.05, vs. vehicle). BMS at the top dose of 0.4+0.8 (mg/kg+mg/kg/h) did not increase KBT when compared to vehicle (109±6 sec vs. 111±23 sec in vehicle). BMS at 0.4+0.8 (mg/kg+mg/kg/h) increased ex vivo aPTT by 2.9±0.2-fold without changing PT (1.0±0.0-fold). In companion monkey studies, the standard of care antiplatelet agent clopidogrel at a clinically-equivalent dose (0.3 mg/kg/day given orally for 3 days) reduced thrombus weight by 49±6%, but increased KBT by 7.4±0.4-fold (756±44 sec vs. 103±5 sec in vehicle, n=6/group).

Conclusions: BMS produces strong antithrombotic activity with limited impact on hemostasis as compared to clopidogrel in monkeys. These results support that FXIa inhibition is a safer antithrombotic target, and a small-molecule FXIa inhibitor is currently under clinical investigation (ClinicalTrials.gov Identifier: NCT02902679).

OC 40.5 | New Implications of the Deleterious Role of Furin Processing on Factor VIII Biology

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Background: We recently reported that processing by furin has deleterious effects on B-domain deleted Factor VIII (FVIII-BDD) biological activity (Siner *et al* JCI Insight 2016). FVIII-BDD lacking the furin recognition motif (FVIII-ΔF) demonstrated 1) increased secretion from mammalian cells and transduced hepatocytes after liver-directed gene therapy (LDGT) in hemophilia A (HA) models and 2) increased pro-coagulant activity. These results were the opposite of what was previously assumed, but not tested, for decades. Based on this experience, we have now re-examined two related aspects of FVIII biology. First, we investigated the only reported HA causing missense mutation (R1645C) within the furin recognition motif. Second, since dicoumarol, which is structurally related to warfarin, is a known inhibitor of furin, we explored the role of dicoumarol and warfarin on FVIII levels.

Aims: To determine if R1645C changes the expression or specific activity of FVIII-BDD. To test if warfarin/dicoumarol increase FVIII levels due to furin inhibition.

Methods: We used mammalian cell culture and HA mice expressing FVIII variants after LDGT.

Results: FVIII-BDD-R1645C and FVIII-BDD exhibited comparable expression and specific activity, which it is unlikely to cause HA. We observed that the amount of FVIII-BDD secreted from BHK cells increased in a dose-dependent manner with increasing amounts of dicoumarol. The amount of circulating FVIII-BDD in mice fed warfarin decreased after warfarin was discontinued. The relative magnitude of this decrease was significantly larger in mice expressing FVIII-BDD than FVIII-ΔF. This result suggests that this decrease was partly due to the inhibitory effect of warfarin on furin being relieved.

Conclusions: Mutations in the furin site are unlikely to cause HA. Initial results support the hypothesis that warfarin inhibition of furin may be at least partially responsible for the transient increased FVIII levels in patients receiving warfarin and may not impose safety concerns after warfarin therapy.

OC 43.1 | A Non-circulating Pool of Factor XI

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[1]*Vanderbilt University School of Medicine, Pathology, Microbiology, and Immunology, Nashville, United States,* [2]*Tomsk Polytechnic University, Bioengineering and Organic Chemistry, Tomsk, Russian Federation,* [3]*Oregon Health & Science University, Biomedical Engineering, Portland, United States,* [4]*Oregon Health & Science University, Division of Hematology / Medical Oncology, Portland, United States*

Background: The homologous plasma proteins prekallikrein (PK) and factor XI (FXI) circulate as complexes with high molecular weight kininogen (HK). While it is clear that the PK-HK complex interacts with the vascular endothelium, there is conflicting information regarding HK-dependent or HK-independent FXI binding to endothelium.
Aims: To study the distribution of FXI in the vasculature.
Methods: C57Bl/6 wild-type (WT) mice, or FXI deficient ($FXI^{-/-}$) mice in which human FXI was expressed by hydrodynamic tail injection (HTI), received intravenous infusions of saline (0.9% NaCl), unfractionated heparin, polyphosphate (60-100 phosphate units), or an enzyme that digests glycosaminoglycans (GAGs - heparinase I/III or chondroitinase ABC). Blood was collected at intervals after infusion and plasma was analyzed by western blot for FXI, PK, HK, and factor XII (FXII).
Results: There was significant (>3-5 fold) increases in plasma FXI antigen in WT mice within five minutes of heparin or polyphosphate infusion, but not saline. There were similar increases 30 min after infusion of heparinase or chondroitinase. Levels of PK, FXII and HK did not change with any treatment. Treatment of whole blood ex vivo in a similar manner did not increase plasma FXI, indicating that the increase in FXI in mice is due to release of protein associated with the blood vessel. The releasable non-circulating FXI fraction could be reconstituted in $FXI^{-/-}$ mice by expression of human FXI using HTI. Immunohistochemical analyses of murine saphenous arteries with anti-mouse FXI IgG (14E11) showed FXI associated with the vessel endothelium in WT, but not $FXI^{-/-}$ mice.
Conclusions: Most of the FXI in the vasculature of mice appears to form a non-circulating pool associated non-covalently with blood vessel endothelium. The ability to release FXI with polyanions or by digestion of GAGs suggest that binding is mediated by the interaction between anion binding sites on FXI and endothelial cells surface GAGs.

OC 43.2 | Complex Formation with Pentraxin-2 Regulates Factor X Plasma Levels and Macrophage Interactions

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Background: Recently, we have identified scavenger receptor-AI (SR-AI) as a receptor for coagulation factor X (FX), mediating the formation of a FX-reservoir at the macrophage surface (Muczynski, Blood 2016 127:778). However, a third component was suspected to play a role in the formation of this complex as well.
Aims: We aimed to identify the third component that contributes to the formation of the FX-reservoir.
Methods: Protein- and cell-biological approaches were combined with animal experiments and analysis of human plasmas.
Results: We identified pentraxin-2 as a partner for FX and SR-AI. PTX2 prevents internalization of FX by SR-AI, while it also interferes with SR-AI-mediated internalization of PTX2. Binding studies reveal that FX, SR-AI and PTX2 independently bind to each other (Kd,app: 0.2-0.7 microM). Surprisingly, immune-precipitation experiments revealed that FX and PTX2 circulate as a complex in plasma, and complex formation involves the FX activation peptide. No binding of PTX2 to other vitamin K-dependent proteins was observed. shRNA-mediated inhibition of PTX2 levels in mice resulted not only in reduced levels of PTX2, but also in similarly reduced FX levels. Moreover, PTX2 and FX levels were correspondingly reduced in SR-AI-deficient mice. Analysis of 71 human plasma samples uncovered a strong correlation between FX and PTX2 plasma levels. Furthermore, plasma samples of patients with reduced FX levels (congenital/acquired FX deficiency or after anti-vitamin K treatment) were characterized by concomitantly decreased PTX2 levels.
Conclusions: In conclusion, we identified PTX2 as a novel partner for FX, and both proteins cooperate to prevent their SR-AI-mediated uptake by macrophages. Interestingly, their respective plasma levels are inter-dependent. These findings seem of relevance in perspective of ongoing clinical trials, in which plasma-depletion of PTX2 is used as a therapeutical approach in the management of systemic amyloidosis.

OC 43.3 | Role of the Thrombomodulin - Endothelial Protein C Receptor System in New Vessel Formation Following Ischemia

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Background: Coagulation proteases play crucial roles during the acute response to endothelial cell injury, chronic vascular healing processes and neovascularization. The role of coagulation signaling in angiogenic processes is less well understood.
Aims: To study the effect of coagulation receptors in ischemia-induced new vessel formation.
Methods: The mouse model of unilateral hindlimb ischemia (HLI) was used to induce new vessel formation and perfusion of the distal hindlimbs was monitored over four weeks using Laser Doppler perfusion imaging (LDPI). Gastrocnemius (GC) muscles of the ischemic and contralateral control hindlimb were analyzed.
Results: Reperfusion of the injured limb was increased in mice hypercoaguable due to a point mutation in the thrombomodulin (TM)

gene ($TM^{Pro/Pro}$ mice) at all time points examined (n=10; P< 0.05). CD31-positive endothelial cells and F4/80-positive macrophages were elevated already in uninjured $TM^{Pro/Pro}$ mice (P< 0.01; P< 0.05) and further increased following ischemia (P< 0.0001 and P< 0.05 respectively). Total cellularity and the number of PCNA-positive proliferating endothelial cells was also significantly upregulated in $TM^{Pro/Pro}$ animals compared to controls (P< 0.0001). Because $TM^{Pro/Pro}$ mice are deficient in thrombin binding and Protein C (PC) activation, we next analyzed mice with endothelial cell deletion of the PC signaling co-receptor endothelial PC receptor (EPCR). $EPCR^{flfl}$/Tie2.Cre mice (n=8) showed reduced hindlimb reperfusion following ischemia, indicating that increased thrombin generation rather than loss of PC signaling the likely cause for enhanced angiogenesis of $TM^{Pro/Pro}$ mice. Consistently, mice lacking the thrombin receptor protease activated receptor-1 (PAR1) showed reduced hindlimb re-perfusion under ischemic conditions (n=5; P< 0.001).

Conclusions: Our results indicate that increased thrombin-PAR1 signaling contributes to the pro-angiogenic phenotype of hyperthrombotic $TM^{Pro/Pro}$ mice.

OC 43.4 | Protective Function of Tissue Factor in Diabetic Nephropathy

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Background: Diabetic nephropathy (dNP) is considered to be an inflammatory disease. dNP is closely linked with coagulation activation, which is promoted by tissue factor (TF). TF promotes factor X activation (which aggravates dNP) and signalling *via* PAR2. In addition, TF may regulate cellular function *via* its cytoplasmic domain. Of note, deficiency of TF's cytoplasmic tail (TFCT) protects from experimental glomerulonephritis. Thus, TF may convey disruptive or protective effects in dNP.

Aims: To evaluate the role of TF in dNP.

Methods: First, we analysed expression of TF in control and diabetic (DM) mice (immunoblotting; immunohistochemistry). To evaluate the role of TF in podocytes, we depleted TF in podocytes ($TF^{loxP/loxP}$ x Pod^{cre+}) mice and induced persistent hyperglycemia by injecting streptozotocin in $TF^{loxP/loxP}$ x Pod^{cre+} and wild type (WT) mice. Glomerular injury was analysed by PAS staining, electron microscopy, immunohistochemistry, immunoblotting, and qPCR.

Results: In diabetic $TF^{loxP/loxP}$ x Pod^{cre+} mice albuminuria is increased compared to WT DM. Increased glomerular damage is supported by increased mesangial matrix accumulation, loss of podocytes (Wilms´ Tumor 1 - positive), and increased glomerular basement membrane thickness in diabetic $TF^{loxP/loxP}$ x pod^{cre+} mice. Interestingly, expressions of podocin and nephrin were not altered, suggesting that TF impairs podocyte function independent of these important regulators of podocyte function. Indeed, in dNP the interaction of ß1 integrin and TF was reduced while phosphorylation of TFCT was increased. Loss of TF in podocytes increased active ß catenin and expression of epithelial-mesenchymal transition markers.

Conclusions: Loss of podocyte TF-expression aggravates glomerulopathy in dNP. The current data suggest a critical role of TF in podocytes, which is required for normal podocyte function. Further studies have been initiated to determine the significance of TFCT and the TF-ß1 integrin interaction in podocytes in health and disease.

OC 43.5 | Factor XII Protects Neurons from Serum Deprivation-induced Apoptosis

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Background: Factor XII (FXII) is a serine protease that participates in the intrinsic coagulation pathway. Several studies have shown a deleterious role for plasmatic FXII in cerebral ischemia and traumatic brain injury. However, the impact of FXII on neuronal cell death remains unknown.

Aims: We investigated the role of FXII and its active counterpart (FXIIa) in neuronal apoptotic cell death.

Methods: We used a model of apoptotic death of murine primary neuronal cultures through serum deprivation.

Results: We observed that both FXII and FXIIa protect primary cortical neurons against apoptotic cell death induced by serum deprivation in a dose-response manner. This effect is blocked by corn trypsin inhibitor (CTI), an inhibitor of the FXII proteolytic domain, suggesting that the antiapoptotic effect of FXIIa is dependent on its proteolytic activity. As potential effectors of this anti-apoptotic effect, we focused on two members of the Erb family of receptors which are expressed by neurons and have been shown to interact with other serine proteases: EGFR and c-Met. EGFR is able to interact with other serine proteases presenting an EGF domain (such as FXII) and c-Met is known to display anti-apoptotic effect in neurons. Interestingly, the anti-apoptotic effect of FXII was blocked by an EGFR inhibitor (AG1478) and the anti-apoptotic effect of FXIIa by a blocking antibody against c-Met. Further experiments demonstrated that the anti-apoptotic effect of FXIIa can be blocked by an antibody against the main ligand for c-met, hepatocyte growth factor. This suggests that FXIIa activates the latent HGF secreted by stressed neurons, thereby leading to c-met activation and anti-apoptotic effects.

Conclusions: Altogether, our results demonstrate a potent anti-apoptotic effect of FXII and FXIIa involving EGFR, HGF and c-MET. Therefore, inhibition of FXII in neurological disorders may have deleterious effects by preventing its beneficial effects in neuronal survival.

OC 55.1 | Multi-ethnic Genome-wide Association Study Identifies New Loci Regulating Factor VIII and von Willebrand Factor

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Background: Factor VIII (FVIII) and its carrier protein von Willebrand factor (vWF) are key players in hemostasis. Plasma levels of these factors have been associated with risk of arterial and venous thrombosis and with hemorrhagic disorders.

Aims: To identify novel genes regulating plasma levels of FVIII and vWF.

Methods: We meta-analyzed genome-wide association results from up to 46,232 individuals of European, African, East Asian, and Hispanic ancestry. All studies performed linear regression analysis according to an additive model of inheritance between genome-wide 1000 Genomes imputed variants and natural log transformed phenotype levels, with adjustments for age, sex, and ancestry-informative principal components. Inverse variance weighted meta-analysis was performed within each ancestry group and then combined for a trans-ancestry meta-analysis. Additionally, in order to reveal genes of pleiotropic effect and increase analytical power, a joint analysis of FVIII and vWF was performed using the aSPU model. Finally, *in vitro* gene silencing in cultured endothelial cells was performed for the main candidate genes in order to elucidate the functional gene in each region.

Results: We identified significant associations with vWF at 8 known loci and 9 new loci, including *ST3GAL4*, *C2CD4B*, *TAB1/SYNGR1*, *PDHB*, *FCHO2*, *HLA-DGA1*, *GIMAP7*, *OR13C5*, and *DAB2IP*. Five known and 6 new loci were associated with FVIII, including *FCHO2*, *HLA-DQA1*, *TC2N*, *RPL3*, *SOX17/RP1*, and *LINC00583*. Of these, *SOX17/RP1* and *LINC00583* were uniquely associated with FVIII. Joint analyses of FVIII and vWF data identified 2 newly associated loci near *RAB5C* and *ARSA* genes.

Conclusions: This represents the largest meta-analysis to date to identify genetic determinants regulating FVIII and vWF plasma levels, and new associations were identified. *In vitro* functional follow-up preliminary data contribute to elucidate the role of these novel findings in regulating plasma levels.

OC 55.2 | Exome Enriched Genome-wide Association Analysis Identifies Novel Influence from The *ADCY2* Locus on Circulating Levels of Factor VII Activating Protein (FSAP) Activity

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Background: Factor VII activating protein (FSAP) plays a role in haemostasis and vascular remodeling. The minor allele of the G534E SNP (Marburg-1; MAF ~0.05 in Caucasians) in the FSAP encoding gene hyaluronan binding protein 2 (*HABP2*) is associated with reduced FSAP activity, and associations have also been reported with stroke, carotid stenosis, and liver fibrosis.

Aims: We sought to identify novel genetic determinants of circulating FSAP activity.

Methods: Plasma concentrations of FSAP activity were measured in samples from 3,260 subjects from the Sahlgrenska Academy Study on Ischemic stroke (SAHLSIS) and the Malmö Diet Cancer study (MDC). For stroke cases (n=600) samples were collected 3 months post-stroke. Genotypes from Illumina chips enriched with 240k exome variants were imputed using the UK10K reference. Genome-wide association scans were conducted to identify genetic variants associated with FSAP activity using age, sex, cohort, and case-control status as covariates. Gene-based tests were used to analyze directly typed rare variants. *Habp2* gene expression was evaluated in primary mouse hepatocytes.

Results: The strongest association was found at the *HABP2* locus with the known Marburg-1 variant as the lead SNP (P value=7.0×10^{-142}). Additional genome-wide significance was found at 5p15 with the lead SNP upstream of adenylate cyclase 2 (*ADCY2*) (rs35510613; P value=1.3×10^{-8}). *HABP2* was significant also in the rare variant analysis. *ADCY2* encodes a protein that catalyzes cAMP formation. Therefore, hepatocytes were treated with the cAMP modifiers, forskolin and 8-CPT-cAMP, and a significant increase in *Habp2* gene expression was observed.

Conclusions: In addition to the *HABP2* locus, the *ADCY2* locus was identified as a potential regulator of FSAP activity, and this finding is supported by *in vitro* data showing that cAMP modifiers influence *Habp2* gene expression. The identification of a novel regulator of FSAP expression will help to further understand the role of FSAP in diseases.

OC 55.3 | High Levels of Latent Antithrombin in Plasma from Patients with Antithrombin Deficiency. New Conformational Mutations and Pleiotropic Deficiency

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Background: Antithrombin (AT) is an anticoagulant serpin that efficiently inhibits multiple procoagulant proteases. The cost for the structural flexibility required for this function is the vulnerability to mutations that impact its folding pathway. Most conformational mutations identified in serpins cause polymerization. Only 3 mutations in *SERPINC1* affecting 2 residues have been found to favor the transformation to the latent conformation, another hyperstable non-anticoagulant form with strong antiangiogenic activity, which constitutes 3% of plasma AT in healthy subjects.

Aims: To identify mutations causing AT deficiency by favoring the transition to latent conformation.

Methods: 141 unrelated patients with congenital AT deficiency were evaluated by sequencing (Sanger and NGS), electrophoretic assays able to quantify latent AT in plasma and functional methods. Recombinant expression (HEK-EBNA) of selected mutants was also done.

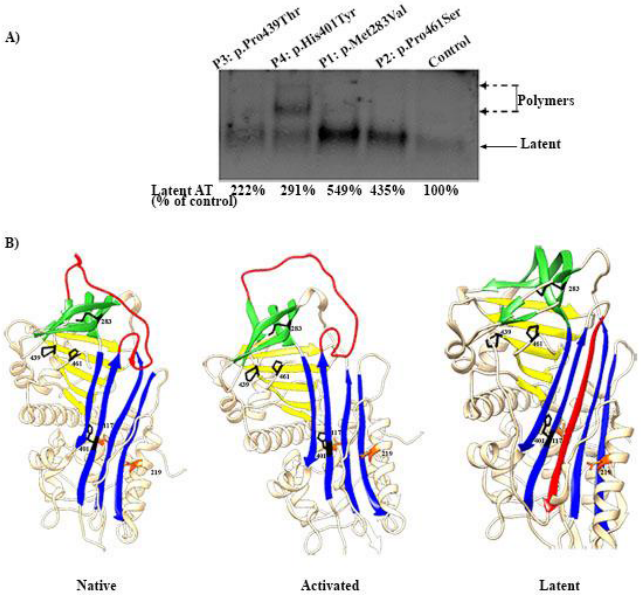

FIGURE 1 Variants associated with high latent levels. A)PAGE of plasma latent AT. B)Residues(black) whose variant favors latent AT(orange, yet described)

Results: 4 cases (2.8% of patients and 4.5% of mutations) had higher levels of latent AT than controls: p.Pro439Thr, p.Pro461Ser, p.Met283Val, and p.His401Tyr (Fig 1), the last also with circulating polymers.

Heating of plasma at 42°C exacerbated the transformation to latent conformation in p.Pro439Thr and p.Pro461Ser.

The conformational effect of p.Met283Val, the mutation associated with the highest levels of latent AT (689%), was verified in a recombinant model.

AT deficiency in these cases should be classified as pleiotropic based on the impaired reactivity and the low heparin affinity of the variant.

Despite high levels of latent AT (up to 80 µg/mL in p.Met283Val), no vascular defects were described in carriers of these mutations.

Conclusions: Our study identifies new residues involved in the structural stability of AT and potentially of other serpins. High levels of endogenous latent AT seem to play a minor antiangiogenic effect. Finally, pleiotropic deficiencies may be caused by mutations inducing transformation to the latent conformation.

PI15/00079;CB15/00055;SETH,19873/GERM/15;GATRA.

OC 55.4 | Spatio-temporal Analysis of Hemostasis by Immunofluorescence Microscopy Following Transection of the Murine Caudal Vein

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Background: Hemostasis is an orchestrated process aiming to minimize blood loss. Previously, microscopical analysis of skin biopsies from healthy persons and hemorrhagic patients provided a first insight into this process. However, novel microscopical techniques now allow us to visualize the involvement of individual proteins during clot formation that seals the vessel wall.

Aims: We analyzed sections of transected mouse tails via immunofluorescence microscopy using antibodies against proteins relevant for hemostasis.

Methods: Tail sections were prepared at different time-points (20sec-20min) after transection of the caudal vein in wt-, FVIII-deficient & VWF-deficient mice. Sections were stained with antibodies against fibrinogen/fibrin (Fn), VWF, platelet-integrin CD41 & the red blood-cell antigen Ter119. Analysis included widefield and confocal microscopy.

Results: Both VWF and platelets could be observed in tissues-sections taken 20 sec after the tail transection, underscoring their role in primary hemostasis. Surprisingly, Fn was also present, indicating that VWF-driven primary hemostasis and coagulation-dependent Fn formation act in parallel immediately following injury. In VWF-deficient mice (which do not stop bleeding), platelets were more dispersed within the wound, forming instable clumps seemingly attached to the Fn-network. Fn formation was delayed in FVIII-deficient mice, but seemed to reach normal levels within minutes compared to wt-mice. Unexpectedly, we observed a near complete co-localization between

VWF and Fn in both wt- and FVIII-deficient mice, pointing to a previously unrecognized, physiologically relevant interaction between VWF and Fn.

Conclusions: We used a standardized bleeding model to monitor the involvement of specific proteins in a spatio-temporal manner during clot formation. This approach reveals that primary hemostasis and the coagulation cascade proceed in parallel to limit blood loss, and two main components (VWF and Fn) co-localize in this process.

OC 55.5 | Water Channel Aquaporin-1 Regulates the Platelet Procoagulant Response and *in vivo* Thrombus Formation

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Background: We recently showed that the mechanism of platelet procoagulant membrane dynamics is dependent upon a coordinated system of fluid entry. We now hypothesise that the water channel aquaporin-1 (AQP1) is a molecular mediator of this event.

Aims: To establish the role of AQP1 in platelet procoagulant response and thrombosis *in vivo*.

Methods: Western blotting, immunocytochemistry and immunogold labelling, super-resolution microscopy, 4D live-platelet imaging and FLIM-FRET, thromboelastometry and $FeCl_3$ model of arterial thrombosis.

Results: We found AQP1 expression in human and mouse platelets was localised to internal tubular membrane structures, likely the open cannanicular system. Interactions between AQP1 and ERM proteins (Ezrin, Radixin, Moesin) were minimal, but the ablation of AQP1 significantly altered moesin phosphorylation. While membrane ballooning in response to fibrillar collagen was unaffected by AQP1 deletion, $AQP1^{-/-}$ platelets showed a marked reduction in procoagulant-spreading (64.1±2.3% vs 14.14±3.9%) and microvesiculation (356±58 vs 142±16/view) under this condition. Furthermore, haemostasis after tail bleeding remained unaffected, but thrombus formation *in vivo* was markedly suppressed by 79.3±8.8% in $AQP1^{-/-}$ mice after $FeCl_3$ injury to carotid arteries. In addition, *in vitro* platelet exchange experiments showed a platelet-specific delay in clotting time and clot formation time.

Conclusions: AQP1 is a key regulator of the platelet procoagulant response. Our data suggest AQP1 may modulate coagulation after injury or pathologic stimuli without affecting normal haemostasis, making it a suitable target for the control of thrombotic events. Thus, AQP1 inhibitors may represent a novel class of antiprocoagulant antithrombotics.

OC 71.1 | The Phosphate Transporter XPR1 Regulates Platelet Polyphosphate Metabolism

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Background: Platelet polyphosphate (polyP) is a procoagulant inorganic molecule that drives thrombosis. PolyP functions as storage pool for phosphate and intracellular levels of polyP and phosphate are linked in prokaryotes. Xenotropic and polytropic virus receptor 1 (XPR1) regulates phosphate homeostasis in mammalians.

Aims: We hypothesized that XPR1 regulates platelet polyP levels with implications for thrombosis.

Methods: PolyP levels and chain length were analyzed in cells including human megakaryocytes dependent on knockdown or overexpression of XPR1. We produced XPR1 gene-floxed mice and crossed them with animals expressing Cre recombinase under the control of the platelet factor 4 promoter to ablate XPR1 expression in platelets.

Results: Antibodies against the N- and C-terminal region of the protein detected XPR1 in membranes of human and mouse platelets. XPR1 overexpression dose-dependently significantly reduced polyP levels in HEK293 and MEG-01 cells, respectively. Vice versa, down-regulation of the transporter with XPR1-specific siRNA largely increased polyP levels. Modulation of XPR1 affected long chain but not short chain polyP. Based on the cellular data transgenic mice with platelet specific deletion of XPR1 will be phenotyped in platelet-mediated murine thrombosis models to unravel the role of XPR1 in thrombosis.

Conclusions: XPR1 expression modulates intracellular polyP levels and alters polyP size distribution with implications for thrombosis. Thus XPR1 is a promising anticoagulant drug target.

OC 71.2 | Lack of FXI on Top of Platelet Depletion Does Not Increase Gastrointestinal Bleeding in Mice

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Background: Gastrointestinal (GI) bleeding is one of the most prominent bleeding complications in patients at high risk for thrombotic events who are treated with anti-coagulants in combination with anti-platelet drugs. Therefore, better anti-coagulants showing strong anti-thrombotic potency without GI bleeding complications are needed. Factor XI (FXI) inhibition is regarded as potent and safe with respect to thrombosis prevention and acute bleeding. However, the effects of FXI depletion on GI bleeding especially in combination with platelet depletion have not yet been tested.

Aims: Therefore, our aim was to investigate the impact of FXI deficiency, platelet depletion, and a combination thereof on GI bleeding in mice.
Methods: For this purpose, we used wild type (WT) or FXI knockout mice (FXI-ko). To mimic platelet inhibition, the platelet depleting antibody R300 (0.3 mg/kg i.v.) was applied. GI bleeding was induced by oral treatment with 20 mg/kg indomethacin (IMC). To quantify GI blood loss hemoglobin (HB) levels were measured 24 hours after IMC application. Haemoccult tests of gastric content and feces were used to identify blood loss via gastrointestinal lesions.
Results: Upon GI bleeding induction with IMC, FXI-ko mice showed no additional HB decrease compared to WT mice (-10% HB and -9.1% HB, respectively). In WT mice, platelet depletion (>95%) dose-dependently promoted blood loss upon IMC application to up to 40% HB reduction. Even on top of platelet depletion, blood loss via GI bleeding was not increased in FXI-ko mice compared to WT mice.
Conclusions: To develop potent and safe new anticoagulants, we evaluated the effect of FXI inhibition in combination with platelet depletion in a newly developed GI bleeding model in mice. FXI inhibition did not show increased GI bleeding tendency, even on top of platelet depletion. Therefore, targeting FXI might represent a potent and safe new anti-thrombotic treatment regime.

OC 71.3 | Defining Differential Effects of Direct fXa *versus* fIIa Inhibition on Coagulation and Inflammation

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Background: Thrombin is the key protease in regard to thrombus formation. However, in regard to protease dependent signaling other coagulation proteases, including fXa, likewise convey cellular effects.
Aims: We postulate that inhibition of fXa or fIIa convey different effects in regard to inflammation despite comparable anticoagulant efficacy.
Methods: WT mice were treated with different doses of dabigatran (direct fIIa inhibitor) or rivaroxaban (direct fXa inhibitor) or PBS (control) for 1 week. We then conducted tail-bleeding assay, arterial injury induced thrombosis formation, or LAD ligation (ischemic/reperfusion) induced myocardial infarction.
Results: Using *in vivo* assays we determined doses of rivaroxaban and dabigatran conveying comparable effects in regard to bleeding and thrombosis. Using - in regard to hemostasis - equally potent dosages of fIIa and fXa inhibitors we analyzed the impact of direct fIIa and fXa inhibition on myocardial infarction. Direct fIIa and fXa inhibition resulted in comparable infarct size. However, while an anti-inflammatory was observed following direct fXa inhibition, this was not apparent following direct fIIa inhibition. Furthermore, fXa inhibition, but not fIIa inhibition, reduced expression of pro-inflammatory cytokines (IL-6, TNF-alpha), NF-κB activation, and macrophages infiltration in infarcted heart tissue. Mechanistically, fIIa inhibition, but not fXa inhibition, resulted in lower endogenous protein C (PC) activation. Importantly, the protective effects following fXa inhibition were lost following inhibition of endogenous aPC (mAb MPC1609).
Conclusions: Taken together, these results demonstrate that inhibition of fIIa and fXa can convey distinct anti-inflammatory effects despite equal anticoagulant efficacy. Following direct fXa inhibition sufficient thrombin to promote protein C activation appears to be available, resulting in preserved thrombin-dependent protein C activation and cytoprotection.

OC 71.4 | Characterization of Activated Protein C (APC) Antibodies: Preferential Inhibition of APC's Anticoagulant Activity over Cytoprotective Activity

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Background: APC is a major endogenous anticoagulant, making it an attractive target for hemophilia bypass therapy when factor replacement is not possible. APC also mediates beneficial cytoprotective activities that require endothelial protein C receptor-dependent activation of protease-activated receptor (PAR) 1. A complication for targeting APC is the deleterious effects associated with inhibition of its cytoprotective effects.
Aims: To characterize the selectivity of the humanized anti-APC antibody (Ab) 4885 and its parental murine Ab HAPC1573 for inhibition of APC's anticoagulant versus cytoprotective activities.
Methods: Anticoagulant activity was measured using APTT and thrombin generation (TG) assays and cytoprotective activity by PAR1 cleavage assays and endothelial barrier function using iCelligence.
Results: The Abs specifically bound APC (Kd 3-7 nM) and inhibited APC anticoagulant activity in APTT and TG assays (>95% inhibition at 1:4 APC:Ab molar ratio [mr]). Both Abs prevented APC-mediated degradation of FVIIIa (>90%) and FVa heavy chain (~86%) and protected FVa activity (~75%) in prothrombinase assays. APC-mediated PAR1 peptide cleavage at both Arg41 and Arg46 was inhibited by ~50%. Titration of Ab 4885 showed a reduction of APC-mediated protection against thrombin-induced endothelial permeability to a plateau of ~55% of normal at ≥1:15 mr APC:Ab. Nevertheless, PAR1 cleavage on EA.hy926 endothelial cells as measured by disappearance of the Arg46-sensitive ATAP2 epitope was not significantly reduced by either Abs at a 4-fold mr Ab:APC. APC barrier protection was also unaffected under these conditions. Thus, Ab 4885 has ≥10-fold selectivity for inhibition of APC anticoagulant versus barrier protective activities.
Conclusions: The APC anticoagulant pathway is activated disproportionally in hemophilia and APC may directly contribute to bleeding. We demonstrate that Ab 4885 and HAPC1573 are functionally selective for preferential inhibition of anticoagulant activity versus cytoprotective activities of APC.

OC 71.5 | Establishment of a Reporter Cell Line for the Functional Screening of Vitamin K Cycle Enzymes

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Background: Vitamin K-dependent (VKD) carboxylation is a post-translational modification that is essential for the function of coagulation factors. Defects in carboxylation cause mainly bleeding disorders. VKD carboxylation requires three membrane proteins: gamma-glutamyl carboxylase (GGCX), vitamin K epoxide reductase (VKOR), and vitamin K reductase (VKR). Although the genes encoding GGCX and VKOR are known, the identity of VKR remains elusive. This is mainly because traditional biochemical approaches for protein identification are unsuccessful for identifying membrane proteins.

Aims: The goal of this study is to establish a reporter cell line that can accommodate the genome-scale loss-of-function screening of vitamin K cycle enzymes.

Methods: We fused the gla domain of factor IX (FIXgla) and an HPC4 tag to a single transmembrane domain protein. We expressed the chimeric protein, which functions as a cell surface reporter protein, in mammalian cells, and used immuno-staining, fluorescence confocal imaging, and fluorescence-activated cell sorting (FACS) to distinguish the carboxylation status of the reporter cells.

Results: We cultured reporter cells with vitamin K or warfarin, and stained them with fluorescence labeled antibodies of anti-carboxylated FIXgla and HPC4 tag. Figure 1A shows that the chimeric reporter protein is properly expressed on the cell surface, and the two-color antibody staining distinguishes the cells' carboxylation status. Flow cytometry analysis shows that carboxylated or uncarboxylated cells can be clearly separated from the unstained cells (Figure 1B). Furthermore, when a mixture of carboxylated and uncarboxylated reporter cells were co-stained by these two fluorescence labeled antibodies, cells that lost their carboxylation function are clearly separated from the carboxylated cells.

Conclusions: Our results suggest that the established reporter cell line, in combination with FACS, is a powerful tool for high-throughput loss-of-function screening for enzymes of the vitamin K cycle.

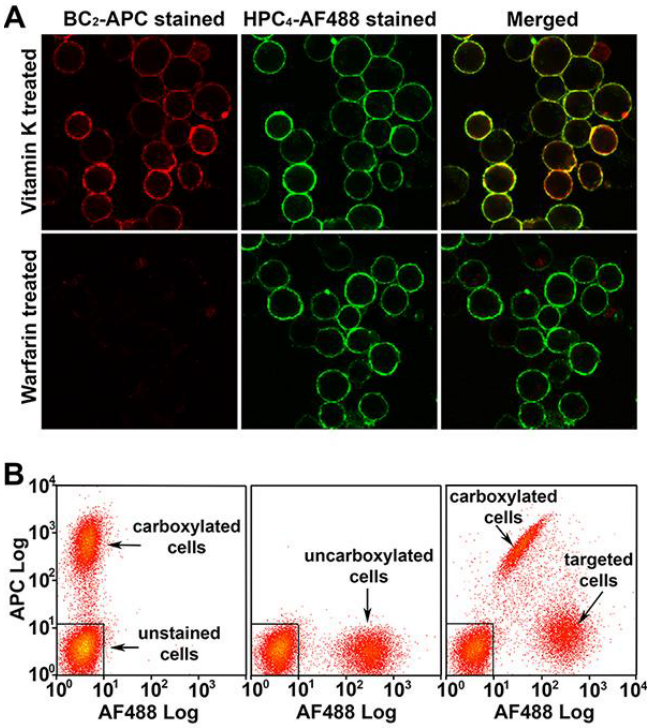

FIGURE 1 (A) Confocal images of carboxylated (top) and uncarboxylated (bottom) reporter cells. (B) FACS analysis of the immune-stained reporter cells

OC 72.1 | The Role of Fibrinogen's αC Regions in the Early Stages of Fibrin Polymerization

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Background: The C-terminal parts of fibrinogen's (Fg's) Aα chains called the αC regions are ≈400-residues long, mostly disordered and flexible. They are thought to mediate intermolecular interactions during fibrin formation, although many aspects of their structure and functions remain unknown.

Aims: To study the involvement of the Fg's αC regions in the early stages of fibrin formation and structural consequences of their presence or absence.

Methods: We used high-resolution atomic force microscopy to image Fg monomers and fibrin oligomers made from a plasma-purified full-length human Fg or a Fg sub-fraction I-9 lacking most of the αC regions. To correlate structural and functional data, we used concurrent dynamic turbiditimetry, confocal microscopy, and scanning electron microscopy.

Results: The incidence of the αC regions was 50% in the full-length Fg compared to 19% in Fg I-9. The residual αC regions in Fg I-9 were shorter than in the full-length Fg. More αC regions were seen in fibrin oligomers than in corresponding Fg monomers; but in the full-length Fg, unlike Fg I-9, the αC regions' length increased upon oligomerization, suggesting exposure and involvement of the αC regions. Dynamic turbidity correlated with confocal and scanning electron microscopy imaging of clots and revealed a significant effect of the αC regions on fibrin polymerization kinetics and the final clot structure. Specifically, in Fg I-9 an average lag time was longer than in full-length Fg and the rate of lateral aggregation was smaller indicating impaired protofibril formation and lateral aggregation. The lower maximum turbidity of I-9 clots corresponded to confocal and scanning electron microscopy data showing that fibrin fibers formed from Fg I-9 were thinner than those formed from the full-length Fg.

Conclusions: These results provide a direct structural basis for the functional role of the αC regions in the early stages of fibrin polymerization, with important implications for clot structure and thrombus properties in vivo.

OC 72.2 | The Role of Thrombin Exosites 1 and 2 in the Activation of Factor XI by Thrombin

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Background: There is mounting evidence that the contact pathway is important for thrombus stabilization and growth. Thrombin activation of factor (F) XI is one mechanism by which the contact pathway is bypassed in thrombosis.

Aims: To examine the role of the exosites of thrombin in activating FXI using exosite-binding aptamers and exosite-impaired thrombin variants.

Methods: FXI was incubating with α-thrombin, exosite-1-deficient thrombin (γ-thrombin), or exosite-2 mutant thrombin (RA-thrombin) for 30 minutes at 37°C and FXIa generation was monitored by chromogenic activity after hirudin addition. Studies were done in the absence or presence of dextran sulfate with molecular weights of 500,000 (DS-500) or 5,000 (DS-5) or exosite 1 or 2 directed DNA aptamers (HD1 and HD22, respectively).

Results: Titration of DS-500 in the FXI activation by α-thrombin resulted in a bell-shaped curve with the peak at 2 μg/ml. This suggests that DS serves as a template to promote FXI activation by formation of a ternary complex. Similar profiles were observed when activation was catalyzed by γ-thrombin and RA-thrombin, suggesting that either exosite can mediate ternary complex formation. The DS-5 titration using α-thrombin also showed a bell-shaped curve, however, γ-thrombin and RA-thrombin did not promote activation. This suggests that longer DS chains are required to mediate ternary complex formation. In the presence of DS-500, HD22 attenuates FXI activation by α-thrombin 2-fold more than HD1. This suggests that while both exosites are necessary in the activation of FXI, exosite 2 has a more significant role. In the presence of DS-5, HD22 attenuated FXI activation, whereas HD1 had only a modest effect.

Conclusions: Although either exosite of thrombin can mediate the ternary complex formation necessary for FXI activation by thrombin, exosite-2 appears to be more important. Involvement of the exosites is also dependent on the template length.

OC 72.3 | Clot-bound Thrombin Characterization of Plasma Clots in a Flow Reactor

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Background: Thrombin binds to fibrin via exosite mediated interactions, remaining a functional enzyme that stabilizes clots. The amount of thrombin incorporated in the clot is a function of both thrombin generation intensity and clot structure.

Aims: To characterize clot-bound thrombin activity in a plasma-based system under venous flow conditions.

Methods: Pooled citrated normal human plasma was diluted (1:4) with citrate containing buffer and clotting was initiated with either tissue factor or aPTT reagent under static conditions in the bottom of a flow chamber (50 mm x 7 mm x 0.25 mm; 87.5 μL). A fluorescent, thrombin-specific substrate (SN-59; 100 μM) containing buffer was flowed through the chamber (92 sec^{-1}) and the effluent collected dropwise into 2% SDS for 1000 sec. The fluorescence intensity of the effluent directly correlates with the amount of active/available clot-bound thrombin. In some experiments, antithrombin (0.5 μM; AT) and/or unfractionated heparin (0.5-4 U/mL; UFH) was included in the flowing solution. To induce hemophilia B, an inhibitory α-factor IX mAb (0.1 mg/mL) was incubated with the plasma.

Results: Fibrin-adhered thrombin formed under all conditions was stable for 1000 sec. UFH alone did not inhibit clot-bound thrombin while AT with UFH, inhibited 60-70% of thrombin activity. AT alone in the flowing solution decreased thrombin activity by 30%, suggesting the constant resupply of AT from the flowing phase can have a more rapid impact on clot-bound thrombin activity than expected from plasma based closed systems. Induced hemophilia B clots had 25% less clot-bound thrombin activity than normal clots, consistent with discordant fibrin structure and thrombin generation.

Conclusions: Under flow, the majority of thrombin activity associated with plasma clots is not derived from α_2-macroglobulin-thrombin complexes and is thus available to interact with macromolecular substrates. This pool of fibrin-associated thrombin appears to be more susceptible to AT inhibition than previously expected.

OC 72.4 | Thrombospondin 1 is Required for Fibrin Accumulation during Thrombus Formation

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Background: Thrombospondin 1 (TSP1) is a homo-trimeric matricellular glycoprotein previously implicated in the pathogenesis of arterial thrombus formation. While TSP1 is considered an important mediator of platelet activation, a role for TSP1 in the regulation of fibrin formation has yet to be established.

Aims: To determine whether TSP1 is required for fibrin accumulation during thrombus formation.

Methods: Laser-injury induced thrombus formation was assessed in cremaster muscle arterioles of TSP1 knockout mice or mice infused with an anti-TSP1 antibody (A6.1) by wide-field fluorescence microscopy. Accumulation of TSP1 at the site of vascular injury was assessed in the presence or absence of the GPIIb/IIIa antagonist eptifibatide.

Results: Supportive of a role in thrombus formation, TSP1 was found to accumulate at the site of vascular injury ($P < 0.0001$). Localization of TSP1 by confocal microscopy revealed accumulation predominantly

occurred at the interface of the platelet thrombus and vessel wall. The proximity of TSP1 to the vascular wall led us to investigate whether TSP1 could regulate fibrin generation at the site of injury. TSP1 knockout mice demonstrated impaired accumulation of fibrin (60%) and platelets (80%) after laser-induced injury (P< 0.05). Similar findings were obtained after infusion of the C-terminal binding anti-TSP1 antibody A6.1 (P< 0.05); confirming that TSP1 is required for the generation of fibrin *in vivo*. Treatment with eptifibatide, a selective platelet inhibitor, partially reduced TSP1 accumulation
(60%, P< 0.01) indicating that platelets, although a major source of injury resident TSP1, may not be the only cellular contributor.
Conclusions: These data suggest that fibrin accumulation occurs in a TSP1 dependent manner during thrombus formation.

OC 72.5 | A Critical Role of Factor XI Feedback Activation for Placental Hemostasis

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Background: Blood coagulation factor XI (FXI) can be activated by factor XII or thrombin. While factor XII-mediated FXI activation has a crucial role for thrombosis, a potential *in vivo* function of thrombin-driven FXI activation (feedback activation) has remained enigmatic.
Aims: We aimed to analyze the *in vivo* role of FXI-feedback activation.
Methods: To target the FXI-feedback loop, low tissue factor (TF) mice were bred with FXI- and FXII-null animals.
Results: We found that combined deficiency in TF and FXI was associated with embryonic lethality. In contrast, mice with combined deficiency in TF and factor XII were viable and did not differ from low TF animals. $FXI^{-/-}$/low TF embryos develop normally and were found within the expected Mendelian distribution at late gestation but suffered from morphological signs of degeneration. Bioinformatics of RNA-seq data from $FXI^{-/-}$/low TF embryonic tissues revealed defective thrombin signaling but normal vessel homeostasis in the placenta. Histological analyses of $FXI^{-/-}$/low TF placentas showed enlarged maternal blood spaces, disruptions in trophoblast cell layer and hemorrhage of maternal blood in the labyrinth, a pathology more severe than in placentas of low TF mice. Expression of FXI in pregnant mice prevented bleeding in $FXI^{-/-}$/low TF placentas and rescued the lethal phenotype of $FXI^{-/-}$/low TF mice.
Conclusions: Our study suggests a critical role for FXI feedback activation for placental hemostasis and development. The data identify an unexpected role for FXI in reproduction with possible implications for pharmacological targeting of the protease.

COAGULATION SIGNALING & IMMUNITY

OC 05.1 | The Lipid-raft Organizer Tetherin Negatively Regulates Platelet Receptor Function

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Background: Tetherin (also known as CD317 or BST2) is a lipid raft-associated transmembrane protein that regulates host response to viral infection by inhibiting viral particle release (1). This integral membrane protein, reported to play a role in lipid raft organization (2), is expressed in platelets although its physiological function is unknown. The function of a number of platelet receptors appears to be dependent upon their residence in lipid rafts although little is known about the mechanisms regulating their surface compartmentalisation.
Aims: To characterize the physiological relevance of tetherin in relation to platelet function.
Methods: Platelet receptor function was assessed in tetherin (-/-) and wild-type (WT) mouse platelets as previously described (3).
Results: Studies in tetherin (-/-) platelets established that this protein negatively regulates platelet aggregation downstream of a number of platelet receptors. CRP, and thrombin-stimulated platelet aggregation was significantly enhanced in tetherin (-/-) platelets with greatest effects evident at sub-maximal agonist concentrations. This effect was maintained in the presence of $P2Y_{12}R$ antagonists indicative of a more direct effect on GPVI and PAR4 receptor activity. Subsequent study established that reduced tetherin expression enhanced signalling downstream of GPVI and PAR4. Importantly there was no significant change in the surface expression of either GPVI or PAR4 as assessed by FACs. Initial studies in cell lines suggest that both GPVI and PAR4 receptor have the potential to interact with tetherin.
Conclusions: We demonstrate for the first time the physiological relevance of tetherin showing that it negatively regulates the function of a number of lipid raft associated platelet receptors.

OC 05.2 | bPAR-1 Protects Mice from Influenza A Virus Infection by Regulating Vascular Permeability and the Innate Immune Response

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Background: Protease-activated receptor 1 (PAR-1) is activated by a variety of proteases and exhibits biased signaling. Activation of PAR-1

on endothelial cells by high doses of thrombin increases permeability whereas activation by APC or low doses of thrombin decreases permeability. One study proposed that interaction of PAR-1 with EPCR promotes barrier protective signaling.

Aims: We investigated the role of PAR-1 in influenza A virus (IAV) infection of mice.

Methods: Wild-type (WT) and *Par1*$^{-/-}$ mice were infected intranasal with 0.02 HAU H1N1/PR8 virus. Survival, immune cell infiltration, inflammation and vascular permeability in the lung was analyzed up to 14 days after infection. We also determined the effect of deleting PAR-1 in different cell types, including lung epithelial cells (*Par1*$^{fl/fl}$,SPC-Cre), myeloid cells (*Par1*$^{fl/fl}$,LysM-Cre) or endothelial cells and hematopoietic cells (*Par1*$^{fl/fl}$,Tie2-Cre). *Par1*$^{fl/fl}$ served as controls.

Results: *Par1*$^{-/-}$ mice exhibited a lower survival rate (34.3%) compared to WT control mice (75.8%) after IAV infection ($P< 0.05$). This was associated with higher neutrophil counts and CXCL1 levels at day 3, and increased vascular permeability in the lungs at day 7 in *PAR1*$^{-/-}$ compared to WT mice. The survival rate of *Par1*$^{fl/fl}$,SPC-Cre mice (68.2%) after infection was not different to controls (73.3%). *Par1*$^{fl/fl}$,LysM-Cre mice had similar survival after infection compared to controls (65.0% vs. 68.2%). Interestingly, *Par1*$^{fl/fl}$,Tie2-Cre mice had lower survival rate (36.4%) compared to littermate control mice (66.7%) after infection, although it did not reach statistical significance (P=0.28). Importantly, only *Par1*$^{fl/fl}$, Tie2-Cre mice exhibited increased neutrophil numbers, CXCL1 levels and vascular permeability after IAV infection as observed in *Par1*$^{-/-}$ mice.

Conclusions: Our results suggest that activation of PAR-1 on the endothelium may limit the increased permeability caused by IAV infection. PAR-1 also contributed to the innate immune response to IAV infection.

OC 05.3 | PAR-1 Limits Coxsackievirus B3 Hepatitis in vivo and in vitro

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Background: Coxsackievirus B3 (CVB3) can cause severe myocarditis and hepatitis. Recently, we showed that protease-activated receptor 1 (PAR-1) enhances anti-viral responses and reduces CVB3 myocarditis. Also, CVB3 uses autophagy to increase its replication.

Aims: We investigated the role of PAR-1 on hepatocytes during CVB3 infection in innate immune responses, cellular infectivity and viral replication.

Methods: CVB3 hepatitis was analyzed in wild-type (WT), *Par1*$^{-/-}$ and mice with PAR-1 deficient hepatocytes (PAR1$^{fl/fl}$,Alb-Cre). PH5CH8 hepatocytes were used for in vitro experiments. PH5CH8 cells were stimulated with poly I:C or infected with CVB3 to analyze immune responses, virus adhesion, uptake, replication, and cytopathology with or without PAR-1 activation. Last, we analyzed different pathways to assess how PAR-1 affects virus life cycle.

Results: *Par1*$^{-/-}$ mice exhibited reduced innate immune responses and increased virus load in the liver after CVB3 infection. PAR1$^{fl/fl}$,Alb-Cre mice also showed an increased virus load compared to controls. In vitro experiments revealed that PAR-1 stimulation increased the poly I:C induction of interferon responses, and reduced uptake but not adhesion of CVB3 in PH5CH8 cells. Further, PAR-1 stimulation reduced CVB3 replication and cytopathology in PH5CH8. As expected, the CVB3 cytopathic effects were dependent on pathways associated with autophagy. PAR-1 stimulation enhanced CVB3-dependent p44/42 activation. Interestingly, treatment with PI3K inhibitors or the microtubule inhibitor increased whereas inhibition of Ca^{2+} or MEK1 signaling abolished PAR-1 mediated cytoprotection.

Conclusions: PAR-1 activation enhances TLR3-dependent innate immune responses, and reduces CVB3 uptake and replication in PH5CH8 hepatocytes. The observed PAR-1-mediated effects on the CVB3 life cycle were due to an enhancement of Ca^{2+} and MEK1-p44/42 signaling, and possible reduced PI3K activation, autophagy and microtubules rearrangement subsequently improving hepatocyte viability after CVB3 infection.

OC 05.4 | Protease Activated Receptor-1 (PAR-1) Impedes Tumor Progression by Promoting Tumor Cell Apoptosis

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Background: Thrombin-mediated activation of Protease Activated Receptor-1 (PAR-1) has been implicated as a mechanism coupling thrombin to tumor progression, particularly in the prostate and intestine. However, the role of PAR-1 in these contexts has never been directly evaluated.

Aims: To directly determine the role of PAR-1 in prostate and intestinal tumor progression

Methods: PAR-1$^{-/-}$ mice were interbred with TRAMP mice, which carry prostate epithelia-specific expression of SV40T, or APC$^{Min/+}$ mice, which carry a mutation in the adenomatous polyposis coli gene resulting in intestinal adenomatosis.

Results: TRAMP/PAR-1$^{-/-}$ mice evaluated at 30 weeks of age had dramatically larger prostate tumors than control mice. PAR-1$^{-/-}$ prostate tumors also demonstrated more frequent neuroendocrine (NE) differentiation, an established marker of advanced disease in this tumor model. To evaluate tumorigenesis prior to the NE switch, prostates were harvested from 12 week old mice. At this early time point the degree of dysplasia and carcinoma *in situ* was similar between genotypes, as was quantitation of prostate epithelial proliferation. However, transformed prostate epithelia from PAR-1$^{-/-}$ mice demonstrated significantly less evidence of apoptosis. Analyses of the role of PAR-1 in APC$^{Min/+}$ intestinal tumorigenesis paralleled these results. Quantitative analyses of the intestines revealed that

PAR-1 deficiency resulted in a 3-fold increase in intestinal adenomas. Markers of adenoma proliferation were similar between genotypes, but apoptosis was considerably decreased in PAR-1$^{-/-}$ adenomas.

Conclusions: These studies challenge the prevailing view that PAR-1 drives cancer progression. These studies reveal a surprising role for PAR-1 in *inhibiting* tumor progression in the context of spontaneously arising tumors in the prostate and intestinal epithelia. Here, loss of PAR-1 resulted in diminished tumor cell death, suggesting that PAR-1 signaling promotes apoptosis in certain contexts.

OC 05.5 | PAR2 Mutant Mice Provide New Insights into Protease Signaling Specificity in the Tumor Microenvironment

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Background: Tissue factor (TF) signaling through protease activated receptor (PAR) 2 plays a pivotal role in promoting tumor progression. TF-associated protease activate PAR2 in two distinct signaling complexes, i.e. the TF-FVIIa complex directly cleaving PAR2 and the TF-FVIIa-FXa coagulation initiation complex in which FXa cleaves PAR2 following recruitment of the endothelial protein C receptor (EPCR). It remains unclear which proteases in the tumor microenvironment (TME) contribute to PAR2-mediated tumor progression.

Aims: We generated PAR2 mutant mice that are resistant to cleavage by FXa (PAR2 G37I) or by all proteases (PAR2 R38E) to provide new insight into candidate coagulation proteases contributing to tumor development.

Methods: We analyzed tumor growth effects of PAR2 mutation with transplanted tumors and the PyMT model of spontaneous breast cancer. We characterized the composition of the TME by flow cytometry and mRNA expression profiling.

Results: Cleavage resistant PAR2 R38E mice recapitulated the delayed tumor progression and growth of PAR2-/- mice in the PyMT model, whereas tumor growth was selectively attenuated in FXa-resistant PAR2 G37I mice. Attenuated tumor growth was also seen with tumors transplanted onto PAR2 G37I mice, demonstrating that host PAR2 signaling promoted tumor progression. Immuno-suppressive myeloid-derived suppressor cells (MDSC) were reduced in PAR2 mutant mice relative to WT. We show that FVII and FX are expressed by tumor-associated macrophages (TAM) in advanced tumors. While overall numbers of TAM were unchanged in advanced tumors of PAR2 mutant mice, markers of alternative macrophage activation, including the neutrophil and MDSC attracting chemokines, were significantly reduced. Conversely, the tumor stroma of PAR2 mutant mice was characterized by an expansion of CD8$^+$ T cells of relevance for tumor surveillance.

Conclusions: These data provide evidence that FXa signaling through PAR2 modulates macrophage polarization and promotes tumor evasion through immune suppression.

OC 20.1 | Contributions of Complement-Dependent Membrane Perturbations to Myeloid Cell TF Activation in Venous Thrombosis

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Background: Genetic dispositions leading to hyperactive complement are known contributors to vascular and thromboembolic diseases, such as paroxysmal nocturnal hemoglobinuria and antiphospholipid syndrome. Although C5-inhibitors are being used to treat these diseases, the links between complement and hemostatic systems are poorly understood.

Aims: To evaluate in a venous thrombosis model the previously demonstrated contributions of PDI-catalyzed thiol-disulfide exchange and complement to TF activation and fibrin formation.

Methods: We characterized C3$^{-/-}$ and C5$^{-/-}$ mice in tail bleeding assay, in vitro platelet activation and the flow-restriction IVC thrombosis model. Since thrombosis is dependent on both leukocytes and platelets, we quantified vessel wall platelet and leukocyte interaction and fibrin formation at by intravital microscopy. We quantified leukocyte procoagulant phosphatidylserine (PS) exposure by imaging annexin 5 staining on GR1$^+$ leukocytes. To explore TF activation in human blood, we measured procoagulant activity in LPS-stimulated whole blood in vitro.

Results: C3$^{-/-}$ mice showed prolonged initial and total bleeding times, reduced thrombus size, fibrin and platelet deposition in the IVC model, and less platelet activation in vitro. Early fibrin formation at the vessel wall was dependent on PDI and TF expression by myeloid cells, but not NETs. In contrast, C5$^{-/-}$ mice had no apparent defect in initial hemostasis and vessel wall platelet deposition in vivo or platelet activation in vitro. However, fibrin formation, PS exposure on adherent leukocytes, and thrombus sizes measured 48h after induction of IVC were significantly reduced in C5$^{-/-}$ mice. In vitro LPS stimulation of human monocytes showed that TF procoagulant activity was markedly reduced with the PDI inhibitor Rutin or αC5 and αC7, but not αC9.

Conclusions: These data show that complement is crucial for leukocyte PS exposure and myeloid cell TF- and PDI-dependent fibrin formation, but C3 is specifically required for platelet activation.

OC 20.2 | Deep Vein Thrombosis Suppresses Peripheral T Cell Effector Function

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Background: Recent experimental studies shed light on the role of the immune system in the pathophysiology of venous thrombosis. However, data from human studies investigating the effects of deep vein thrombosis (DVT) on T cell effector function is currently limited.
Aims: To investigate the functional state of T lymphocytes in DVT patients compared to healthy subjects.
Methods: A total of 20 healthy individuals and 18 DVT patients were enrolled in a translational study, which comprised clinical and laboratory phenotyping. For immunological characterization, T cell surface marker expression and cytokine competence were assessed. The frequency of regulatory T cells was determined as CD4+CD25highIFN-gneg cells in stimulated samples. For the analysis of clinically-relevant subgroups, information on etiology, site and thrombotic burden of DVT were integrated.
Results: Overall, DVT patients showed significantly reduced production of IFN-g in CD4+ (DVT: 7.03±4.06 vs. controls: 12.68±6.29; p=0.002) and CD8+ cells (DVT: 19.03±10.82 vs. controls: 29.70±11.34; p=0.005) in comparison to controls. Reduced effector cytokine competence was associated with an increase in regulatory T cells (p=0.011) and C-reactive protein (CRP_{DVT}: 11.00 (4.18/25.67) vs. $CRP_{controls}$: 1.15 (0.48/4.76); p< 0.0001). The difference in IFN-g production was most pronounced for proximal DVT, provoked etiology, and high thrombotic burden (for all, p< 0.05) compared to healthy subjects. In linear regression analysis with adjustment for age, sex and CRP, an independent negative effect of DVT on IFN-g production (beta-estimate: -5.98, 95%CI -10.0/-1.96; p=0.006) was confirmed.
Conclusions: In this study, DVT was associated with lower peripheral T cell inflammatory competence and increased levels of inflammation and regulatory T cells. Significant T cell suppression was observed in provoked but not unprovoked DVT suggesting that alterations in T cell function may rather precede than result from thrombosis.

OC 20.3 | IFNγ Induces the Formation of NETs in Venous Thrombosis

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Background: Recent studies demonstrate that innate immune cells promote venous thrombosis through the activation of the coagulation cascade and platelets. Neutrophils contribute to venous thrombosis through the release of neutrophil extracellular traps (NETs) but the mechanism triggering their formation remain unknown. Recent data demonstrate that IFNγ induces the formation of NETs.
Aims: We hypothesize that IFNγ promotes venous thrombosis.
Methods: We used IFNγ, Tbet (the transcription factor regulating the expression of IFNγ) deficient (-/-) or wild type (WT) mice. Venous thrombosis was induced using the flow restriction model in the inferior vena cava, as has been previously published.
Results: Absence of Tbet or IFNγ decreases the formation of thrombi after venous thrombosis induction, suggesting that the Tbet+ /IFNγ producing cells are required for the early development of venous thrombosis. The number of NETs formed during thrombosis was significantly lower in Tbet-/- and IFNγ-/- mice. NET formation was also decreased in WT mice treated with an IFNγ blocking antibody. Injection of IFNγ in IFNγ-/- mice rescued the phenotype. Natural killer (NK) cells are the main producers of IFNγ. Thus, NK cells were specifically depleted with an antibody prior to venous thrombosis induction. NK cell depletion results in smaller thrombi suggesting that NK cells are required for thrombus development. Additionally, NK cell depletion results in decreased NET formation. In vitro, we show that WT neutrophils release fewer NETs when co-cultured with IFNγ-/- NK cells.
Conclusions: We demonstrate that IFNγ production is crucial for thrombus development by promoting the formation of NETs by neutrophils and that NK cells are key effector cells in this process.

OC 20.4 | Natural IgM Binding Oxidation-specific Epitopes Protect from Microvesicle-driven Thrombosis

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Background: Natural IgM are germline encoded antibodies that exhibit crucial homeostatic housekeeping functions. A large part of natural IgM has been found to bind oxidation-specific epitopes, such as malondialdehyde (MDA)-adducts. Microvesicles (MVs) are a subtype of extracellular vesicles and important mediators of coagulation. We have recently shown that 50% of circulating MVs are recognized by MDA-specific natural IgM antibodies.

Aims: To investigate whether MDA-specific natural IgM influence the procoagulatory properties of MVs.

Methods: To study the effect of the MDA-specific IgM LR04 on coagulation in vitro, rotational thromboelastometry (ROTEM), platelet aggregation, fibrin and thrombin generation (FG, TG) activated partial thromboplastin time (aPTT) and prothrombin time (PT) were used. Murine pulmonary embolism (PAE) and tail bleeding models were used to assess effects of LR04 in vivo.

Results: While LR04 caused a delay of all clotting parameters in ROTEM, it had no influence on washed platelet aggregation or aPTT and PT, which are MV-insensitive. In contrast, LR04 significantly delayed TG and FG in platelet poor MV-containing plasma. This effect was blocked by the P1 peptide, a mimotope of MDA that is bound by LR04. Neither a control IgM, nor an MDA-specific IgG, nor F(ab´)2 fragments of LR04 displayed anticoagulatory properties. LR04 also delayed MV-induced TG in factor VII and XII deficient plasma, indicating that the effect is independent of intrinsic or extrinsic activation. Importantly, in PAE, co-injection of LR04 significantly increased 30-minute survival of mice injected with thrombogenic MVs. On the other hand, injection of LR04 had no effect on tail bleeding.

Conclusions: Our study identifies a hitherto unknown protective role of MDA-specific natural IgM antibodies in MV-induced coagulation and thrombosis. These anti-thrombotic effects require the full IgM structure and are exerted on the common pathway of the coagulation cascade. Our findings may open new avenues in the prevention of thrombosis.

OC 20.5 | Hemodynamic Force Triggers NETosis within Sterile Thrombotic Occlusions

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Background: Neutrophil extracellular traps (NETs) are released by neutrophils encountering infectious pathogens, especially during sepsis. Additionally, NETosis occurs intravascularly during venous and arterial thrombosis, disseminated intravascular coagulation, trauma, and autoimmune vasculitis.

Aims: We tested if hemodynamic forces trigger NETosis during sterile thrombosis.

Methods: We imaged NETs with Sytox-Green during microfluidic perfusion of Factor XIIa-inhibited or thrombin-inhibited human whole blood over collagen (± tissue factor).

Results: For constant pressure-drop perfusions at initial inlet venous or arterial wall shear rates (100 or 1000 s^{-1}), platelets rapidly accumulated in the microchannels at either flow condition, however subsequent neutrophil infiltration with concomitant NETosis was detected only at the arterial condition. Shear-induced NETs (SINs) at 30 min were >150-fold greater at arterial conditions in the absence of thrombin and >80-fold greater in the presence of thrombin, relative to the venous condition. With or without thrombin, venous perfusion for 15 min generated no NETs, but an abrupt shift-up to arterial perfusion triggered NETosis within 2 min, eventually reaching levels by 30 min that were 60-fold greater than microchannels without perfusion up-shift. Aspirin inhibition of platelet deposition led to attenuated neutrophil accumulation at venous flow, but did not block SINs upon shift-up to arterial flow. SINs contained citrullinated histone H3 and were DNase-sensitive, but were not blocked by inhibitors of PSGL-1, CD18, or HMGB1.

Conclusions: The interstitial shear stress on clot-entrapped neutrophils is sufficient to drive NETosis. This is the first demonstration that hemodynamic forces trigger NETosis during sterile occlusive thrombosis.

OC 42.1 | Functional Characterization of Characterization of RIG-I Like Receptors (RLRs) in Megakaryocytes and Platelet

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Background: Beyond their central role in hemostasis, platelets are an important component of the innate immune system contributing to detection and effector responses to microbial pathogens through Toll-like and NOD-like receptors. Hemorrhagic viral infections are commonly associated with virus-induced thrombocytopenia. Apart from TLRs, viruses can be sensed by cytoplasmic RLRs.

Aims: We asked whether RLRs are present in megakaryocytes and platelets and how their activity contributes towards host responses during virus infections.

Methods: As a cell culture model, we used the megakaryocytic cell lines DAMI and Meg01. In addition, mouse megakaryocytes were isolated from bone marrow. Vesicular stomatitis virus (VSV), VSV-M51N and Sendai virus were used to infect cells. Differentiation of DAMI cell into polyploid megakaryocytes was determined by FACS analysis. Expression of interferon-stimulated genes (ISGs) was analyzed by RT-PCR and secretion of the cytokine was measured by ELISA.

Results: We could detect RIG-I and MDA5 along with other PRRs in DAMI, Meg01, and murine megakaryocytes. Stimulation of megakaryocytic cell lines with RLRs-specific agonists polyI:C and pppRNA, as well as viral infections, elevated secretion of IP10 and expression of ISGs indicating that RIG-I and MDA5 are functional in these cells. Unlike type-I interferon, Sendai virus did not inhibit the PMA-induced polyploidy in DAMI cells suggesting that viruses may not directly inhibit megakaryocyte differentiation. In vivo, treatment of mice with polyI:C showed an increased RIG-I and MDA5 expression in megakaryocytes and platelets.

Conclusions: Our results show that megakaryocytes do have functional RLRs and that they potentially participate in host innate immune responses against viruses. During viral infections, upregulated RLRs in megakaryocytes are transferred to their progeny, platelets. We hypothesize that platelets after acquiring RLRs from megakaryocytes are able to detect viruses and contribute to host responses during virus infections.

OC 42.2 | SERPINE2 Expression and Function in Neutrophils

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Background: Serpin E2 orProtease Nexin-1 (PN-1) is a tissue serpin, known as the most potent endogenous inhibitor of thrombin. PN-1 can regulate coagulation, tissue remodelling, cell behaviour by inhibiting proteases like coagulation factors or furin. Vascular cells, platelets and inflammatory cells express PN-1. However its potential role in regulating inflammatory processes is not known. PN-1 has been detected in monocytes/macrophages, but no data are available concerning its expression and potential function in neutrophils (PMNs).

Aims: We studied PN-1 expression in PMNs and its potential role in their inflammatory functions.

Methods: PMNs isolated from human blood or from Wild-Type (WT) or PN-1 Knock-Out (KO) mice bone marrow were activated by different agonists. Expressions of PN-1 and surface integrins were quantified and analysed by western blot and flow cytometry. The production of reactive oxygen species (ROS) as well as myeloperoxidase (MPO) and furin activities were quantified using specific fluorometric substrates. Vascular recruitment of PMNs was analysed by intravital microscopy in mesenteric vessels stimulated with leukotriene B4 (LTB4).

Results: We demonstrated the presence of PN-1 in PMNs in the cytosol, in the azurophilic granules as a degraded form and in the specific granules as a covalent complex. PN-1 was overexpressed after LPS stimulation of PMN. No difference was observed in MPO activity and integrin expression between WT and KO activated PMNs. ROS generation was significantly decreased in KO *vs* WT PMNs. PMNs recruitment was much less important in inflamed mesenteric vessels of KO mice than in those of WT mice. Fig 1. Diminish... Interestingly, PMNs from KO mice exhibit a higher furin activity than WT PMNs.

Conclusions: PN-1 is a serpin regulating positively PMNs functions. Because furin mediates the activation of proteins involved in the expression of adhesion molecules, PN1 may regulate neutrophil adhesion and recruitment via its ability to inhibit furin.

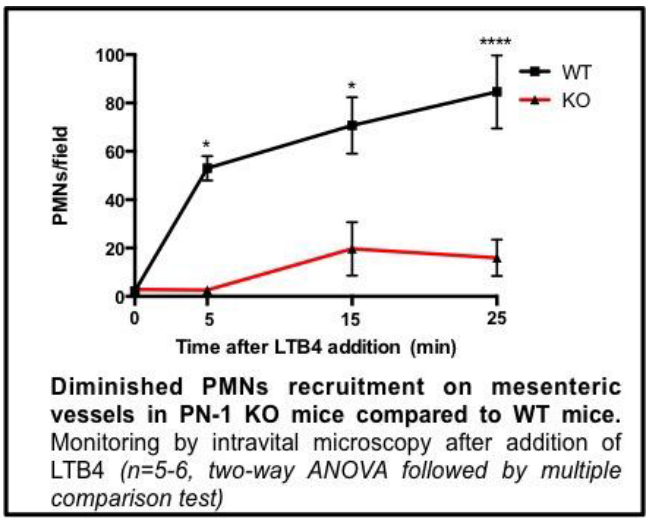

FIGURE 1 Diminished PMNs recruitment on mesenteric vessels in PN-1 KO mice compared to WT mice

OC 42.3 | Biophysical Regulation of Leukocyte Recruitment by the 3-Dimentional Structure of Platelet Thrombi

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Background: Biochemical signals (pro-inflammatory molecules) generated at sites of tissue injury are considered the dominant factor regulating the efficiency of leukocyte recruitment to sites of endothelial injury. The importance of biophysical elements in regulating leukocyte recruitment is largely unknown.

Aims: To investigate how the 3D structure of microvascular thrombi regulates the efficiency of leukocyte recruitment to sites of vessel injury.

Methods: To examine leukocyte-platelet thrombi recruitment *in vivo* we used a murine model of thrombosis induced by localized needle injury, and in a model of intestinal ischemia reperfusion (I/R) injury. *In vitro* studies involved perfusing neutrophils over platelet thrombi on microfluidic devices that incorporated 3D, platelet coated posts.

Results: During intestinal I/R injury and localized needle injury there was a direct correlation between the size of platelet thrombi and the extent of intravascular leukocyte recruitment. Computational fluid dynamics analysis revealed a central role for local blood flow changes around the margins of thrombi (low-shear pockets) in facilitating leukocyte arrest. These localized flow disturbances increased intravascular leukocyte accumulation to thrombi by up to 40-fold relative to inflamed endothelium alone. Similarly enhanced leukocyte recruitment also occurred in the low shear pockets around *in vitro* thrombi. Studies using 3D posts confirmed a critical role for the luminal extension of platelets (thrombus height) in regulating the efficiency of leukocyte recruitment, with tall (40μm) platelet-coated posts enhancing leukocyte recruitment >6-fold relative to shorter (10μm) posts.

Conclusions: These studies demonstrate the existence of a biophysical mechanism of leukocyte recruitment, mediated by the 3D structure of platelet thrombi, which plays a major role in enhancing leukocyte recruitment *in vivo*. This biophysical leukocyte recruitment mechanism may help explain the potent pro-inflammatory properties of microvascular platelet thrombi.

OC 42.4 | Plasmin and Plasminogen Induce Macrophage Reprogramming and Regulate Key Steps of Inflammation Resolution via Annexin A1

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Background: Participation of the plasminogen/plasmin (Plg/Pla) system in the productive phase of inflammation is well known, but its involvement in the resolution phase remains unclear.

Aims: To investigate the potential role of Plg/Pla in key events during the resolution of acute inflammation and its underlying mechanisms.

Methods: BALB/c mice received an intrapleural (i.pl.) injection of Plg or Pla (2µg/cavity) and, after different time points cells were recovered from the pleural cavity to have their profile investigated. Also, an acute pleurisy model was performed by the i.pl. injection of LPS (250ng/cavity). We also applied a therapeutic protocol by i.pl. injection of 2 µg of Plg or Pla after inflammation was established.

Results: The injection of Plg/Pla into the pleural cavity of mice induced a time-dependent influx of mononuclear cells that were primarily macrophages of anti-inflammatory (M2 - F4/80^{high} Gr1^{-} CD11b^{high}) and pro-resolving (Mres - F4/80^{med} CD11b^{low}) phenotypes, without changes in the number of macrophages with the pro-inflammatory profile (M1 - F4/80^{low} Gr1^{+} CD11b^{med}). Pleural injection of Plg or Pla also increased M2 markers (CD206 and Arginase-1) and M2 secretory products (IL-6 and TGF-β) without affecting expression of iNOS (M1 marker). During the resolving phase of LPS-induced inflammation, when M2 and Mres macrophages predominate, we found increased Plg expression and Pla activity, further supporting a link between the Plg/Pla system and macrophage reprogramming. Indeed, Plg or Pla given at the peak of inflammation promoted resolution by decreasing neutrophil numbers and increasing neutrophil apoptosis and efferocytosis. Next, we have confirmed the ability of Plg/Pla system to promote efferocytosis and override the pro-survival effect of LPS, both via AnxA1.

Conclusions: The results demonstrate a key role for the Plg/Pla system in the resolution of the inflammatory response whereby the effects induced by Plg are mediated by the anti-inflammatory and pro-resolving molecule AnxA1.

OC 42.5 | Alternative Proteolytic Activation of Human Prothrombin by a Bacterial Serine Protease

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Background: Factor Xa (FXa) converts inactive prothrombin (ProT) to active α-thrombin (αT) by cleaving ProT at R271-T272 and R320-I321. The signature of αT activation is the formation of a buried salt bridge between the N-terminus of the I321 and the negative D524. Exogenous proteases, such as ecarin from the viper snake venom, proteolytically activate ProT by cleavage at R320-I321. Non-proteolytic activation of ProT can be achieved by staphylocoagulase, a protein from *S. aureus* interacting with D524 through its N-terminal Ile-Val dipeptide. Little is known about the possibility that bacterial proteases can activate ProT.

Aims: Investigate blood clotting induced by subtilisin, a serine protease from *B. subtilis*, taken as the prototype member of the subtilase superfamily.

Methods: Proteolysis of ProT by subtilisin (2000:1, w/w) was monitored by SDS-PAGE. Hydrolytic activity was determined on S2238 substrate. The cleavage sites were identified by mass spectrometry. Fibrin generation and platelet activation were monitored by turbidimetry or impedance aggregometry.

Results: As with FXa, subtilisin cleaves ProT at R271-T272 but, at variance with FXa, it attacks ProT at A470-N471 in the autolysis loop, generating σPre2 (Fig. 1). Purified σPre2 hydrolyzes S2238 with a K_m identical to that of αT but with a k_{cat} 120-fold lower. The raise of thrombin-like activity is caused by the formation of the N471-D524 salt bridge. σPre2 is able to generate fibrin clot from fibrinogen, either purified or in human plasma, even though with a 150-fold lower efficiency. A similar trend is observed for the aggregation of platelets, either isolated by gel-filtration or in whole blood. Strikingly, addition of subtilisin (50 nM or 2 µM) to whole blood samples induced clot formation.

Conclusions: Subtilisin converts ProT to the active σPre2, which is able to generate fibrin and aggregate platelets. These results are consistent with clinical studies demonstrating a positive relation between bacterial infections and thrombotic risk.

OC 56.1 | Different Roles for Platelet Glycoprotein VI and CLEC2 during Gram Negative Pneumonia Derived Sepsis

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Background: Glycoprotein (GP)VI and C-type lectin-like receptor (CLEC-)2 are platelet receptors implicated in platelet activation. Their role in sepsis is unknown.

Aims: To assess the role of GPVI and CLEC2 in the host response and prevention of bleeding during pneumonia derived sepsis.

Methods: Mice treated with platelet GPVI antibody JAQ1, CLEC2 antibody INU-1 or IgG control were infected with Klebsiella pneumoniae via

the airways and sacrificed after 12, 36 or 42 hours. Additionally, mice were treated with platelet depleting antibody a-GPIb and sacrificed 42 hours after Klebsiella infection. Human whole blood or isolated neutrophils were stimulated with GPVI ligand CRP-XL and assessed by flow cytometry.

Results: GPVI depleted mice showed increased bacterial growth in lung and bronchoalveolar lavage fluid (BALF) compared to control mice 36 and 42 hours after infection. CLEC2 depletion did not influence Klebsiella growth, despite abundant presence of podoplanin in the lung. GPVI depleted mice showed increased activation of neutrophils and coagulation, and higher levels of chemokines and cytokines in their lungs. GPVI depletion also reduced platelet influx, platelet-neutrophil complex formation and platelet activation in BALF, which was not seen in CLEC2 depleted mice. In human whole blood, GPVI signalling via platelets increased platelet-neutrophil complexes and neutrophil activation. GPVI depleted mice displayed some bleeding during infection, but not to the extent as observed in platelet depleted mice. CLEC2 depleted infected mice had no bleeding and in thrombocytopenic mice neutrophil depletion did not abrogate bleeding upon infection.

Conclusions: These results suggest that GPVI enforces local host defense during pneumonia derived sepsis and mediates local platelet activation and platelet-neutrophil complex formation. Vascular integrity during sepsis is partially regulated by GPVI, but CLEC2 or neutrophils are not crucially involved herein.

OC 56.2 | Inhibition of Factor XI Activation by Factor XIIa Blocks Coagulopathy and Provides Organ Protection and Survival Benefit in a Baboon Model of *S. aureus* Sepsis

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Background: *S. aureus* sepsis can induce severe inflammatory response syndrome (SIRS) even during successful antibiotic therapy. Some forms of sepsis are characterized by fulminant disseminated intravascular coagulation (DIC). Systemic anticoagulants have long been tested or used to treat DIC in large trials, without evidence of overall outcome benefit. Differences in microbe-specific host responses contributing to the failure of anticoagulation trials in humans remain unclear.

Aims: We hypothesized that contact activation of FXI contributes to the pathogenesis of S. aureus-induced SIRS and DIC. We aimed to determine whether inhibition of FXI activation by FXIIa improves the outcome of SIRS and DIC in a model of acute fulminant sepsis.

Methods: We used a monoclonal antibody, xisomab 3G3, to selectively inhibit FXI activation by FXIIa in a baboon model of severe S. aureus sepsis. Baboons were challenged with 10^{10} heat-inactivated

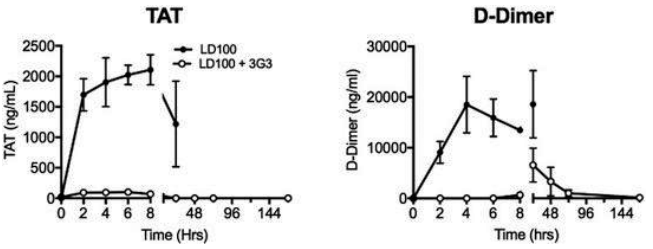

FIGURE 1 Time course of TAT and D-dimer levels in plasma

S. aureus by intravenous (i.v.) infusion over 2 h, with or without pretreatment with 1 mg/kg of intravenous xisomab 3G3. Animals were monitored for 7 days; during this time, blood samples and vital sign data were collected.

Results: Pretreatment with xisomab 3G3 drastically decreased heat-inactivated *S.aureus*-induced coagulopathy as reflected by clotting times, thrombin-antithrombin (TAT) complexes, fibrinogen and platelet consumption, fibrin deposition in tissues and D-dimers.

The antibody also protected key organs (liver, pancreas and kidney), as shown by reduced changes in transaminases, amylase, glycemia, creatinine and urea. Similarly, serum lactate dehydrogenase, nucleosomes and myeloperoxidase were significantly decreased showing lower inflammation and cytotoxicity in the treated group. All four animals pretreated with xisomab 3G3 survived while all three non-treated animals developed terminal organ failure.

Conclusions: Contact activation of FXI plays a key role in the pathogenesis of *S. aureus*-induced SIRS, DIC and multiple organ failure.

OC 56.3 | Targeting Coagulase Activity in *S. aureus* Bacteremia: A Prospective Randomized Controlled Trial of Staphylothrombin Inhibition

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Background: *S. aureus* coagulases trigger blood clotting. The coagulase-prothrombin complex, i.e. „staphylothrombin", is not inhibited by standard anticoagulants, but can be targeted by direct thrombin inhibitors (DTIs). In preclinical models, DTIs improve outcome of *S. aureus* infection.

Aims: To study the feasibility and safety of staphylothrombin inhibition with DTIs in patients with *S. aureus* bacteremia. Secondary outcomes: D-dimers as a marker of coagulation activation; inflammatory and microbiological parameters; clinical outcomes.

Methods: Between March 2013 and April 2016, a single-centre, randomized controlled, open-label trial was conducted in the University Hospital Leuven, comparing DTIs to standard enoxaparin in patients with *S. aureus* bacteremia. The trial was approved by the Ethical Committee; informed consent was obtained for all patients. (NCT01911624).

Ninety-four consecutive patients with *S. aureus* positive blood cultures and without contra-indication for thromboprophylaxis were randomized to either DTI (oral dabigatran 110 mg bid or IV argatroban) for 7-10 days, or to standard enoxaparin 40 mg od SC.

Results: Randomization was feasible for one third of *S. aureus* bacteremic patients. Levels of dabigatran inhibited staphylothrombin. We observed similar clinically relevant bleeding and thrombotic complications in both groups. Adjunctive treatment with DTIs was associated with a faster decrease in D-dimers (-783 +- 234 ng/mL vs. -189 +- 156 ng/mL) (p=0.04) and a trend towards a lower number of persistent positive blood cultures. No differences in inflammatory parameters or other clinical outcomes were noted.

Conclusions: Targeting coagulase activity with DTIs is feasible in a subset of patients with *S. aureus* bacteremia; with comparable safety to standard thromboprophylaxis. The more rapid resolution of D-dimers warrants further study of staphylothrombin inhibition in *S. aureus* bacteremia, preferably aided by rapid microbiological diagnostics and using strategies without concomitant anticoagulant effect.

OC 56.4 | PF4 Binds and Stabilizes Neutrophil Extracellular Traps: Implications in Sepsis

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Background: In response to inflammation, neutrophils release NETs, histone-decorated DNA that ensnares pathogens but also damages host tissue. We found that platelet factor 4 (PF4) binds to released NETs, inducing NET compaction and enhanced resistance to nuclease digestion. We also previously observed that mice that overexpress PF4 have improved survival in a model of sepsis.

Aims: Using in vitro and in vivo models, we examined whether PF4 interacts with NETs in a way that mitigates their toxicity.

Methods: Neutrophils were treated with LPS to induce NET release and then exposed to recombinant PF4 or buffer without PF4. Samples where then flowed through human endothelial umbilical vein cell (HUVECs) lined microfluidic channels that had been stimulated with tumor necrosis factor (TNF) α. EC viability was assessed 24-hours post NET exposure. PF4-deficient mice (mPF4$^{-/-}$) and wildtype (WT) controls were injected with LPS and euthanized 2-8 hours post injection. Plasma levels of DNA, citrullinated histones (cit-His), and myeloperoxidase (MPO) were quantified via ELISA and Western blot.

Results: In a microfluidic model, the presence of PF4 led to NET compaction and decreased NET adhesion to ECs. At 24 hours, channels incubated with NET+ PF4 contained higher numbers of viable ECs compared to channels incubated with NETs alone (Figure 1). Following LPS injection, both mPF4$^{-/-}$ and WT mice had similar increases in levels of TNFα. However, mPF4$^{-/-}$ mice had higher levels of DNA, cit-His and MPO.(Figure 2).

Conclusions: In vitro, the presence of PF4 protected ECs from NET associated damage. In a murine model of endotoxemia in which we previously showed improved survival in mice expressing PF4, the presence of PF4 did not alter the initial inflammatory response, but did lead to decreased plasma markers of NET release. Together, these findings suggest that the survival advantage observed in PF4$^{+/+}$ mice is in part due to PF4-induced NET compaction and EC protection.

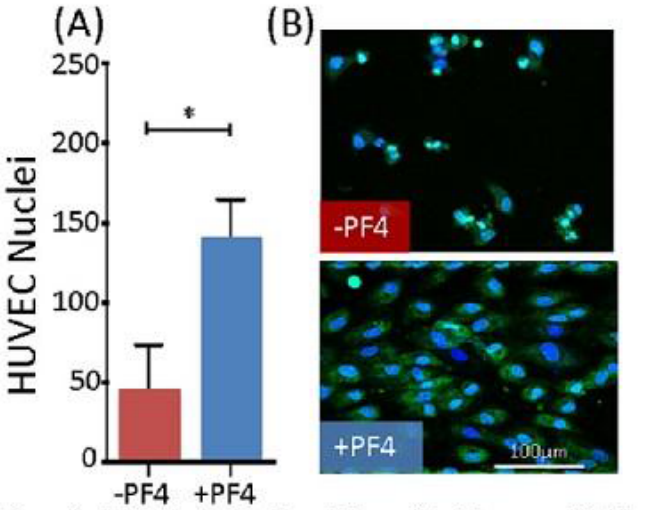

Figure 1. PF4 protects ECs from NET mediated damage. (A) LPS stimulated NETs with and without PF4 25µg/mL, flowed through microfluidic channels lined with TNFα exposed HUVECs and incubated for 24 hours at 37• Celsius. Residual adherent HUVEC nuclei counted using ImageJ (NIH). 2 channels per arm, 3 10x hpf counted per arm. *p = 0.0002 using a two tailed student T test **(B)** Representative confocal image of HUVEC cells following NET incubation. Blue=DNA, Green=Calcein AM

FIGURE 1 PF4 protects ECs from NET mediated damage

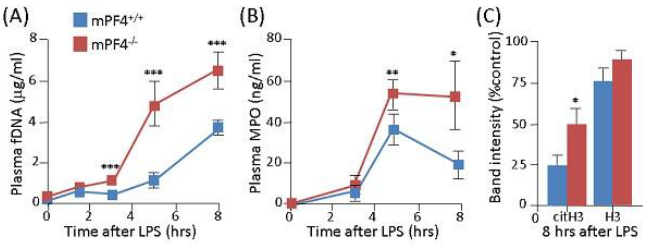

Figure 2: PF4$^{-/-}$ mice have increased plasma levels NET markers following LPS injection. (A) PF4-deficient mice (mPF4$^{-/-}$) and wildtype (mPF4$^{+/+}$) controls were injected with LPS and euthanized at different time points. Plasma levels of DNA were measured using a fluorescent plate assay with SYTOX green staining. Mice expressing PF4 had significantly lower plasma levels of DNA compared to mPF4$^{-/-}$ mice. ***=p<0.005 using a two-tailed student T test **(B)** Following LPS injection, mPF4$^{-/-}$ animals had lower levels of plasma MPO compared to WT controls. These difference reached statistical significance 5 and 8 hours post LPS exposure. *=p<0.05 **p<0.001 **(C)** Measuring Western Blot band intensity, mPF4$^{-/-}$ animals had increased levels of plasma histones H3 as compared to WT controls 8 hrs after LPS injection. This difference was statistically significant with citrullinated histones (citH3) *=p<0.05.

FIGURE 2 PF4-/- mice have increased plasma levels of NET markers following LPS injection

OC 70.1 | Von Willebrand Factor-platelet Microthrombi Phagocytosis by Macrophages as a New Homing Mechanism for Tissue Entry and Fibrosis

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Background: In cancers, tumor fibrosis constitutes a risk factor for metastasis. For their growth, tumors are dependent on a sustained influx of cytokines-releasing leukocytes, mostly macrophages. Another consequence of this chronic inflammatory state is the release of von Willebrand factor (VWF) by tumor blood vessels.

Aims: Because blood platelets are known to mediate the entry of immune cells into inflamed tissues and because VWF has been linked to fibrosis, we have hypothesized that VWF would support the formation of microthrombi which and act as homing signal for fibrosis-causing tumor-associated macrophages (TAMs).

Methods: Immunohistological analysis was performed on biopsies taken from cancer patients to assess the presence of macrophage-platelet-VWF clusters. Complementary in vitro studies were performed to elucidate the molecular mechanisms underlying macrophage binding to VWF-bound platelets.

Results: Immunohistological studies performed revealed the existence of a distinct TAM population, one that presents with strong VWF and integrin αIIb co-staining. Strikingly, those VWF+/αIIb+ TAMs were only found in highly fibrotic regions. In vitro studies confirmed the importance of VWF-borne microthrombi for macrophage homing as well as the importance of integrin αMβ2. These studies also yielded a novel finding: the ability of macrophages to transition from arrest to extravasation only takes place after internalization of VWF-platelet clusters has occurred.

Conclusions: We have identified a novel mechanism through which macrophages infiltrate tumor territory, one that is dependent on their binding and engulfing of VWF-borne microthrombi. Furthermore this TAM sub-population appears to bear strong pro-fibrotic potential. These observations suggest that the disruption of VWF-platelet-leukocyte interactions may help curtail tumor inflammation and fibrosis and, ultimately decrease the metastatic potential of certain tumors.

OC 70.2 | Interferon-induced Transmembrane 3 (IFITM3) on Megakaryocytes and Platelets Regulates Fibrinogen Endocytosis and Thrombosis During Inflammation

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Background: IFITM3, an interferon (IFN) responsive gene, restricts pathogen replication through vesicular trafficking mechanisms. IFITM3 in megakaryocytes (MKs) and platelets has not been examined.

Aims: We examined the expression, regulation, and function of IFITM3 in MKs and platelets under inflammatory conditions.

Methods: Cultured murine MKs were left alone or stimulated with IFNα. IFITM3 expression and localization was determined in MKs and developing proplatelets. Fibrinogen (Fgn) endocytosis, a clathrin-mediated event requiring $\alpha_{IIb}\beta_3$, was measured in IFN-stimulated MKs and platelets from wild type (WT) and *Ifitm*$^{-/-}$ mice. Integrin $\alpha_{IIb}\beta_3$ activation, aggregation, and thrombosis was determined in WT and *Ifitm*$^{-/-}$ mice basally and upon IFN-stimulation. Co-immunoprecipitation identified putative interaction partners for IFITM3. IFITM3 expression, Fgn content, and platelet aggregation was also measured in platelets from patients with systemic inflammation and healthy control subjects.

Results: Upon IFNa stimulation *in vitro*, IFITM3 surface expression and Fgn endocytosis in MKs significantly increased. IFITM3 upregulation was STAT1 dependent. Super-resolution microscopy demonstrated that IFITM3 co-localized with $\alpha_{IIb}\beta_3$. IFNα stimulation *in vivo* concordantly increased platelet Fgn endocytosis, $\alpha_{IIb}\beta_3$ activation, aggregation, and death from thrombosis in WT mice. In contrast, *Ifitm*$^{-/-}$ mice were completely rescued from IFN-induced platelet hyperreactivity and thrombosis ($p< 0.05$ vs. WT for all comparisons). Clathrin and α_{IIb} were confirmed to closely interact with IFITM3. Platelets from patients with systemic inflammation mirrored findings in murine models, with significant increases in IFITM3 expression (~20-fold increase in protein), $\alpha_{IIb}\beta_3$ expression, Fgn content, and aggregation.

Conclusions: IFITM3 is a novel regulator of MK and platelet Fgn endocytosis under inflammatory stimuli in humans and mice. Moreover, IFITM3 is necessary for IFN-induced platelet hyperreactivity and thrombosis.

OC 70.3 | Platelet exosomes Promote Vaso-occlusive Thrombosis in Sickle Cell Disease

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Background: Acute painful vaso-occlusive crisis (VOC) is the predominant reason for emergency medical care among Sickle Cell Disease (SCD) patients. VOC involves microvascular thrombosis across

multiple organs, but the mechanism that promulgates widespread thrombosis in SCD is largely unknown.

Aims: To determine whether caspase-1-mediated shedding of IL-1β-containing platelet exosomes promotes vaso-occlusive thrombosis in SCD.

Methods: We used our experimental model of lung vaso-occlusion in transgenic, humanized SCD mice, which is triggered by intravenous challenge with nanogram levels of the TLR4 ligand, lipopolysaccharide (LPS). Thrombosis was visualized in real time *in vivo*, using multi-photon-excitation microscopy of intact lung in live SCD mice. SCD or control human blood was perfused through microfluidic channels *in vitro* and neutrophil-platelet aggregation was visualized using fluorescence microscopy. Platelet derived exosomes were characterized using nanoparticle tracking and biochemical approaches.

Results: Vaso-occlusive thrombosis in the lung involved blockade of pulmonary arterioles by neutrophil-platelet aggregates, which was associated with an increase in peripheral blood levels of platelet exosomes. *In vitro* microfluidic studies revealed increased neutrophil-platelet aggregation in LPS-treated SCD patient blood compared with healthy controls, and this correlated with increased numbers of IL-1β containing platelet exosomes. The shedding of exosomes was secondary to the activation of platelet NLRP3-ASC-Caspase-1 inflammasome. Inhibition of caspase-1 attenuated shedding of platelet exosomes and neutrophil-platelet aggregation in SCD human blood *in vitro* and lung vaso-occlusion in SCD mice *in vivo*.

Conclusions: Inflammasome-mediated caspase-1 activation in platelets promotes shedding of IL-1β-containing exosomes in SCD. These exosomes trigger vaso-occlusive thrombosis through the formation of neutrophil-platelet aggregates in the lung.

OC 70.4 | Visualising LPS-induced Platelet Adhesion and Activation in Lungs Using Immunohistochemistry and Intravital Microscopy

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Background: Platelets can accumulate in lungs as a result of exposure to bacterial lipopolysaccharides (LPS), but the mechanisms underlying this response are incompletely understood.

Aims: To measure platelet activation and adhesion in the mouse lung microcirculation following LPS inhalation using immunohistochemistry and intravital microscopy.

Methods: Frozen lung sections were collected from PBS control mice (n=6) or mice treated with 5 mg/kg LPS (n=7, O55:B5, i.n., +48h). Immunohistochemistry and image analysis were used to measure the number of CD41+ platelets, activation status (platelet CD41 expression), and platelet/neutrophil interactions by evaluating platelet association with neutrophil elastase. Using the same LPS challenge protocol, platelets in the lungs of *Pf4-cre×mTmG* mice (n=4, cells not expressing *Pf4* are tomato fluorescent protein+, platelets expressing *Pf4* are green fluorescent protein+) were imaged using a thoracic window and multiphoton microscopy in order to test the effect of LPS on platelet adhesion in the living lung. Comparisons are t tests (*), or 2-way ANOVA with repeated measures and Holm's *post hoc* test ($^{\#}$), *=p< 0.05, **=p< 0.01, ***=p< 0.001.

Results: LPS increased platelet numbers (60%**) in lung sections and the CD41 expression of platelets (38%**) on both platelets associated with neutrophils (39%$^{\#\#}$) and platelets not associated with neutrophils (37%$^{\#\#}$). Platelets associated with neutrophil elastase had higher CD41 expression in both PBS (12%$^{\#\#}$) and LPS treated mice (16%$^{\#\#\#}$). Frame-to-frame tracking of intravital video microscopy revealed an increased number of adherent platelets per frame in the pulmonary microvasculature following LPS inhalation (47%*).

Conclusions: LPS-induced platelet activation and adhesion in the pulmonary circulation can be measured using two imaging technologies. Spatial patterns of platelet activation are supportive of the existence of both neutrophil independent and neutrophil associated platelet activation following inhalation of LPS.

OC 70.5 | PLD1 Regulates TNF-mediated Protection against Responses to LPS

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Background: Phospholipase D1 (PLD1) is an important cell signaling enzyme in inflammatory cells, platelets and other cells that hydrolyzes phosphatidylcholine to phosphatidic acid (PA) and choline. PA is known to act as second messenger because it can bind and activate a multitude of proteins including kinases, phosphatases etc. to control cell migration, proliferation and cell survival. Deficiency of PLD1 protects mice against arterial thrombosis because thrombus formation under high shear is impaired in these mice. Moreover, PLD1 is important for the inflammatory response including cytokine release; however, nothing is known about the impact of PLD1 in sepsis.

Aims: The aim of this project is to analyze the role of PLD1 in sepsis as a systemic inflammatory response.

Methods: In vitro and in vivo analysis of PLD1 deficient mice after injection of LPS.

Results: LPS induced sepsis led to an increase of acute phase cytokines such as TNF-a and interleukin (IL)-6 in plasma of wildtype mice while levels were reduced in PLD1 deficient mice. Western blot analysis revealed that PLD1 is important for the phosphorylation of Mek1/2

and Erk1/2 upon LPS-induced inflammation pointing to a crucial role for PLD1 as regulator of TNF-a expression beside reduced TNF-a release. Moreover, PLD1 deficiency prevented LPS-induced liver and lung damage as shown by reduced cell apoptosis and decreased fibrin deposition in lungs. PLD1 deficient platelets contributed to preserved outcome of PLD1 deficient mice after sepsis. Defective platelet integrin activation and reduced thrombin generation was shown to be causative for reduced thrombosis in lungs and liver thereby protecting these mice from ischemic organ damage.

Conclusions: This study reveals an important role for PLD1 in the regulation of TNF-a expression and release, cell apoptosis and organ damage after LPS-induced sepsis in mice.

DIAGNOSTICS AND OMICS

OC 35.1 | Exome Sequencing as a First Tier Genetic Test for Hemostatic Diseases

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Background: Hemostatic diseases are characterized by a susceptibility to develop hemorrhages because of genetic defects in one or more components of the hemostatic balance. At least 135 genes/loci are known as genetic causes for hemostatic disorders. We recently implemented Whole Exome Sequencing (WES) allowing analysis of a large panel of genes in one single test.

Aims: We performed WES analysis in patients with a severe bleeding phenotype with an ISTH BAT score above 8 and compared the data with the patients' phenotype.

Methods: WES started with the screening of genetic abnormalities in a predefined haemostatic panel. The hemostatic gene panel contains 135 genes proven to be involved in hemostatic disease including genes involved in primary hemostasis and secondary hemostasis. Second, if the haemostatic panel was negative, exome wide analysis was performed. The patients' phenotype was analysed using standard diagnostic assays.

Results: 43 patients were tested with the panel. 9 patients carried a pathogenic mutation causative for the disease explaining the laboratory/clinical phenotype; 6 with an autosomal dominant mutation (*VWF*, *RUNX1*, *MYH9*), 2 with a homozygous autosomal recessive mutation (*ITGA2B*, *P2RY12*) and 1 with an X-linked hemizygous mutation (*F8*).12 patients were heterozygous carriers of mutations genes involved in primary/secondary hemostasis either a combination of mutation in *VWF* gene and autosomal recessive genes or two autosomal recessive genes.

Conclusions: WES is a fast and cost efficient approach to detect genetic abnormalities in a large set of genes. Since the overall yield to detect pathogenic mutations was approximately 34% with the conventional targeted gene analysis approach, we implemented WES as a first tier diagnostic test for hemostatic abnormalities. Based on our experience we expect to resolve more hemostatic disorders due to the detection of combinations of autosomal recessive abnormalities particularly in primary hemostasis, a domain with a large number of unresolved clinical cases.

OC 35.2 | A Role for Chromatin Architecture in the Expression of the Fibrinogen Gene Cluster

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Background: Fibrinogen is a hexameric protein encoded by a coordinately regulated, evolutionarily conserved three-gene cluster. Fibrinogen expression is thought to be regulated by a combination of proximal promoters and enhancer elements. Intriguingly, there are interaction sites for proteins involved in chromatin looping flanking the locus. These regions may act as insulators, demarcating a chromatin domain boundary and serving to loop regulatory elements into proximity of the three fibrinogen genes, facilitating their co-regulation.

Aims: To further our understanding of fibrinogen gene regulation, our study aims to uncover new enhancer elements, detect chromatin interactions in and around the fibrinogen gene cluster, and study how such interactions influence fibrinogen gene expression.

Methods: Cell-based luciferase gene reporters and a transgenic zebrafish assay were used to test regulatory sequences *in vitro* and *in vivo*, respectively. Chromatin interactions were assessed by chromosome conformation capture (3C). The importance of chromatin looping sites for fibrinogen expression was tested using CRISPR-Cas9 genome editing in fibrinogen-expressing cells. Fibrinogen mRNA expression was measured by qRT-PCR and protein levels by ELISA.

Results: We identified two new liver enhancers flanking the fibrinogen gene cluster. We demonstrated that the fibrinogen gene cluster is looped into a chromosomal domain, with proximity-based interactions detected between sites flanking the locus. Further sub-looping was detected, particularly in cells that express fibrinogen. After targeted removal of chromatin interaction sites flanking one side of the locus, previously detected interactions were disrupted and de novo interactions detected. In cells with targeted disruption of the fibrinogen locus looping, fibrinogen mRNA and protein production were halved.

Conclusions: We have assessed the chromatin conformation of the fibrinogen gene cluster and demonstrated that chromatin architecture contributes to fibrinogen gene expression.

OC 35.3 | A Survey of *F8* and *F9* Variants in *my Life, our Future* for Evidence of Alternative Splicing in Hemophilia

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Background: Hemophilia A and B are rare X-linked bleeding disorders. *My Life, Our Future* (MLOF) is a U.S. national collaboration to clinically genotype patients with hemophilia A and B and establish a research repository.

Aims: We hypothesized that aberrant splicing is an under-recognized pathogenic mechanism in hemophilia. We studied *F8* and *F9* gene variants in MLOF to predict alternative splicing *in silico* in hemophilia A and B.

Methods: We studied *F8* and *F9* gene variants along with disease status, gender, and baseline factor level in the first 3000 subjects enrolled in MLOF. We used splice prediction tools (dbscSNV, Jian et al. 2014; SPIDEX, Xiong et al, 2015) to assess annotated splice variants and to seek signatures of other DNA variants predicted to impact *F8* and *F9* splicing.

Results: A total of 893 *F8* and *F9* gene DNA variants, excluding inversions, large insertions and deletions, were found in the first 3000 subjects enrolled in MLOF. Of these, 824 were classified as likely causative (629 in *F8;* 195 in *F9*). 51 were clinically categorized as splice variants (38 in *F8;* 13 in *F9*). Most of these variants were also identified by splice prediction algorithms (32/38 in *F8*; 11/13 in *F9*). Additionally, 50 other candidate splice variants were identified (34 in *F8*; 16 in *F9*). Interestingly, 30 variants were positioned distant (>10bp) from canonical splice sites. Collectively, the predicted splice variants were annotated as intronic (n=44), synonymous (n=5), missense (n=34), in-frame deletion (n=1), frameshift (n=6), and nonsense (n=3). Predicted splice variants were associated with half the baseline factor levels of variants not predicted to impact splicing (hemophilia A, FVIII: 3.6 +/- 7.5 *vs.* 6.2 +/- 9.0, p = 0.001; hemophilia B, FIX: 2.3 +/- 4.7 *vs.* 4.7 +/- 7.3, p < 0.01).

Conclusions: These data suggest that aberrant splicing is diverse and more common in hemophilia than previously suspected, and that predicted splice variants are associated with more severe disease.

OC 35.4 | Development of an Antidote-controlled RNA Probe for Molecular Thrombi Imaging

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Background: Vascular thrombosis is both a major underlying factor in many cardiovascular diseases worldwide and a major post-surgical complication. Early detection and treatment of thrombi improves outcomes for patients as the responsiveness of thrombi to fibrinolytic treatment decreases with thrombus age. The serine protease, thrombin, plays a central role in thrombogenesis and remains associated with the thrombus thereby facilitating further activation of coagulation factors and platelets after initial clot formation. Therefore, targeting and imaging clot-bound thrombin may provide a way of distinguishing newly formed thrombi from older, constituted ones.

Aims: The goal of this study is to develop the thrombin-targeting RNA aptamer Tog25t into a diagnostic probe for detecting blood clots via subtraction imaging.

Methods: The RNA aptamer Tog25t was conjugated to the near-infrared dye AF680 to detect clot-bound thrombin and a complementary antidote oligonucleotide was used to remove clot-bound aptamer, creating a subtraction image. Tog25t-AF680 was assessed for its in vitro binding capability to newly formed human plasma clots using fluorescence reflectance imaging and for its in vivo binding capability to a murine jugular clot using small animal near infrared fluorescent imaging.

Results: In comparison to a labeled non-binding control RNA, we observed a greater than 6-fold increase in near-infrared fluorescence (NIRF) target-to-background ratio (TBR) with Tog25t-AF680 in human plasma clots. Upon treatment with the antidote oligonucleotide (AO4), approximately 57% of the fluorescent signal disspated within 10 minutes of incubation. Tog25t-AF860 also targets jugular thrombi *in vivo* in an antidote-reversible fashion.

Conclusions: Our results suggest that thrombin is a suitable target for imaging newly formed clots. This thrombin-targeting near-infrared probe has the potential to be used as a diagnostic tool for arterial and venous thrombosis and may further advance our understanding of the role of thrombin in vivo.

OC 35.5 | The Design of a Novel Catheter to Deliver and Monitor Heparin Anticoagulation Therapy: Validation in a Small Cohort

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Background: Heparin anticoagulation therapy has a narrow therapeutic window and is the second most common ICU medication error. The aPTT monitors heparin, but suffers from long turnaround times and a

variable reference range. Here, we describe a novel catheter to deliver heparin and monitor activity in real-time with potential applications for patients on perfusion and extracorporeal membrane oxygenation.

Aims:

Aim 1. Validate ultrasound-based measurement of heparin levels in human samples.

Aim 2. Build and validate smart catheter to deliver heparin and monitor anticoagulation.

Methods: This technique uses novel photoacoustic ultrasound with a heparin-sensitive phenothiazinium molecule in tandem with an embedded catheter to monitor anticoagulation (**Fig. 1a**). First, we used this phenothiazine to measure the photoacoustic signal of whole human blood with different concentrations of heparin and correlated the signal to the aPTT. We then created a hydrogel on the surface of the catheter via silica nanoparticles, agarose, and the phenothiazine and measured heparin (0-50 U/mL).

Results: Clinically relevant heparin concentrations (aPTT: 30 -250 s) were measured in blood with a detection limit of 0.28 U/mL. We validated this imaging approach by correlation to the aPTT (Pearson's r = 0.86; p< 0.05) as well as with protamine sulfate (**Fig. 1b**). This technique also has good utility with low molecular weight heparin (enoxaparin) including a detection limit in blood of 72 µg/mL.. We tuned the surface charge of the hydrogel on the catheter surface to -15 mV, and this material retained >90% of the phenothiazinium reporter (**Fig. 2a**). The detection limit of the catheter was 1 U/mL with good reversibility via protamine sulfate (**Fig. 2b**). The catheter´s response time is < 30 s.

Conclusions: This is the first report of non-invasive imaging to monitor heparin. This utility offers an instantaneous response and will be coupled to smart infusion pumps for real-time control of heparin therapy.

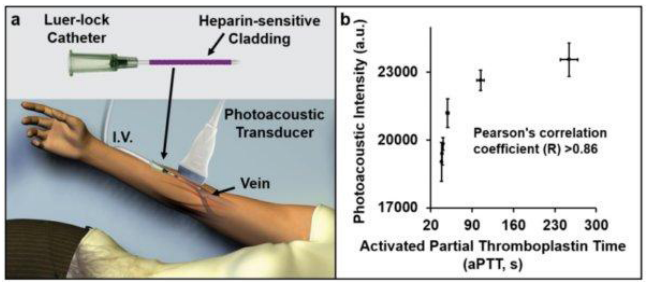

FIGURE 1 a) Schematic of device. b) Correlation of imaging signal with aPTT in six human whole blood samples with different heparin concentrations

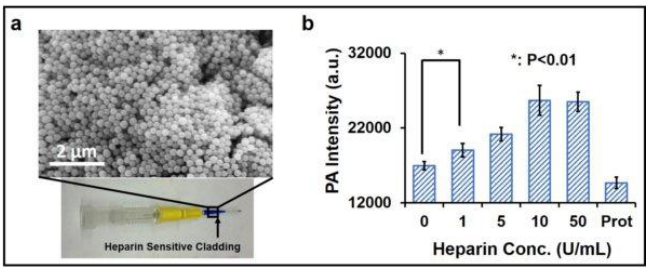

FIGURE 2 Catheter is coated with a hydrogel (a) containing the phenothizinium to measure heparin (b) with protamine sulfate reversibility (Prot.)

OC 51.1 | Impact of Genetic Variation on the Plasma Proteome in the Tromsø Study

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Background: Plasma proteins are involved in normal hemostasis and may be risk biomarkers of thrombotic diseases. It is, however, unclear how genetic variations contribute to protein levels estimated by mass spectrometry and whether these variants affect plasma protein level estimates through biological or technical mechanisms.

Aims: Our aim was to identify and characterize common and rare genetic variants associated with plasma protein levels assayed by mass spectrometry.

Methods: We performed exome sequencing or exome array genotyping of 200 participants recruited from the general population (the Tromsø Study). Additionally, EDTA-anticoagulated plasma was isolated and profiled for protein levels using TMT-multiplexed mass spectrometry. Mass spectra were mapped to peptides and proteins using Proteome Discoverer and the Uniprot protein sequence database. Mixed models were used to test for associations between common genetic variants near the protein's locus (cis) and peptide or protein levels. Analysis of rare genetic variation is ongoing.

Results: We quantified levels of 6,734 peptides that map to 748 Uniprot protein IDs (PIDs) and 692 gene loci. Common genetic variation was associated with 63 of the 748 PIDs as well as 230 of the 6,734 peptides. By examining the coding impact of the most associated SNP, we found that almost 30% of the variants associated with peptide levels were missense mutations that disrupted the peptide sequence and caused mis-mapping of the peptide. In some cases, these missense mutations had functional effects on the peptide, such as rs854560 (L55M) in Paraoxonase 1 (PON1), which decreases PON1 mRNA and plasma levels, while others may not be functional and confound mass spectrometry protein level estimation.

Conclusions: We identified cis common variation associated with plasma peptide and protein levels and showed that genetic variation often alters mass spectrometry estimates of peptide levels that may

be important to take into account when identifying or assaying protein biomarkers.

OC 51.2 | Effect of Endogenous Sex Hormone Levels Overall, and as Markers for Polycystic Ovary Syndrome and Primary Ovarian Insufficiency on Risk of Venous Thromboembolism

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Background: The risk of venous thromboembolism (VTE) in women of fertile age can largely be attributed to exogenous hormone exposure such as oral contraceptive (OC) use. The influence of endogenous sex hormone levels or disturbances of these hormones, such as in polycystic ovary syndrome (PCOS) or primary ovarian insufficiency (POI) on VTE risk is uncertain.

Aims: To assess the risk of VTE for levels of endogenous sex hormones
1) overall and
2) as markers for PCOS and POI.

Methods: Female participants of the MEGA-study (case-control study on VTE risk factors) ≤45 years who provided a blood sample in the absence of OC exposure or pregnancy were included. Sex-hormone binding globulin (SHBG), Estradiol (E2), Follicle stimulating hormone (FSH) and testosterone (TT) were measured. The free androgen index (FAI), used to determine (abnormal) androgen status, was calculated (100 x [testosterone/SHBG]).VTE risk was assessed for 1) hormones according to quartiles based on control levels, in FAI categories of < 1.5,1.5-3.0,3.0-4.5,>4.5 and 2) on clinical cut-off values used to diagnose PCOS and POI. Logistic regression models were constructed to estimate odds ratios (OR) with 95% confidence intervals (95%CI) adjusting for age and body mass index.

Results: A total of 679 women (376 cases, 303 controls) were eligible for the analyses.
1) We found a dose-response association for increased SHBG levels and VTE risk (Table 1). For FAI we found a U-shaped association with VTE risk (Table 2). There was no apparent association observed between levels of E2 or TT and VTE risk.
2) FSH levels ≥40 U/L, indicating POI, were possibly associated with decreased VTE risk, OR 0.7 (95%CI 0.3-1.3) as compared with levels < 40 U/L. A FAI>4.5, indicating PCOS, was associated with markedly increased VTE risk especially in participants who had not used OC at time of VTE: OR 5.1 (95%CI 1.0-26.2).

Conclusions: Endogenous sex-hormones overall and as markers for PCOS and POI are promising potential indicators of VTE risk.

OC 51.3 | Risk Prediction of Recurrent Venous Thrombosis in All Patients with a First Venous Thrombotic Event: Results from the MEGA Follow-up Study

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Background: The decision on the optimal duration of anticoagulant treatment after a first venous thrombosis (VT) is challenging. The predictive performance of 3 existing models for recurrence in patients with unprovoked VT is suboptimal in external validation studies and is heavily dependent on the definition of unprovoked VT.

Aims: To develop a prediction model for all patients with VT, provoked ánd unprovoked, with inclusion of a large set of clinical and laboratory candidate predictors (n=39) to obtain maximum discriminatory power.

Methods: Patients with first VT without cancer were followed for recurrence between 1999 and 2010 (MEGA follow-up study). Blood was sampled 3m after discontinuation of anticoagulation. Clinical and laboratory parameters were collected for each patient. A prediction model was developed using Cox regression analyses and a backward selection procedure (p< 0.15). Provoked recurrences were censored.

TABLE 1 Odds ratios (OR) with 95% confidence intervals (CI) for sex hormone binding globulin (SHBG) levels per quartile

SHBG, nmol/L	Cases (n)	Controls (n)	OR (95%CI)	OR*(95%CI)	OR** (95%CI)
Total	376	302			
≤P25, <42.9	91	75	1 (reference)	1 (reference)	1 (reference)
P26-P50, 42.9-57.3	88	76	1.0 (0.6-1.5)	1.0 (0.6-1.5)	1.2 (0.8-1.9)
P51-P75, 57.3-80.0	103	76	1.1 (0.7-1.7)	1.2 (0.8-1.8)	1.8 (1.1-2.9)
>P76, >80.0	94	75	1.0 (0.7-1.6)	1.1 (0.7-1.7)	1.9 (1.2-3.1)

* Adjusted for age
** Adjusted for age and BMI Pct=percentile

TABLE 2 Odds ratios (OR) with 95% confidence intervals (CI) for FAI in participants who did not use oral contraceptives at time of VTE

FAI	Cases (n)	Controls (n)	OR (95%CI)	OR* (95%CI)	OR** (95%CI)
Total	72	271			
FAI <1.5	41	135	1 (reference)	1 (reference)	1 (reference)
FAI 1.5-3.0	25	106	0.8 (0.4-1.4)	0.9 (0.5-1.5)	0.8 (0.4-1.4)
FAI 3.0-4.5	2	27	0.2 (0.1-1.1)	0.3 (0.1-1.4)	0.3 (0.1-1.2)
FAI >4.5	4	3	4.4 (0.9-20.4)	6.7 (1.3-34.2)	5.1 (1.0-26.2)

* Adjusted for age
** Adjusted for age and BMI

The performance of the model was estimated by means of Harrell's C-statistic.

Results: 3750 Patients were followed from discontinuation of anticoagulation for a median of 5.7 years (IQR;3.2-7.4), during which 507 unprovoked recurrences were identified. A full model, including 9 clinical and 7 laboratory parameters, showed good discriminative performance with a C-statistic of 0.73 (95%CI;0.71-0.76); 2-yr predicted risks ranged from 0-54% which coincided with observed risks. 13% of patients with provoked first VT had a two-yr predicted risk of >10%. For leaner versions of the full model the predictive performance decreased with each simplification (Table). In choosing a final model, a balance needs to be found between its predictive performance and clinical usefulness. Results from internal and external validation analyses will be available at the congress.

Conclusions: This model, including various clinical and laboratory parameters is able to accurately distinguish patients regarding their risk of recurrent VT, hence allowing refined assessment of the optimal duration of anticoagulant treatment.

OC 51.4 | Discovery of Plasma Biomarkers of Future Incident Venous Thromboembolism by Untargeted MS-Based Proteomics

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Background: Current plasma components associated with risk of venous thromboembolism (VTE) do not adequately predict first VTE at an individual level. Thus, there is a major unmet need for discovery of novel plasma protein biomarkers to improve risk stratification and advocate targeted prevention actions in order to prevent VTE. Identification of predictive plasma biomarkers of VTE in a prospective population-based cohort by an untargeted approach has not previously been undertaken.

Aims: To identify plasma protein biomarkers associated with future risk of VTE by an untargeted mass spectrometry (MS)-based approach on blood plasma sampled prior to VTE events.

Methods: We conducted a nested case-control study of 100 VTE cases and 100 age- and sex-matched controls randomly sampled from the general population (the Tromsø Study 1994-2012). Proteomic profiles of EDTA-anticoagulated plasma samples were generated using Proteome Discoverer (ver. 2.1) and the Uniprot protein sequence database to analyze data from an untargeted TMT-multiplexing LC-MS3 shot-gun proteomics approach. Statistical analysis was done in R. The study was approved by the regional research ethics committee and all subjects gave informed written consent.

Results: A total of 6734 peptides mapping to 708 protein IDs were quantified. Proteins differently expressed in VTE cases and controls were identified by two-sample t-test, non-parametric analysis, and multiple linear regression (significance level 0.05), and revealed 29, 26, and 28 proteins, respectively. In total, 47 unique candidates were identified. Among the most interesting was Transthyretin, which was also differently expressed between groups after Bonferroni correction for multiple testing.

Conclusions: We have, by untargeted MS-based proteomics, identified several candidate proteins that were differentially expressed in plasmas from subjects with and without future development of VTE. Our findings will be validated by targeted proteomics in a large case-cohort study.

OC 51.5 | D-dimer Measured at Diagnosis Improves the Prediction of Major Bleeding Events during the 1-yr after an Incident VTE

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Background: Currently available prediction tools do not provide accurate prediction of bleeding risk in patients on anticoagulant treatment for acute venous thromboembolism (VTE). D-dimer is measured to exclude the suspicion of VTE, but circumstantial evidence suggest that d-dimer is also associated with bleeding risk under various conditions.

Aims: To investigate whether d-dimer measured at the time of VTE-diagnosis is associated with major bleeding (MB) within the first year after diagnosis, and assess whether addition of d-dimer to a well-established prediction model (HAS-BLED) improves prediction of MB.

Methods: Cases with first VTE and d-dimer measured at diagnosis (n=555) aged 28-102 years, were sampled from the general population (The Tromsø study). Information on clinical variables, including HAS-BLED components (hypertension, abnormal renal/liver function, history of stroke or bleeding, age≥65, and drug- or alcohol abuse), was collected from medical records at the time of objectively confirmed VTE. MB-events (according to ISTH criteria) were registered up to one year after VTE-diagnosis. Multivariable Cox-regression models were used to calculate hazard ratios (HR) of MB across categories of d-dimer, and area under the curve (AUC) was used to compare the predictive abilities of a modified HAS-BLED with and without d-dimer.

Results: There were 52 MBs within one year after VTE-diagnosis. The risk of MB increased across quintiles of d-dimer ($p< 0.01$), and patients with d-dimer in the upper 20th percentile (>10.7 µg/ml) had a 2.2-fold (95% CI 1.1-4.6) higher risk of MB compared to the lower 40th percentile (0-2.8 µg/ml) in analyses adjusted for age, sex, duration of anticoagulant treatment, CRP and comorbidity. By adding one point for d-dimer (>10.7 µg/ml) to the HAS-BLED score, AUC increased from 0.60 (0.53-0.67) to 0.64 (0.55-0.72).

Conclusions: D-dimer measured at diagnosis was associated with risk of MB and improved the prediction of MB by HAS-BLED. Our results should be confirmed in larger cohorts.

OC 65.1 | Commutability - The Importance of Valid Material for Proficiency Testing Purposes

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Background: External Quality Assessment (EQA) may identify problems in laboratories, and highlight differences between methods. Spiking of plasma may be employed to mimic clinical samples. Such material should give similar results to clinical samples (ie, be commutable) to ensure appropriate conclusions can be drawn.

Aims: We describe data from UK NEQAS BC exercises where spiked samples were tested alongside matched *ex vivo* samples.

Methods: Normal plasma was spiked with dabigatran, rivaroxaban, enoxaparin or unfractionated heparin (UFH). FVIII deficient plasma was spiked with FVIII concentrate. Samples were sent to participating centres and results for spiked and patient samples were compared.

Results: Similar precision was seen for patient and spiked material for assays of rivaroxaban (CVs 16.5% & 18.2%) dabigatran assays (CV 15.1% & 16.7%). For rivaroxaban, the pattern of results for widely used kits was the same but for dabigatran some kits showed differences between spiked and patient samples. For enoxaparin, agreement was seen between results for samples from a pool of plasma from patients receiving the drug and spiked samples ($r=0.84$); ranking of results was similar between different kits. For a pooled plasma from patients receiving UFH, the median APTT ratio was lower than for a spiked sample with similar anti Xa activity (1.35vs1.90, $p< 0.001$) with no correlation in ranking of reagents. For FVIII assays results with different reagent sets varied by 12% for an Advate-spiked sample, compared to 2% for a post infusion sample; for rFVIIIFc, the difference between methods was < 8% for spiked and *ex vivo* samples.

Conclusions: Spiked material is suitable for EQA only if commutability is demonstrated. Our data show commutability of results for plasma spiked with rivaroxaban, enoxaparin, and some FVIII concentrates. For some tests, notably APTT for UFH, there are marked differences between patient and spiked samples. There is a danger of inappropriate conclusions arising over methods used with non-commutable samples.

OC 65.2 | Chromogenic and One Stage FIX Assays in the Presence of Idelvion (rFIX-FP), Alprolix (rFIXFc) Benefix and Replenine: Data from a UK NEQAS for Blood Coagulation Survey

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Background: Two extended half life FIX concentrates have recently been licensed. Some 1-stage FIX assays underestimate FIX-activity (FIX:Ac) but data is limited for most 1-stage FIX APTT and the chromogenic FIX assays.

Aims: To assess the use of multiple different APTT reagents and 2 chromogenic assays in samples containing FIX concentrates.

Methods: Samples were collected from different severe Haemophilia B subjects after infusion of Replenine or Benefix. Severe haemophilia B plasma was spiked with either Replenine, Benefix, Idelvion or Alprolix. All 6 samples were lyophilised and distributed through the UK NEQAS for Blood Coagulation programme to 68 UK haemophilia centres and 8 centres in Europe for FIX:Ac assay using the method in routine use for post infusion monitoring.

Results: Results with methods used by 3 or more centres are summarised in the table. Mean differences between results with different methods were < 25% for the samples containing Replenine. For samples containing Benefix FIX results were around 1/3 higher by 1-stage than chromogenic. For Idelvion FIX:Ac was markedly underestimated in 1-stage assays using AFS or CK Prest and markedly overestimated

TABLE 1

		n	Post Replenine	Spiked Replenine	Post Benefix	Spiked Benefix	Spiked Idelvion	Spiked Alprolix
Calculated FIX in spiked samples		-	-	50 IU/dL	-	70 IU/dL	60 IU/dL	60 IU/dL
	AFS	19	41.8	51.5	68.8	83	31.5	65.5
	AFSL	4	49	51.5	81.9	89.9	50.7	75.4
One stage methods Median (IU/dL)	CK Prest	5	51	55	76.5	86	32	48
	Pathromtin	4	42.7	46.5	57.6	66.5	52.6	46.9
	PTT Auto	3	46.3	50.7	80.5	82.6	52	40
	SynthaSIL	32	45	48.1	74	79.9	65.5	60.7
Chromogenic Median (IU/dL)	Hyphen	10	44	46.7	47.9	54.4	99.4	54.0
	Rossix	13	45	45	55	63	116	66.0

using either chromogenic assay. For Alprolix FIX activity was markedly underestimated in assays using CK Prest , Pathromtin SL or PTT Auto, although numbers of centres were low so more data are needed for robust conclusions to be drawn. For Benefix and Replenine there was an excellent correlation between results obtained on the post infusion and spiked samples indicating that the two materials were behaving in a similar way.

Conclusions: Our data suggest that chromogenic FIX or 1-stage FIX assays with Actin FS or CK Prest should not be used to monitor Idelvion. Either chromogenic assay could be used to monitor Alprolix. Based on our limited data one stage assay with CK Prest, APTT Auto or Pathromtin SL are expected to underestimate Alprolix activity.

OC 65.3 | The ECAT External Quality Assessment Programme: Larger Variation in FVIII Activity < 0.05 IU/ml with One-stage Assay Compared to Chromogenic Assay

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Background: Currently, both one-stage (OSA) and chromogenic substrate assay (CSA) are used to measure factor VIII (FVIII) activity and therefore to diagnose and monitor treatment in hemophilia A patients. Consequently, it is of clinical importance to identify analytical variation in OSA and CSA.

Aims: To quantify and specify the variation in FVIII activity when testing by OSA and CSA.

Methods: Seventeen samples distributed by the ECAT foundation between 2010-2016 with FVIII values between < 0.01 IU/ml and 1.94 IU/ml were analyzed by >200 laboratories worldwide. Measured FVIII levels and coefficient of variation (CV) for both OSA and CSA were compared and subgroup analysis was performed for most commonly used kits (n>10).

Results: Only minimal differences were observed between measured FVIII levels for OSA and CSA; for example in plasma from a healthy subject the measured FVIII activity (median±IQR) was 1.00±0.14 IU/ml for the OSA and 0.97±0.11 IU/ml for the CSA (p=0.10) and a hemophilia A patient 0.01±0.02 IU/ml OSA and 0.01±0.01 IU/ml CSA (p=0.29). The CV of both tests was negatively associated with FVIII levels (Figure 1). In addition, the CV was larger in the OSA when FVIII< 0.05 IU/ml with a maximal CV of 75% for the OSA and 48% for CSA. Subgroup analysis for OSA for different equipment, type of deficient plasma and calibration techniques was not possible, as most laboratories obtained these products from the same company. Comparison between activators did no show differences in FVIII results (p>0.05). For CSA, the measured FVIII levels were similar for the different kits.

Conclusions: The coefficient of variation in FVIII OSA measurements is larger compared to CSA when FVIII is < 0.05 IU/ml. Consequently, larger variation in FVIII testing might increase the risk on misclassification of hemophilia severity.

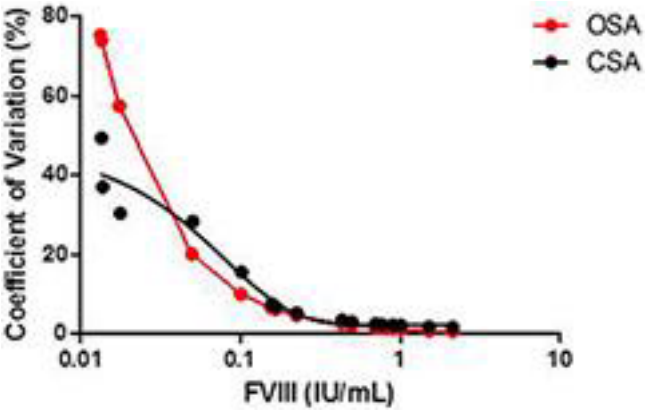

FIGURE 1 Coefficient of variation of FVIII activity measurements in the ECAT program (n>200)

OC 65.4 | North American Special Coagulation Laboratories (NASCOLA): Laboratory Practices for aPTT Based One-stage Factor Assays and their Outcomes on Results: A Survey

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Background: Inter-laboratory variability has been observed when reviewing international proficiency test (PT) results for one-stage factor assays. The greatest variability is seen in aPTT based assays at lower ranges (< 10% /< 0.01U/mL).

Aims: To survey members of NASCOLA to determine current practices for performing one-stage factor assays and if certain laboratory practices can be identified that contribute to PT variability.

Methods: A survey of 22 questions was sent to NASCOLA labs inquiring how they performed one-stage aPTT factor assays. Laboratories provided information regarding type of instrumentation, number of factor dilution run; calibration curve questions included type, number of points, number of curves used, upper and lower limit of detection and how results were reported. Responses were anonymous prior to analysis. PT results (2012-15) were reviewed to determine if specific practices could be identified that contributed to variability of results.

Results: Thirty NASCOLA laboratories responded. Five different families of coagulation analyzers were used. A minimum of three dilutions are run in 93% of labs; the highest dilution at 1:320, and the lowest at 1:2.5. Most common starting dilution is 1:10 (48%). Calibration curve fits were polynomial (61%) with 57% running 6 or more points; 50% included a zero point. Forty percent of laboratories construct a low curve in addition to a standard calibration curve. Results were extrapolated by 13% of laboratories, however all laboratories reported very low levels as < the lowest point.

Conclusions: This NASCOLA survey confirms wide variability in laboratory practices of factor assays. Lack of consensus on how calibration curves are prepared is likely a contributing factor. Variability includes number of points; curve fit and whether multiple curves are used. and extrapolated results. Adapting recently published consensus guidelines for the performance of one stage factor assays (CLSI; 2016) may help to minimize variability in practice and ultimately in PT results.

OC 65.5 | Efficiency of Bayesian Logic in Detecting and Characterizing Loss of Precision, and or Loss of Accuracy: Application to aPTT Statistical Process Control

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Background: A preliminary study („Use of prior manufacturer specifications with Bayesian logic eludes preliminary phase issues in quality control: An example in a hemostasis lab", Blood Coagul Fibrinolysis 2015, 26: 590-596) showed how the conventional preliminary phase can be avoided using Bayesian logic. The first Bayesian tool used to this end is the Predictive Control Chart (PCC), intended to detect outliers. Holistic monitoring will require to identify not only outliers but trends in the mean and the inter assay SD of the process. Two new Bayesian tools (Location and Scale Predictive Residual CUSUM (PRC)) charts are introduced, aiming to identify such trends in the process without any preliminary phase.

Aims: To illustrate the reliability of mathematical simulations with real-life med lab out-of-control (OOC) case studies especially at the early method startup phase.

Methods: From Instrumentation Laboratory (IL), Bedford, MA, USA: ACL TOP 750 analyzer, HemosIL Synthasil and HemosIL Normal Control Assayed: aPTT (activated partial thromboplastin time) prior target = 29 seconds. Calibration of Bayesian tools: PCC with Alarm as with 1_3s ; Location PRC: Alarm for a change in

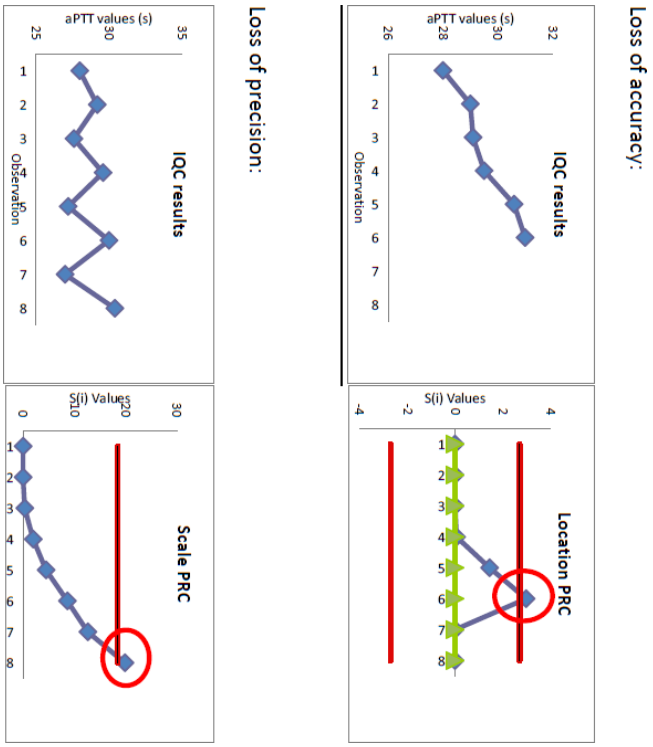

FIGURE 1 loss of accuracy scenario and loss of precision scenario

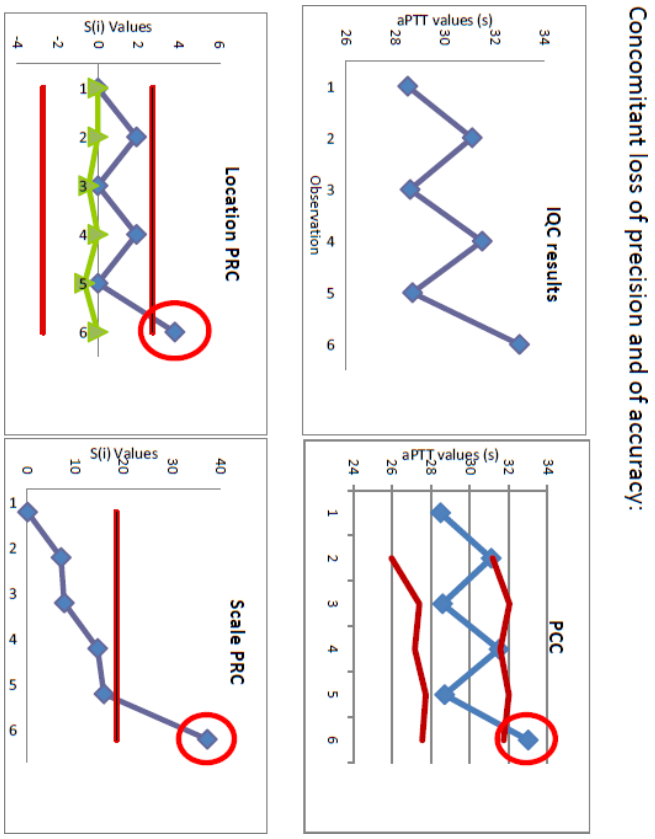

FIGURE 2 Concomitant loss of accuracy and precision scenario

terms of 1.5 SD shift (corresponding to aPTT uncertainty of measurement) ; Scale PRC: Alarm for 50% increase in inter-assay SD (50% with respect to our proper inter-assay SD value and what it is required for unfractionated heparin (UFH) treatment monitoring with aPTT in terms of precision).Three OOC scenarios were tested as of observation 2.

Results: The different kinds of scenario were detected and categorized very early and in online fashion, without any preliminary phase as required in conventional approach (figure 1 and 2).

Conclusions: Using Bayesian logic, laboratory IQC management becomes central to very efficient patient management, with a focus on quality in the spirit of the 2012 version of the ISO 15189 norm.

FIBRINOLYSIS & PROTEOLYSIS

OC 01.1 | The Plasminogen Receptor Plg-R_{KT} is Expressed on Platelets and Co-localises with Platelet-derived Plasminogen upon Stimulation

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Background: Binding of plasminogen to cell surfaces dramatically accelerates rates of activation. We have shown binding of exogenous plasminogen to PS-exposing and spread platelets. Platelet α-granules are reported to contain plasminogen but mechanisms of endocytosis and retention are unclear. Recently, a novel transmembrane lysine-dependent receptor for plasminogen, Plg-R_{KT}, has been described on macrophages.

Aims: To examine expression of Plg-R_{KT} on platelets and determine whether platelet-derived plasminogen associates with this receptor upon endocytosis.

Methods: Plasminogen in releasate from thrombin-stimulated platelets ± εACA was quantified by ELISA. Platelets were separated by ultracentrifugation into soluble protein and membrane fractions and the presence of Plg-R_{KT} identified by Western blotting. Exposure of platelet-derived plasminogen and Plg-R_{KT} was investigated using flow cytometry and confocal microscopy after stimulation of platelets with thrombin and convulxin (CVX) or collagen.

Results: Plasminogen was detected in the releasate of activated platelets (0.08 nmol/10^8 plts), but was significantly augmented by pre-treatment of platelets with εACA (0.33 nmol/10^8 plts). εACA is a lysine analogue that competes for plasminogen binding sites, suggesting that platelet-derived plasminogen associates with the membrane via a lysine-dependent mechanism. Membrane accumulation of platelet-derived plasminogen on activated platelets was confirmed by flow cytometry. A 17 kDa band, consistent with Plg-R_{KT}, was detected in lysate and membrane fraction of platelets. Confocal microscopy revealed the presence of platelet-derived plasminogen on both spread and balloon shaped PS-exposing platelets which concentrated in ‚caps' on the PS-exposing platelets. Plg-R_{KT} co-localized with platelet-derived plasminogen on the activated platelet-membrane.

Conclusions: Platelets express Plg-$R_{KT,}$ which potentially functions in retention of platelet-derived plasminogen and may facilitate cell-surface mediated plasminogen activation.

OC 01.2 | Dysregulation of Metabolic Homeostasis in Plasminogen Receptor$_{KT}$ (Plg-R_{KT}) Deficient Mice

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Background: Inhibition of the plasminogen activation system is linked to adipose fibrosis and insulin resistance in response to a high fat diet. However, the role of molecules that promote plasminogen activation has not been studied extensively. Plg-R_{KT} is a novel transmembrane plasminogen receptor that promotes plasminogen activation and localizes plasmin activity on cell surfaces.

Aims: To test the hypothesis that Plg-R_{KT} regulates systemic metabolic homeostasis and promotes healthy adipocyte function.

Methods: Plg-R_{KT} null mice and wild-type littermates were fed a high fat (60%) diet (HFD) for 16 weeks.

Results: Plg-$R_{KT}^{-/-}$ mice gained significantly more weight than Plg-$R_{KT}^{+/+}$ littermates (final wt., 54±0.9 g vs 39± 3.6 g, respectively, P< 0.01 n=8). Echo MRI measurements showed that total fat mass was significantly greater in HFD- Plg-$R_{KT}^{-/-}$ mice compared with HFD-Plg-$R_{KT}^{+/+}$ littermates (20.8±0.7 g vs 14.7±2.7 g, respectively, P< 0.05, n=8), epididymal adipose tissue weight was significantly less, while liver weights were significantly greater. Histological analysis showed dramatically greater levels of lipid accumulation in liver in HFD- Plg-$R_{KT}^{-/-}$ mice, consistent with decreased ability of Plg-$R_{KT}^{-/-}$ adipose tissue to store lipid, and enhanced ectopic fat accumulation, a hallmark of Type 2 Diabetes. Furthermore, greater fibrin and collagen deposition and greater accumulation of inflammatory cells was observed in adipose tissue of Plg-$R_{KT}^{-/-}$ mice. And Plg-$R_{KT}^{-/-}$ mice exhibited more severe blunting of glucose tolerance and less efficient insulin-mediated suppression of plasma glucose. In insulin signaling studies, Akt phosphorylation was 80% lower in adipose tissue of Plg-$R_{KT}^{-/-}$ mice.

Conclusions: Plg-R_{KT} coordinately regulates multiple aspects of adipose function and metabolic homeostais by maintaining an anti-fibrotic adipose environment, promoting insulin sensitivity and adipogenesis and maintaining an anti-inflammatory adipose environment.

OC 01.3 | Neutrophil Extracellular Traps In Thrombi from Patients with Acute Ischemic Stroke

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Background: Little is known about the exact composition of thrombi causing ischemic stroke. Yet, such information is essential to improve current thrombolytic therapy. For unknown reasons, t-PA mediated thrombolysis often fails, suggesting the presence of unknown components that resist classical fibrinolysis and could be involved in thrombus stabilization. Recently, neutrophil extracellular traps (NETs) have been reported in various settings of thrombosis but their presence in ischemic stroke thrombi remains unknown.

Aims: To determine the presence of neutrophils and more specifically NETs in ischemic stroke patient thrombi.

Methods: Sixty-eight thrombi retrieved from ischemic stroke patients following endovascular treatment were characterized by immunostaining for citrullinated histone H3 (H3Cit), CD66b and neutrophil elastase. Extracellular DNA was visualized via DAPI. Per thrombus, H3Cit and neutrophil amount was quantified and correlated with stroke etiology and thrombus age. Extracellular DNA was targeted by ex vivo lysis with DNase1 and t-PA on eight patient thrombi.

Results: Neutrophils were detected extensively throughout all thrombi. H3Cit, a hallmark of NETs, was observed in almost all thrombi and H3Cit-positive area varied up to 13.45% within thrombi. Co-localization of H3Cit with extracellular DNA released from neutrophils confirmed the presence of NETs. H3Cit presence was significantly higher in thrombi from cardioembolic origin compared to other etiologies (3.07 % ± 2.21 % vs 1.57 % ± 1.23 %, p< 0.05). Older thrombi contained significantly more neutrophils as well as H3Cit compared to fresh thrombi (p< 0.001 and p< 0.05). Interestingly, ex vivo lysis of patient thrombi was more successful when adding DNase1 to standard t-PA (p< 0.01).

Conclusions: Our study demonstrates the presence of neutrophils, and for the first time, the presence of NETs in human stroke thrombi. Initial evidence is provided that targeting NETs with DNase 1 might open novel treatment avenues for acute ischemic stroke therapy.

OC 01.4 | A Hepatocyte Transcriptional Repressor DACH1 Exacerbates Defective Fibrinolysis in Obesity

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Background: Increased risk of thrombosis accounts for enhanced clinical events observed in patients with metabolic syndrome. While recent work describes impaired fibrinolytic activity as a main driver for increased risk of thrombosis, the mechanisms linking defective fibrinolytic activity to metabolic syndrome has yet to be fully elucidated. Previously, we showed a transcriptional co-repressor DACH1 positively correlates with increasing BMI in human liver biopsies. Elevated DACH1 in hepatocyte activates the ER stress and causes defective insulin signaling in obese mice.

Aims: To explore the mechanisms whereby hepatic DACH1 signaling exacerbates the dysfunction of fibrinolysis in obesity.

Methods: Diet-induced obese mice with hepatocyte-*Dach1* deletion were subjected to bleeding, fibrinolytic activity assays, and FeCl3-induced arterial thrombotic injuries. Then we explore the mechanism of how DACH1 regulates the transcription of genes involved in fibrinolysis in human hepatocytes.

Results: Hepatocyte-*Dach1* deletion in obese mice significantly
1) elongates the tail bleeding time, and
2) time to occlude carotid in FeCl3-induced arterial thrombosis model,
3) increases liver tissue plasminogen activator (tPA, or Plat) mRNA, circulating tPA, tPA enzymatic activity, and fibrin-degradation products, consistent with hyperfibrinolysis.

Specifically silencing hepatic *Plat* gene in hepatocyte-DACH-knockout mice reversed these effects. We found a striking correlation between higher human liver DACH1 protein levels and lower plasma tPA activities from very lean to extreme obese individuals.

Conclusions: This study provided a novel pathway linking obesity with defective fibrinolysis through hepatic DACH1-tPA signaling. DACH1 has never before been implicated in blood coagulation and fibrinolysis.

Moreover, the findings reveal that regulation of tPA in hepatocytes, a cell type previously unappreciated as playing a major role in tPA biology, is a key determinant of the tPA/PAI1 balance in blood and its effects on bleeding and thrombosis.

OC 01.5 | Enhanced Fibrinolysis Using Magnetically Driven Functionalized Microwheels

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Background: The efficacy of fibrinolytic agents is limited by delivery to and penetration into an occlusive embolus or thrombus. These transport limitations are particularly acute for occlusions of small arteries that are not accessible to catheters and rely on diffusion of fibrinolytics.
Aims: Use magnetic powered microwheels (µwheels) functionalized with tissue type plasminogen activator (tPA) to achieve faster lysis than comparable concentration of free tPA.
Methods: Under a 9 mT magnetic field rotating at 100 Hz, superparamagnetic beads (1 µm) conjugated to biotinylated recombinant tPA form wheel-like assemblies that roll along the surface in unidirectional and corkscrew motions. Fibrin gels were formed using recalcified plasma and thrombin (4.5 nM) in a microfluidic device between two reservoirs of plasma (Fig. 1A). Fibrinolysis by tPA-wheels with an effective tPA concentration of 3.6 ug/mL was compared to 1 and 10 ug/mL soluble tPA.
Results: High µwheel translation speeds (4.5 ± 3.8 µm/s) relative to lysis rates caused accumulation at the gel interface, resulting in a local tPA concentration of up to 180 ug/mL. µWheels driven with unidirectional and corkscrew motions lyse fibrin gels at rates of 4.8 ± 0.3 and 9.6 ± 1.5 µm/min, compared to rates for 1 and 10 ug/mL tPA of 0.81 ± 0.25 and 5 ± 1.5 µm/min (Fig. 1B-D).

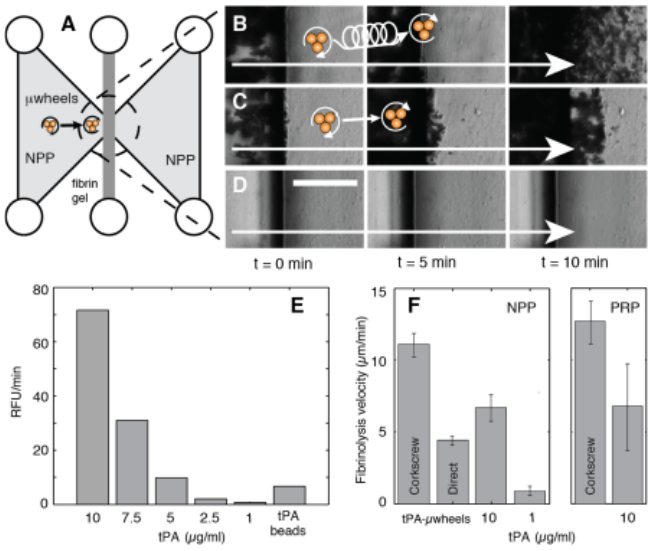

FIGURE 1 Colloids bearing tPA (3.6 ug/mL) lyse fibrin gels faster than 10 ug/mL free tPA when driven in a corkscrew motion by a magnetic field

Conclusions: tPA-functionalized µwheels controlled by an external programmable magnetic field enhance fibrinolysis compared to soluble tPA. Increases lysis is due to the high localized concentration of tPA, and penetration of tPA-loaded beads, especially those with a corkscrew motion, into fibrin gels.

OC 19.1 | Fibrinolysis with Clot-specific Thrombin-cleavable Microplasminogen is a Potential Alternative to Plasminogen Activators

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Background: Thrombolytic therapy is currently exclusively provided by plasminogen activators (PAs). Despite proven benefits, their use is limited by haemorrhagic/neurotoxic side effects and thrombolysis failure. We developed a novel thrombolytic molecule composed of microplasminogen engineered to be activated by thrombin (HtPlg) fused to an activation-specific anti-GPIIb/IIIa single-chain antibody (SCE5).
Aims: We studied the thrombolytic capacities and bleeding side effect of SCE5-HtPlg, evaluating its potential to overcome limitations of PAs.
Methods: We tested the efficacy of SCE5-HtPlg to lyse human blood clots *in vitro* under conditions which mimic thrombolysis resistance from mature thrombi (4 h) *versus* immature clots (30 min). We further measured the thrombolytic capacities in a mouse model of pulmonary embolism and evaluated the safety profile with tail bleeding and intracranial haemorrhage (ICH) measurements.
Results: The SCE5-HtPlg yielded efficient clot degradation in the *in vitro* thrombolysis assay. Clot maturation time significantly delayed urokinase (uPA) induced lysis, but did not affect the thrombolysis profile of SCE5-HtPlg ($p < 0.05$, n=4). In the *in vivo* lung embolism model, SCE5-HtPlg treatment at 4 µg/g bodyweight (BW) and uPA at 500 U/g BW resulted in similar 4-fold reduction of pulmonary fibrinogen deposition compared to saline ($p < 0.01$, n=3). At these therapeutic doses, uPA significantly prolonged the bleeding time compare to saline ($p < 0.001$, n=3) whereas the SCE5-HtPlg did not. We did not observe accumulation of haemoglobin or albumin in brain samples from mice treated with 4 µg/g SCE5-HtPlg compared to saline control ($p=0.77$ and $p= 0.79$, respectively, n=3), suggesting a lack of ICH and blood-brain barrier breakdown.
Conclusions: Thrombolysis provided by the novel thrombin-activated fibrinolytic drug SCE5-HtPlg has the potential to overcome limitations of PAs currently in clinical use. Future studies will confirm these results using *in vivo* models of thrombosis resistant to lysis by PAs.

OC 19.2 | Coagulation Factor XIIIa Is Inactivated during Thrombolytic Treatment

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Background: Coagulation factor XIIIa (FXIIIa) is a transglutaminase that covalently crosslinks fibrin and several other coagulation factors to stabilize blood clots and reduce blood loss. Although the activity and the mechanism of activation had been well characterized, the mechanisms of inactivation are less clear.

Aims: Determine the extent that FXIIIa is inactivated by fibrinolytic enzymes and the implications in thrombolytic therapy.

Methods: Using blood collected from both healthy and patients treated for deep vein thrombosis, the inactivation of FXIIIa by plasmin and tPA was measured using Western blotting.

Results: Here we show that plasmin can cleave FXIIIa. FXIIIa was efficiently cleaved by plasmin in buffer and in plasma, whereas its zymogen, FXIII, was not. We have preliminary data suggesting that FXIIIa is degraded in a subset of patients treated with thrombolytics. In blood collected prior to tPA administration, FXIIIa was stable in all patients; however, in a subset of patients, FXIIIa was degraded in blood collected immediately after the completion of thrombolytic therapy.

Conclusions: These results indicate that the fibrinolytic system can regulate the coagulation system's crosslinking agent, and this provides an additional point of regulation of clotting. The potential implications are that pathophysiological or therapeutic conditions that increase fibrinolysis may inhibit crosslinking and stabilization of fibrin.

OC 19.3 | Fibrinolytic System Components Uniquely Affect the Resolution of Deep Vein Thrombosis: Comparison of α2-antiplasmin and Tissue Plasminogen Activator

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Background: Patients with deep venous thrombosis are treated with anticoagulants to prevent thrombus extension. However, thrombus resolution (fibrinolysis) is minimal, which may lead to recurrent embolism and post-thrombotic syndrome.

Aims: To determine the potential causes of impaired fibrinolysis, we examined the expression of fibrinolytic system components in experimental venous thrombi and compared the effects of tissue plasminogen activator (tPA) and α2-antiplasmin (α_2AP) inhibition on thrombus resolution.

Methods: Venous thrombi were examined in mice one and seven days after inferior vena cava ligation. Fibrinolytic components were detected by immunostaining. Plasmin generation and clot lysis were assessed. Data were analyzed by Student's t-test or a one way ANOVA.

Results: Occlusive venous thrombi were comparable in size at day one and seven post-IVC ligation (p=0.68). Urokinase and PAI-1 expression were significantly increased in 1 day thrombi as compared to sham mice (p< 0.01), tPA expression was minimal-absent. Plasminogen (Pg) and α_2AP were significantly increased (p< 0.001) throughout the thrombus. We examined whether insufficient Pg activation or plasmin inhibition were responsible for impaired thrombus dissolution. Mice were treated with recombinant (r-) tPA or an antibody that inhibits α_2AP, 1 day post IVC ligation. A therapeutic r-tPA dose and a higher dose (1.2 and 5 mg/kg) had no significant effect, but α_2AP inhibition significantly reduced thrombus weight, 7 days after treatment (p< 0.001). Plasma clot lysis showed that α_2AP inhibition enhanced plasmin activity and fibrinolysis.

Conclusions: Despite the presence of fibrinolytic components, fibrinolysis is suppressed in venous thrombi. Inactivation of α_2AP was more effective at dissolving existing thrombi than pharmacologic doses of r-tPA. We conclude that α_2AP contributes to persistence of venous thrombi in vivo and α_2AP inhibition may have therapeutic value.

Funded in part by NIH (NINDS NS089707 and by NHLBI HL092750) to Guy Reed.

OC 19.4 | CVD-risk Associated Lipid Levels Associate Not Only with Increased Fibrinogen Concentration but also with Altered Plasma Clot Properties in a Healthy Population

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Background: Blood lipid levels are known to be related to fibrinogen concentration. *In vitro* and clinical studies have also indicated effects on fibrin clot structure and lysis. It is less clear whether the relationship between blood lipids and fibrinogen also extends to altered clot properties in a population setting.

Aims: To investigate the relationship between total (TC), low-(LDL-C) and high density lipoprotein (HDL-C), non-HDL cholesterol and triglycerides (TG) with total and gamma' fibrinogen concentration as well as plasma clot properties.

Methods: This cross-sectional study included 1250 healthy participants. Blood lipids were measured (Cobas Integra 400 plus) and LDL-C calculated. Clot properties [lag time, slope, maximum absorbance and clot lysis time (CLT)] were determined (turbidimetry) and total fibrinogen (Clauss assay) and gamma' (ELISA) measured. Lipids were divided into healthy and cardiovascular disease (CVD) risk categories and differences in fibrinogen and clot properties investigated using t-tests and ANCOVA.

Results: Increased LDL-C, non-HDL-C and decreased HDL-C were associated with increased total fibrinogen concentration and a prothrombotic clot phenotype. Adjustment for total fibrinogen nullified the association with clot properties except for CLT which remained

TABLE 1 Direction of associations between risk related lipid categories and clot properties

CVD-risk lipid category	Total fibrinogen	Fibrinogen γ´	Lag time	Slope		Maximum absorbance		CLT	
↑TC	-	-	-	↑	-	↑	-	↑	↑
↑LDL-C	↑	-	-	↑	-	↑	-	↑	↑
↑Non-HDL-C	↑	-	-	↑	-	↑	-	↑	↑
↓HDL-C	↑	-	-	↑	-	↑	-	↑	↑
↑TG	-	-	-	↓	-	↓	-	↑	↑

- No association; ↑ Increase; ↓ Decrease; Second column for slope, maximum absorbance and CLT present associations after adjustment for total fibrinogen

increased in all CVD-risk lipid categories. There was no association between any of the lipids and gamma´ fibrinogen. Increased TG were however associated with decreased slope and maximum absorbance.

Conclusions: CVD-risk associated lipid levels were not only associated with increased total fibrinogen concentration but also with a pro-thrombotic clot phenotype in this healthy population. Additional factors other than increased fibrinogen, contributed to slower lysis in individuals with CVD-risk lipid levels. Fibrinogen gamma' levels showed no association with any of the blood lipids. The relationship of TG needs to be investigated further.

OC 19.5 | Recombinant ADAMTS-4 Enhances tPA-mediated Fibrinolysis in Ischemic Stroke

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Background: Thrombolysis using intravenous administration of tissue-type plasminogen activator (tPA) remains the only approved pharmacological treatment to induce arterial recanalization in acute ischemic stroke. Notably, a growing body of evidence suggests that some metalloproteases play a role in fibrinolysis regulation and could accordingly be used to increase tPA efficiency.

Aims: To investigate the interactions between a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4, a metalloprotease mainly involved in the degradation of chondroitin sulfate proteoglycans) and the fibrinolytic system and its potential use as an ischemic stroke treatment.

Methods: Clot lysis and substrate cleavage experiments were performed to explore the effects of ADAMTS-4 on the fibrinolytic system. Then, to investigate the effects of ADAMTS-4 on tPA efficiency as a stroke treatment, we performed an experimental model of thromboembolic stroke in mice allowing tPA-induced reperfusion, including experiments with ADAMTS-4 knockout mice. Lastly, we investigated the interactions between plasmatic ADAMTS-4 levels and different stroke outcomes in a cohort of ischemic stroke patients.

Results: We demonstrated that ADAMTS-4 increases tPA fibrinolytic activity in plasma by cleaving plasmin(ogen) in a dose and time-dependent manner, thereby increasing the resulting proteolytic activity of plasmin. Accordingly, concomitant administration of ADAMTS-4 with tPA improved stroke outcome in mice. In contrast to exogenously administered recombinant ADAMTS-4, endogenous ADAMTS-4 did not play a key role in stroke outcome according to our human and transgenic mice experiments.

Conclusions: Recombinant ADAMTS-4 enhances tPA-mediated fibrinolysis and reduces ischemic lesion size after thromboembolic stroke.

OC 28.1 | Blocking of Tissue Factor Signaling in Breast Cancer Inhibits Tumor Metastasis

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Background: Tissue Factor (TF) expression in breast cancer is associated with higher tumor grade, metastasis and poor survival. Classically, it has been thought that TF influences cancer progression through two distinct pathways; it facilitates cellular signaling pathways to promote tumor growth and it initiates blood coagulation to promote survival of metastatic cells. However, the role of TF signaling in metastasis has never been addressed.

Aims: To investigate whether TF signaling influences metastasis and to address the cellular processes associated with TF-dependent metastasis.

Methods: Effects of TF signaling in metastasis *in vivo* were studied after graftment of MDA-MB-231-mfp cells, a highly aggressive subclone of the claudin-low breast cancer cell line MDA-MB-231, in a NOD-Scid-gamma (NSG) mouse breast cancer model. Effects of TF on metastasis *in vitro* were studied using a matrigel invasion assay. TF-dependent gene regulation was studied using real time PCR.

Results: We show *in vivo* that the monoclonal TF antibody 10H10, which blocks TF signaling, inhibits metastasis 10-fold, irrespective of its effects on primary tumor growth, and 10H10 reduces invasion by 3-fold. As 10H10 does not inhibit TF-dependent coagulation and thus

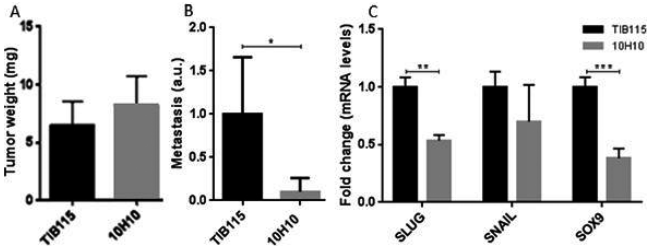

FIGURE 1 A) Tumor weight with 10H10 or control antibody B) Metastasis in the lungs C) Reduced CSC marker gene expression after 10H10 treatment in vitro

survival of metastatic cells, we argued that TF signaling is directly responsible for pro-metastatic events in the primary tumor. TF signaling inhibition by 10H10 led to a reduction in cancer stem cells (CSCs) in mammosphere assays and in mouse models for CSC behavior; and a 2-fold reduction of SLUG and SOX9 expression, the combination of which influences CSC genesis. Finally, in tumor specimens from 574 patients we found an association between TF expression and the CSC marker aldehyde dehydrogenase 1 (ALDH1) (P=0.001);TF expression was also highly associated with metastasis in ER-negative tumors (P< 0.005).

Conclusions: TF signaling inhibition leads to a reduced CSC transcriptional program, a reduction in primary tumor-resident cancer stem cells and thus a reduction in metastasis.

OC 28.2 | Thrombin Cleavage of Osteopontin Plays an Important Role in Melanoma Growth and Progression

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Background: Osteopontin (OPN) is a matricellular multifunctional protein with a highly conserved RGD domain that binds to a wide range of integrins. Thrombin cleavage at Arg153 generates OPN-R and OPN-CTF (C-terminal fragment). OPN-R, with SVVYGLR at its C-terminus, binds to a new subset of integrins ($\alpha4\beta1$and $\alpha9\beta1$) (Fig.1).

OPN expression is markedly increased in inflammatory conditions. It has been reported that growth of B16 melanoma is suppressed in OPN knock-out (KO) mice. However, despite extensive studies on the importance of OPN in cancer progression, the role of thrombin cleavage of OPN is unknown.

Aims: To investigate the role of thrombin cleavage of OPN in B16 melanoma growth.

Methods: Mice with OPN resistant to thrombin cleavage were generated by replacing Arg153 with Ala (OPN_{R153A}; OPN knock in [KI]). B16 cells were inoculated subcutaneously in mice, and tumor growth was

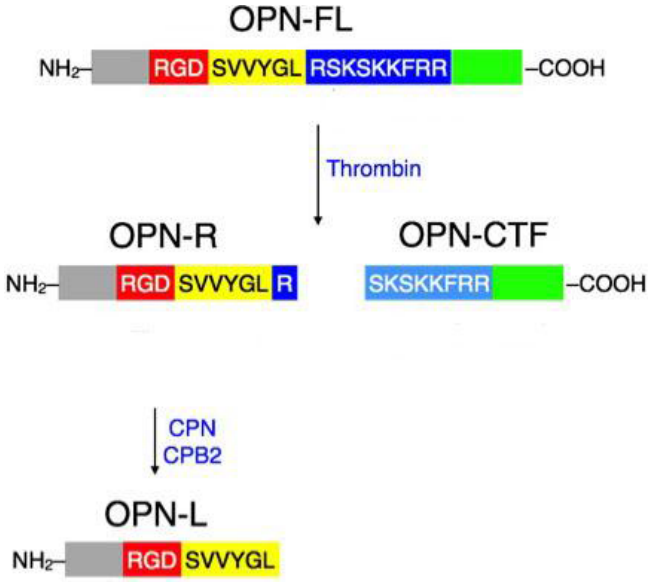

FIGURE 1 Schematic representation of thrombin and CPB2-mediated OPN cleavages

monitored. B16 cell adhesion and migration in response to various forms of recombinant OPN were performed *in vitro*.

Results: Robust tumor growth was observed in wild-type (WT) mice while being suppressed to the same extent in both OPN KO and OPN_{R153A}-KI mice when monitored as volume over time or weight upon sacrifice (Fig.2).

B16 cell adhesion assays showed a 4-fold increase in adhesion to OPN-R compared to OPN-full length (OPN-FL). Migration assays showed a 2-fold increase with OPN-R and OPN-CTF compared to OPN-FL. Preliminary histology showed a trend of more necrosis in OPN KO and KI compared to WT not related to tumor weight.

Conclusions: Our data provides the first *in vivo* demonstration of the importance of thrombin cleavage of OPN in cancer biology. Despite the presence of RGD and the other functional domains in OPN_{R153A}, the OPN KI mice demonstrated diminished B16 tumor growth equal to its absence, indicating that thrombin cleavage of the host OPN plays a critical role in melanoma growth *in vivo*.

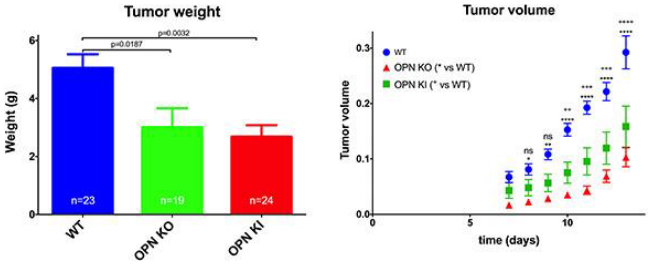

FIGURE 2 Decreased B16 tumor growth in OPN KO and OPNR153A KI mice compared to WT mice

OC 28.3 | Thrombin-PAR-1 Promotes Pancreatic Ductal Adenocarcinoma Through Tumor Cell Expression of Urokinase Plasminogen Activator and Receptor

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Background: Pancreatic ductal adenocarcinoma (PDAC) accounts for greater than 85% of pancreatic cancer cases and is associated with extremely poor patient survival. Analyses of PDAC in patients and animal models have demonstrated that it is associated with robust coagulation system activity driven by high tissue factor (TF) expression in the tumor cells.

Aims: To determine the mechanisms of tumor cell associated thrombin/PAR-1 function in driving PDAC tumor cell growth.

Methods: Tumor cell lines (termed KPC2) were derived from mice in which PDAC was induced by activation of two key pancreatic cancer alleles, *Kras*^{G12D} and *Trp53*^{R172H}. Tumor growth of KPC2 cells were evaluated either (*i*) in mice with genetically imposed deficits in coagulation and fibrinolytic system components or (*ii*) using KPC2 cells in which tumor-derived factors including TF, PAR-1, urokinase plasminogen activator (uPA), and uPA receptor (uPAR) were genetically reduced by shRNA or eliminated using CRISPR-Cas9.

Results: In transplant studies, primary tumor growth and experimental metastasis of KPC2 cells were significantly reduced by reduction of TF expression by the tumor cells or reduction of prothrombin in the microenvironment (*i.e.*, fII^{low} mice which constitutively express 10% of normal prothrombin). Knockdown of PAR-1 in KPC2 cells resulted in significantly diminished tumor growth whereas knockout of PAR-1 completely prohibited tumor growth. PAR-1 deficiency was linked to diminished expression of uPA and uPAR by KPC2 cells. Notably, knockout of either uPA or uPAR in KPC2 cells resulted in a similar significant reduction in tumor growth and metastasis. Analysis of parental KPC2 cells in knockout mice indicated that PDAC tumor cell growth was linked to plasminogen but independent of plasmin-mediated fibrinolysis.

Conclusions: Our results suggest that thrombin/PAR-1 can drive PDAC growth and dissemination in part through mechanisms linked to expression of uPA/uPAR by the tumor cell and plasminogen activation in the microenvironment.

OC 28.4 | Mechanisms Linking Fibrin(ogen) Structure/Function to Tumor Metastasis

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Background: Fibrin(ogen) is a multifunctional protein that can form a polymer and engage multiple cell types, including platelets and immune cells, by serving as a ligand for several cell surface receptors. Fibrinogen is a major determinant of metastasis; however, the mechanisms by which fibrin(ogen) drives metastasis have remained elusive.

Aims: Determine the role of fibrin polymerization and fibrin(ogen) engagement of integrins $\alpha_{IIb}\beta_3$ and $\alpha_M\beta_2$ in metastasis.

Methods: We performed experimental metastasis assays using Lewis lung carcinoma in immunocompetent mice carrying specific fibrinogen structure/function alterations.

Results: Mice carrying a mutant fibrinogen that retains clotting function but lacks the $\alpha_M\beta_2$ binding motif (Fib$\gamma^{390\text{-}396A}$) developed significantly fewer pulmonary metastases than controls, suggesting that fibrin(ogen)-a_Mb_2 interactions drive metastasis. Notably, soluble fibrinogen retains the capacity to interact with numerous ligands, but is a poor ligand for a_Mb_2. Metastatic potential in Fib^{AEK} mice carrying fibrinogen "locked" in the soluble state was diminished relative to control mice. However, Fib^{AEK} mice had elevated metastatic potential relative to mice with complete fibrinogen deficiency. Metastasis in Fib$\gamma^{\Delta5}$ mice, carrying fibrinogen lacking the γ chain $a_{IIb}b_3$ binding motif, was indistinguishable from controls.

Conclusions: These studies suggest that thrombin-mediated fibrin polymerization promotes metastasis, but soluble fibrinogen retains some significant prometastatic capacity. These data also imply a role for fibrin(ogen)-leukocyte interactions mediated by a_Mb_2 in metastasis. Surprisingly, loss of the fibrinogen γ chain $\alpha_{IIb}\beta_3$ binding motif had no impact on metastasis. These results suggest that platelets might bind polymerized fibrin at other sites, and/or fibrin interactions with other matrix proteins capable of binding $a_{IIb}b_3$ (e.g., fibronectin, vWF) are sufficient to support platelet functions required for metastasis.

OC 28.5 | Biomarkers of Inflammation and Coagulation in Long-term Survivors of an Adult Cancer - Results from the Gutenberg Health Study

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Ophthalmology, Mainz, Germany, [8]University Medical Center of the Johannes Gutenberg-University Mainz, Center for Cardiology I, Mainz, Germany, [9]DZHK (German Center for Cardiovascular Research), Partner Site RhineMain, Mainz, Germany, [10]University Medical Center of the Johannes Gutenberg-University Mainz, Institute for Clinical Chemistry and Laboratory Medicine, Mainz, Germany

Background: Cancer is among the leading causes of morbidity and mortality worldwide. The advancements in treatment and improved detection of early cancers resulted in a steady increase of cancer survivors over the years. However, the long term toxic effects of chemotherapy and radiotherapy resulted in increasing incidence of cardiovascular disease (CVD) in survivors.

Aims: To explore the associations of inflammatory and coagulation biomarkers as well as multiple cardiovascular risk factors (CVRFs) in long-term cancer survivors (cancer diagnose ≥ five years) from a large adult population-based study sample.

Methods: Biomarkers and presence of CVRFs and were compared in individuals with (n=723) and without (n=13626) history of cancer from the Gutenberg Health Study.

Results: Long-term cancer survivors showed higher fibrinogen concentration (ß:7.30, 95%CI:1.53-13.1), vWf (ß:5.29, [0.35-10.2]) and FXI levels (ß:2.65, [0.17-5.14]), compared to individuals without cancer history, independently of age, sex, CVRFs, CVD and antithrombotic therapy. Cancer survivors with CVD presented particularly higher vWf (143±48%) and lower protein S levels (94±26%) compared to survivors without CVD (vWF:124±40%; protein S:107±17%), in an age and sex weighted analysis. A history of cancer increases mortality by 73% independent from the presence of traditional CVRFs. Worst survival was found in individuals with history of cancer and presence of CVD.

Conclusions: This is a first population-based study investigating inflammation and coagulation profile in long-term cancer survivors. Cancer survivors showed a worse inflammation and coagulation profile and increased overall mortality, independent of traditional CVRFs, compared to individuals without history of cancer. These results underline the need to further investigate plasma biomarkers as complementary cardiovascular risk predictors in cancer survivors.

OC 54.1 | Intermolecular Interactions in Double-stranded Half-staggered Fibrin Oligomers

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Background: The structural scaffold of the blood clot is provided by the filamentous fibrin network, which ultimately consists of fibrin monomers. Upon polymerization, fibrin forms several important intermediate structures fibrin oligomers, protofibrils and fibrin fibers. Due to their transient nature, the atomic structure of these polymeric structures is nearly impossible to determine using experimental techniques.

Aims: The internal structure of the fibrin polymer is necessary to understand the mechanical properties of the fibrin polymers. The aim of this work was to determine the structure of the fibrin oligomers in an atomistic level of detail.

Methods: We used combined experimental and computational techniques, including atomic force microscopy, detailed analysis of the crystal structures available from the PDB, multiscale MD simulations and Monte Carlo docking.

Results: We obtained the atomic structure of the two-stranded half-staggered fibrin oligomer (Figure 1). We added to this structure the missing residues that were not resolved previously by X-ray crystallography. The resolved structure allowed us to identify the amino-acid residues that are important in formation of the double-stranded fibrin oligomers. We also showed how the formation of inter-monomer contacts change the flexibility of fibrin oligomers.

Conclusions: The atomic model computationally reconstructed and experimentally validated in this work represent a significant development necessary for structure-based understanding of unique biochemical and material properties of fibrin polymers.The structures are availible for downloading in the PDB format at http://faculty.uml.edu/vbarsegov/research/fibrin.html

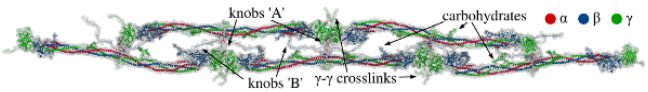

FIGURE 1 Reconstructed structure of double-stranded half-staggered fibrin oligomer

OC 54.2 | Role of Fibrinogen αC Domain in Fibrin Fibre Lateral Aggregation and αC Connector Region In Longitudinal Fibre Growth; Complex Interactions of the αC Region that Regulate Clot Structure and Function

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Background: Fibrinogen (FGN) α-chain is the largest FGN chain, two thirds extend from the D-region (221-610) to form a connector region (221-392) and a globular αC domain (392-610). The αC domain and its connector contain sites for α_2-antiplasmin cross-linking and α-α chain cross linking by FXIII. Previous data on FGN truncated at 251 (middle of connector region) indicated that the αC region is important for lateral aggregation of fibres.

Aims: To investigate the influence of the αC region components on clot structure and function with two new FGN variants deleting the connector region and/or the C-terminal globular domain.

Methods: Two recombinant FGNs truncated at residues 390 (loss of the αC domain) and 220 (removal of connector region + αC domain) and WT FGN were produced in CHO cells. FGNs were characterised by SDS-PAGE for integrity, homogeneity and cross-linking by FXIII. Clot structure was studied by turbidity and confocal microscopy with alexa-488 FGN variants.

Results: Two FGNs were produced with a truncated α-chain of expected size (α390 42kDa and α220 25kDa). Turbidity showed decreased maximum absorbance for α390 (0.27) compared to WT clots (0.49), but higher for α220 (0.73). Lag time was increased (150 α390 and 152 α220 vs 112 WT sec) and clotting rate was reduced in both truncations compared to WT (0.034 α390 and 0.020 α220 vs 0.063 WT OD/min). SDS-PAGE showed reduced α-chain but normal γ-chain cross-linking for both α-truncations. Confocal microscopy showed that FGN α390 formed denser clots with thinner fibres and smaller pores, while α220 produced less dense clots containing thick, short and bundled fibres with large pores.

Conclusions: Our data show a clear role for the αC domain in lateral aggregation, but crucially indicate a new role for the αC connector region in longitudinal fibre growth. These findings highlight the importance and complexity of the FGN αC region in clot structure and function.

OC 54.3 | Pharmacologic Enhancement of the Fibrin(ogen)-integrin αMβ2 Interaction Reduces Acetaminophen-induced Liver Injury in Mice through Induction of Matrix Metalloproteinase 12

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Background: Acetaminophen (APAP) overdose is a leading cause of acute liver failure and is linked to activation of the coagulation cascade and deposition of fibrin in the liver. We have shown previously that fibrin(ogen) engagement of the leukocyte $\alpha_M\beta_2$ integrin stimulates expression of matrix metalloproteinase 12 (MMP12) by liver macrophages and promotes liver repair after APAP overdose.

Aims: Because this particular function of fibrin(ogen) can be targeted, in principle, without causing bleeding, we sought to determine the effect of the novel $\alpha_M\beta_2$ allosteric agonist leukadherin-1 (LA-1) on liver injury and hepatic MMP12 expression after APAP overdose.

Methods: Wild-type (WT) C57BL/6J mice were given a hepatotoxic dose of APAP (300 mg/kg, ip) and then treated with LA-1 (0.4 mg/kg, ip) or vehicle (0.4% DMSO in saline) after liver injury had developed (i.e. at 6 and 12 h later). Extent of liver injury was examined 24 h after APAP challenge.

Results: LA-1 treatment significantly reduced APAP-induced liver injury, reducing both ALT levels and hepatic necrosis. Moreover, LA-1 also dramatically enhanced hepatic expression of MMP12 mRNA relative to APAP-treated mice given vehicle. Importantly, in APAP-treated Fib$\gamma^{390\text{-}396A}$ mice, which express a mutant form of fibrin(ogen) incapable of binding $\alpha_M\beta_2$ integrin, hepatic MMP12 mRNA levels were drastically suppressed. Rescue of APAP-challenged Fib$\gamma^{390\text{-}396A}$ mice with recombinant MMP12 (rMMP12) protein markedly reduced hepatic hemorrhage and congestion, decreased ALT levels and restored hepatocyte proliferation to levels seen in APAP-treated WT mice.

Conclusions: The results indicate that LA-1 treatment attenuates APAP-induced liver injury in mice, in part by inducing MMP12 expression, and administration of rMMP12 improves APAP-induced liver injury phenotype. The results suggest that fibrin(ogen)-$\alpha_M\beta_2$ integrin-dependent pathway can be pharmacologically targeted to drive induction of MMP12 and promote liver repair after APAP overdose.

OC 54.4 | Functional Characterisation of Fibrinogen γ' Truncations

H. McPherson[1], C. Duval[1], M. Domingues[2], S. Baker[1], V. Ridger[3], S. Connell[4], H. Philippou[1], R. Ajjan[1], R. Ariëns[1]

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Background: Fibrinogen (FGN) γ' is a splice variant, in which the C-terminal 4 residues of the γ-chain are replaced with 20 different residues that are more negatively charged. We previously showed that plasma-purified γA/γ' FGN influences polymerisation and protofibril formation resulting in clots with reduced protofibril packing and heterogeneous networks.

Aims: Determine the mechanism by which γ' influences fibrin structure.

Methods: Recombinant FGN with γ'-chain truncations (γ'0, 0-12 and 0-16: deleting all, 8 and 4 residues from the C-terminus respectively), full-length FGN γ' and γA were produced in CHO cells as homodimers. Integrity of the FGN chains was established by SDS-PAGE. Fibrin formation and fibrinolysis was characterised by turbidity and confocal microscopy with alexa 488 FGNs.

Results: SDS-PAGE confirmed different γ' lengths were produced with intact α and β-chains. Turbidity showed marginally decreased maximum absorbance + or - FXIII between γ' truncations and γA. Average clotting rate (10^{-3}OD/sec) was slower with increasing γ' length, both - FXIII (γ' 0.36, γ'0-16 0.37, γ'0-12 0.46) and + FXIII (γ' 0.39, γ'16 0.50, γ'12 0.48) compared to γA (0.87) and (0.92). No differences were seen with fibrinolysis for any truncations + or - FXIII compared to γA. Confocal microscopy showed no differences in the number of fibres between the truncations and γA. However, the truncated fibres were less straight compared to γA, and fibre straightness correlated negatively with γ' length.

Conclusions: Differences in clot structure for plasma purified γA/γ′ are not fully reproduced in this recombinant protein and its truncations. It is unknown what causes this discrepancy but unidentified plasma factors may play a role. Further work is needed to test if γ′ is modified in circulation, the two tyrosine residues in recombinant FGN γ′ chain are sulphated or if differences are due to homodimer versus heterodimer formation.

OC 54.5 | Interaction of Fibrin with the VLDL Receptor: Mapping the VLDL Receptor-binding Site in Fibrin βN-domains

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Background: Our previous studies revealed that interaction of fibrin with the very low density lipoprotein receptor (VLDLR) promotes transendothelial migration of leukocytes and thereby inflammation and localized complementary binding sites to fibrin βN-domains and CR domains of VLDLR. Since this interaction is a potential therapeutic target for controlling fibrin-dependent inflammation, one of the major goals of our current studies is to establish its molecular mechanism and develop its specific inhibitors.

Aims: The major objectives of the present study were to further clarify the molecular mechanism of fibrin-VLDLR interaction and identify amino acid residues in fibrin βN-domains involved in this interaction.

Methods: Recombinant VLDLR(1-8) fragment containing all CR domains of VLDLR and dimeric $(\beta 15\text{-}66)_2$ fragment corresponding to a pair of fibrin βN-domains were expressed in *E. coli*. Various mutants of $(\beta 15\text{-}66)_2$ were generated by site-directed mutagenesis. Interaction of VLDLR(1-8) with $(\beta 15\text{-}66)_2$ and its mutants was studied by ELISA and SPR.

Results: We found that interaction of $(\beta 15\text{-}66)_2$ with VLDLR(1-8) strongly depends on ionic strength and chemical modification of all Lys or Arg residues in $(\beta 15\text{-}66)_2$ results in abrogation of this interaction. To identify which of these residues are involved in the interaction, we mutated Lys and Arg in each of the three positively charged Lys/Arg clusters of $(\beta 15\text{-}66)_2$ and tested affinities of the resultant mutants to VLDLR(1-8). The experiments revealed that the 2nd and 3rd clusters make the major contribution to this interaction while contribution of the 1st cluster is moderate.

Conclusions: The results obtained identified Arg and Lys residues in fibrin βN-domains that are critical for fibrin-VLDLR interaction. They provide valuable information for the development of specific inhibitors of this interaction. They also suggest that this interaction employs the "double-Lys/Arg" recognition mode proposed for the interaction of the LDL receptor family members with their ligands.

HEMORRHAGIC DISORDERS, HEMOPHILIA

OC 07.1 | ThromboGenomics: HTS Diagnosis of Inherited Bleeding, Thrombotic, Coagulation and Platelet Disorders

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Background: Inherited bleeding, thrombotic, coagulation and platelet disorders (BPDs) have a range of frequencies from 1:5000 live male births for haemophilia to rare platelet disorders with only a few cases described.

Aims: ThromboGenomics uses targeted High Throughput Sequencing to provide an efficient and timely molecular diagnosis for patients suspected of having an inherited BPD caused by variants in one of the 78 ISTH-SSC approved Tier 1 BPD genes.

Methods: Over 1,000 samples have been sequenced using ThromboGenomics, with 670 in partnership with the GMC@CUH as a clinical diagnostic service. We have reported clearly pathogenic (class 5) or likely pathogenic (class 4) variants in 60% of coagulation cases, 52% of platelet cases and 54% of thrombotic cases. To assess the proportion of novel pathogenic variants identified through ThromboGenomics we used the Human Gene Mutation Database (HGMD).

Results: In patients with coagulation disorders, 25% of the clearly or likely pathogenic variants reported were not listed in HGMD. For the platelet and thrombotic disorders, 62% and 58%, reported clearly and likely pathogenic variants were not in HGMD. The high proportion of novel variants reflects the molecular heterogeneity of the group of rare diseases under investigation. The relatively high percentage of clearly pathogenic variants in the coagulation disorders is due to the established variant databases for haemophilia and von Willebrand disease. The reported clearly pathogenic and likely pathogenic variants with clinical information (Human Ontology Phenotype [HPO] terms) are deposited in ClinVar to support sharing of data improving the robustness of clinical reporting.

Conclusions: Through ThromboGenomics and BRIDGE-BPD we have succeeded in linking an international gene discovery project with the delivery of an affordable diagnostic service. The high case load of ThromboGenomics has allowed the identification of more than 150 novel pathogenic BPD variants for deposition in a publically accessible database.

OC 07.2 | An International Prospective Cohort of Rare Bleeding Disorders: Results of a Follow-up Study on Patients with Factor XIII Deficiency (PRO-RBDD)

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Background: The PRO-RBDD was designed to collect data on clinical manifestations, laboratory phenotype and treatment (on-demand or prophylaxis) of patients with FXIII deficiency. Data collection began in February 2013 and was organized into two different datasets:

1) historical data, collected at baseline;

2) follow-up data, collected every six months, over three years.

A previous analysis focused on the historical data suggested that 15% of FXIII:C could be needed to prevent spontaneous major bleedings (central nervous system and gastrointestinal tract bleeding, haemarthrosis and haematomas).

Aims: To evaluate benefits and complication of long term treatment, in patients with FXIII deficiency, with particular attention to those with FXIII:C < 15%, during the follow-up period.

Methods: Bleeding incidence and cumulative incidence of the first bleeding treated with replacement therapy were calculated in patients in on-demand therapy and in prophylaxis.

Results: Data on 61 FXIII-deficient patients (31 F; 30 M) from 13 Hemophilia Treatment Centres were registered. Forty patients had FXIII:C < 15%. Patients were followed-up for a median of 926 days (IQR: 721-1099). Table 1 reports the bleeding incidence in patients in on-demand therapy compared to those in prophylaxis (9-59U/Kg/month FXIII concentrate); a significant reduction of bleeding episodes in prophylaxis was observed in all patients and particularly in those with FXIII:C< 15%.

TABLE 1 bleeding incidence

All patients		Patients with FXIII:C <15	
on-demand	on prophylaxis	on-demand	on prophylaxis
0.90	0.26	1.57	0.27
IR=0.29, IC95% 0.23-0.37		IR=0.17, IC95% 0.14-0.23	

Table 2 reports the cumulative incidence of the first bleeding treated with replacement therapy at 1300 days of follow up in patients in on-demand therapy compared to those on prophylaxis; a reduction of bleeding episodes in prophylaxis was observed in all patients and particularly in those with FXIII:C< 15%.

TABLE 2 cumulative incidence

All patients		Patients with FXIII:C <15	
on-demand	on prophylaxis	on-demand	on prophylaxis
36%, 95%CI 15-55	7%, 95%CI 0-15	60%, 95%CI 20-80	8%, 95%CI 0-18
Log-Rank test 0.007		Log-Rank test 0.0001	

No adverse events were observed.

Conclusions: In conclusion, the beneficial effect and the safety of FXIII prophylaxis to prevent major bleeding episodes is confirmed, making this treatment strongly recommendable in FXIII-deficient patients.

OC 07.3 | Profile of Mutations Identified in the 3Winters-IPS Project on a Cohort of European Patients with Previously Diagnosed Type 3 von Willebrand Disease

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Background: Type 3 von Willebrand disease (VWD3) is a rare autosomal recessively inherited bleeding disorder resulting from mutations in the von Willebrand factor gene (*VWF*). VWD affects approximately 1:10^6 outbred populations, but is found in higher proportions of consanguineous families.

Aims: To determine the genetic basis of VWD in a cohort of European patients said to have VWD3.

Methods: Patients classified with VWD3 were recruited into the study following informed consent. DNA was extracted from buffy coat. Venous blood samples provided measurement of VWF levels. PCR and Sanger sequencing/ next generation sequencing and multiplex-ligation dependent probe amplification were used in Hamburg and Sheffield to confirm previously identified mutations or to seek previously unidentified mutations.

Results: 84 individuals from 15 European centres with a VWD3 diagnosis were analysed, comprising 55 (65%) females and 29 (35%) males. Most had 2 mutations, n=73; 43 were homozygous (51%) and 32 compound heterozygous (38%). 9 patients (11%) had a single heterozygous mutation identified. 4 mutation types were common; small deletion (30.2%), nonsense (25.2%), splice (21.4%), and missense (19.5%). Large deletions of ≥1 exon comprised 3.1% and large duplication 0.6%. Disease severity was assessed through VWF:Ag level, with 29 individuals having VWF:Ag < 1IU/dL. 32 had levels ≥1 and < 5, whilst 23 were suspected of being infused with factor concentrate (VWF:Ag levels up to 94 IU/dL). 11 cases were suggested to have severe type 1, 6 severe type 2 and other VWD types, 11. 14 patients had 1 or 2 missense changes, 9/14 lost a cysteine residue of which 3 were classified as VWD3.

Conclusions: Null mutations comprised 81% of variants identified, replicating previous findings in similar cohorts. Only 3 missense alleles were present in the propeptide, the majority were found in exons 36-51.

OC 07.4 | Endoplasmic Reticulum Stress and the Unfolded Protein Response in Congenital FVII Deficiency

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Background: The *F7* gene mutations p.Q160R (FVII-Q100R), p.I289del (FVII-del229I) and p.A354V-p.P464Hfs (FVII-A294V;404delC) are associated with very low circulating factor VII (FVII) levels (FVII deficiency) and a bleeding phenotype. The secretory defects seen in patients can be attributable to misfolded mutant FVII. Accumulation of misfolded proteins within the endoplasmic reticulum (ER) can cause ER stress and activation of the unfolded protein response (UPR). However, little is known about the effects of misfolded FVII on these responses.

Aims: To investigate ER stress and UPR responses in cells expressing FVII-Q100R, FVII-del229I and FVII-A294V;404delC.

Methods: HEK293 cells transiently expressing wild-type or mutant FVII were used as cellular model. FVII antigen was measured by ELISA and intracellular localization was assessed by confocal immunofluorescence microscopy. Binding of chaperones to FVII was investigated by non-reducing SDS-PAGE and Western analysis. ER stress was assessed by luciferase reporter assay. UPR mediators were measured by Western analysis and qRT-PCR.

Results: FVII antigen levels in medium from cells expressing the FVII mutants were severely reduced (FVII-Q100R) or undetectable (FVII-del229I, FVII-A294V;404delC). Increased colocalisation with ER marker PDI and a stronger association to the chaperones GRP-94 and BiP of FVII mutants compared to the wild-type was observed. Elevated ER stress and activation of UPR signalling were detected in cells expressing FVII mutants.

Conclusions: FVII-Q100R, FVII-del229I and FVII-A294V;404delC were poorly secreted, possibly due to a misfolding process resulting in defective intracellular trafficking. Furthermore, FVII mutants had increased association with chaperones indicating retention in ER inducing ER stress and activation of UPR. Small molecules that are known to alleviate ER stress could represent a therapeutic option for FVII deficiency caused by misfolded proteins.

OC 07.5 | Characterization of GGCX Mutations Identified in VKCFD1 Patients

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Background: Vitamin K Dependent Coagulation Factor Deficiency type 1 (VKCFD1) is a rare hereditary bleeding disorder caused by mutations in gamma-glutamyl carboxylase (GGCX) gene. GGCX gamma carboxylates vitamin K dependent (VKD) proteins, including blood clotting factors which is essential for maintaining hemostasis. Until now there are 26 GGCX missense mutations reported to cause VKCFD1.

Aims: The aim of this study is to characterize the effect of all GGCX missense mutations on VKD coagulation factors and to evaluate the effective dose of vitamin K (K) needed for patient treatment.
Methods: A GGCX knockout (KO) HEK293T cell line was generated by CRISPR/Cas9 technology. The cDNAs of GGCX together with F10 or protein C were cloned into a bicistronic vector. GGCXKO cells were transfected with wild-type (WT) and mutant variants and were treated with different K concentrations to determine half maximal effective concentrations (EC50). The amount of carboxylation was measured by ELISA based assay.
Results: CRISPR/Cas9 gene editing resulted in GGCX deficient cells. Compared to WT GGCX, most missense mutations revealed decreased carboxylation levels for F10 and protein C. However, elevated K concentrations rescued the amount of carboxylation for some mutations V255M, R325Q, R476H and mutations R83P, W157R, S300F showed very less recovery in carboxylation levels even at high K concentrations.
Conclusions: Our data suggest that patients with mutations V255M, R325Q, R476H will show reversible phenotype where therapy with K will lead to normal coagulation. The EC50 calculated by our assay will be an indication for the therapeutic dosage. Patients with mutations R83P, W157R, S300F will never reach physiological coagulation under high dose of K treatment.
This suggests that mutations showing recovery with high K treatment can be in regions pertaining to propeptide or K binding. Hence, this approach will elucidate structural regions involved in carboxylation, propeptide or substrate binding.

OC 08.1 | A Discontinuous Autoinhibitory Module Masks the A1 Domain of VWF

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Background: In the absence of shear an isolated fragment containing the A1 domain (defined here as residues 1272-1458) but not full-length VWF binds GPIbα spontaneously, suggesting the presence of an autoinhibitory module (AIM) in VWF, but its structure remains unclear. Two VWF fragments containing residues 1238-1472 (termed longA1) and 1261-1472 (shortA1), respectively, exhibit disparate binding affinities for GPIbα and the platelet.
Aims: To understand why shortA1 binds GPIbα much better than longA1 or full-length VWF.
Methods: Both shortA1 and longA1 proteins are expressed from transfected baby hamster kidney cells. Their binding to purified GPIb-IX and human platelets are detected by ELISA and flow cytometry. Their dynamics are characterized by hydrogen-deuterium exchange (HDX) mass spectrometry.
Results: Both proteins are monomeric in solution as judged by gel filtration chromatography and sedimentation velocity analytical ultracentrifugation, although shortA1 is much more prone to aggregation. ShortA1 binds GPIbα with ~40 nM affinity, while longA1 binding to GPIbα mimics that of full-length VWF. Residues in longA1, particularly those in N- and C-terminal sequences flanking the A1 domain, and in helix a1, loops a1b2 and b3a2 of A1, reported markedly reduced HDX than their counterparts in shortA1. This HDX-protected region in A1 overlaps with the GPIbα-binding interface and is clustered with type 2B VWD mutations. HDX of ristocetin-bound longA1 mimics that of shortA1. Binding of ristocetin to 1458-1472 in longA1 desorbs residues 1238-1271 from A1, as judged by changes in HDX, and enables longA1 binding to GPIbα.
Conclusions: The N- and C-terminal sequences flanking A1 (1238-1271, 1459-1472) form cooperatively an integrated AIM that interacts with the HDX-protected region in A1 and impedes its binding to GPIbα. The masking of the A1 domain by a discontinuous AIM has significant implications for the pathogenesis of type 2B VWD and the shear-induced activation of VWF activity.

OC 08.2 | Allele-specific Inhibition of von Willebrand Factor p.Cys2773Ser Restores a Severe Multimerization Defect

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Background: Treatment of von Willebrand disease (VWD) focusses on increasing von Willebrand factor (VWF) levels through administration of desmopressin or VWF concentrates. Both therapies usually raise VWF sufficiently, however the production of mutant VWF remains uninterrupted. Persistence of mutant VWF could itself lead to hemostatic problems like thrombocytopenia in type 2B VWD or the development of angiodysplasia. Inhibition of mutant *VWF* in dominant-negative VWD might overcome these effects.
Aims: Correct the abnormal multimer pattern of the dominant-negative VWF p.Cys2773Ser mutation applying allele-specific inhibition.
Methods: Allele-specific inhibition of *VWF* was performed by small interfering (si)RNAs. The siRNAs were not developed against the mutation itself, but to single nucleotide polymorphisms (SNPs) in *VWF*. This allows application of the siRNAs to different mutations, provided the patient is heterozygous for the specific SNP. siRNA mediated allele-specific inhibition was studied in HEK293 cells transiently transfected with two different VWF constructs that carried either of the two alleles of a SNP with one of the alleles also carrying VWF p.Cys2773Ser. Multimerization improvements were visualized by multimer analysis.
Results: Efficient and allele-specific siRNAs were identified for four different SNPs. Transfection of normal VWF and VWF p.Cys2773Ser resulted in an abnormal multimer pattern similar to the pattern in a heterozygous patient carrying this mutation. siRNA mediated allele-specific inhibition of the mutant allele by targeting the SNP allele linked to p.Cys2773Ser clearly improved, or for some siRNAs even restored, the multimer pattern.

Conclusions: siRNAs can discriminate *VWF* alleles by discrepancy of one nucleotide of a SNP. The clear phenotypic improvements observed after inhibition of the *VWF* p.Cys2773Ser allele show that siRNA mediated allele-specific inhibition of dominant *VWF* alleles is a promising therapeutic approach for dominant-negative VWD.

OC 08.3 | Altered Sialylation in Patiens with Low von Willebrand Factor Levels

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Background: Von Willebrand disease is the most commonly inherited bleeding disorder. However, the mechanism responsible for VWD in patients with mild reduction of VWF levels (30-50 IU/dL), remains poorly understood. Interestingly, mutations in *VWF* gene occur in less than 50% of these patients.

Aims: To investigate the VWF glycosylation profile in patients with low VWF levels.

Methods: We study a low VWF Irish cohort (n=126) with historically low VWF:Ag, and/or VWF:RCo, and/or VWF:CB. To characterize the glycosylation of VWF, we developed a series of novel lectin ELISAs (SNA, MAAII, RCAI, ECA, UEAI) using a deglycosylated capture antibody coupled with high sensitivity/HRP for detection.

Results: Concerning the clearance of VWF, 94% patients presented a normal VWFpp:VWFAg ratio and it is mirrored with clearance data after DDAVP administration. In contrast, VWF glycosylation profile was significantly different between low VWF patients vs healthy donors. In particular, α(2-6) sialic acids content was significantly reduced and subterminal β-galactoses detection was higher in patients vs controls. Interestingly, we found an inverse correlation between VWF half-life and galactose exposure in patients with shorter VWF half-life. No significant differences were detected in α(2-3) sialic acids, lactosamine extensions or H-antigen. To characterize the physiological modifications on VWF glycans, we studied pre- and post-DDAVP samples and found that VWF presented more α(2-6) sialic acid and less α(2-3) sialic acid 1 hour post-DDAVP infusion, when compared to pre-DDAVP VWF.

Conclusions: In summary, altered α(2-6) sialic acids content is a common feature in patients witn low VWF levels, which might affect VWF clearance through galactose receptors. However, these changes in the glycosylation could also suggest an alteration in the folding and trafficking of VWF. Further, physiological modifications observed in VWF carbohydrates during its secretion and circulation might explain the normal VWF clearance.

OC 08.4 | The N-terminal TIL'-E' Domains of von Willebrand Factor Requires a Structurally Intact VWD3 Domain for Optimal Factor VIII Binding

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Background: Von Willebrand factor (VWF) protects factor VIII (FVIII) from rapid clearance from the circulation. Current view is that the major binding site for FVIII is within the N-terminal TIL'-E' domains of mature VWF. However, multiple point mutations outside these domains have been identified that have been associated with bleedings because of markedly reduced FVIII plasma levels.

Aims: The aim of this study is to assess the role of the N-terminal TIL'-E'-VWD3-C8_3-TIL3-E3 domains of VWF for FVIII binding.

Methods: C-terminal truncated domain variants of the TIL'-E'-VWD3-C8_3-TIL3-E3 fragment were obtained. To prevent dimerization cysteine residues at positions 1099 and 1142 were substituted into serine residues. Solid-phase competition assays and Surface Plasmon Resonance analysis were employed to evaluate the binding efficacy to FVIII. Chemical foot-printing mass spectrometry using lysine directed mass tags was utilized to explore structural implications of the truncations.

Results: Binding studies revealed that deletion of the E3 domain did not affect the binding of the fragment to FVIII. Deletions of the TIL3 followed by the C8_3 domain gradually decreased the FVIII binding efficiency. Notably, the TIL'-E' fragment almost did hardly show any interaction with FVIII suggesting that the VWD3 domain is indispensable for binding of TIL'-E' to FVIII. Chemical foot-printing revealed that lysine residues within the VWD3 domain demonstrated increased exposure to the solvent upon subsequent deletion of the E3, TII3-E3 and C8_3-TII3-E3 domains. This implies structural alterations in the VWD3 upon deletion of the C-terminal domains.

Conclusions: This study shows that subsequent C-terminal deletions of the D3 subdomains decreases ability of the D'D3 fragments to bind FVIII.We propose that the VWD3 domain supports the interaction of TIL-E' with FVIII and that the C-terminal domains are required to maintain the structural integrity of the VWD3 domain.

OC 08.5 | The Redox State of the von Willebrand Factor A2 Domain Disulphide Bond Controls Platelet GPIbα Binding

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Background: Platelet adhesion and thrombus formation is strictly dependent on VWF interaction with platelet GPIbα receptor in the shear forces found in arterioles and stenosed vessels. GPIbα binding to the VWF A1 domain is controlled by the force-sensing A2 domain. A disulphide bond linking adjacent cysteine residues 1669 and 1670 at the C-terminus of the A2 domain acts like a plug that when dislodged by sufficient shear force destabilizes the core and initiates unfolding of the domain and GPIbα and ADAMTS13 access.

Aims: We have quantified the redox state of the Cys1669-Cys1670 disulphide plug in healthy donor plasma VWF and assessed the functions of the different redox forms.

Methods: Redox state measurements were performed by differential cysteine alkylation and mass spectrometry, and GPIbα binding and ADAMTS13 proteolysis by standard assays.

Results: Unexpectedly, cysteines 1669 and 1670 exist in three different redox forms: an oxidised disulphide bond (~25%), S-glutathionylation of Cys1670 (~35%) and a reduced dithiol (~40%). Approximately three quarters of circulating VWF, therefore, contains an inactive A2 domain plug. As anticipated, disabling the plug in recombinant VWF by mutating the cysteines results in enhanced unfolding of the A2 domain and ADAMTS13 cleavage. Contrary to expectations, though, disabling the plug inhibits rather than promotes platelet GPIbα binding.

Conclusions: These results indicate that only one quarter of circulating VWF has an intact disulphide plug and binds GPIbα effectively.

OC 10.1 | An Engineered Serpin for the Treatment of Haemophilia

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Background: Haemophilia caused by deficiency in either component of the intrinsic Xase complex, factor (f) VIII or fIX. A small amount of fXa is initially produced by the extrinsic Xase complex (fVIIa and tissue-factor), but is insufficient to overcome the multiple anticoagulant mechanisms to produce a stable haemostatic clot. Tissue-factor pathway inhibitor (TFPI) and antithrombin (AT) both directly inhibit fXa, and activated protein C (APC) proteolytically inactivates the intrinsic Xase and the prothrombinase complexes. Conventional treatment of haemophilia is replacement of the missing factor, but the short half-life necessitates frequent intravenous infusions and renders prophylaxis incomplete. A fraction of haemophilia sufferers also develop inhibitory antibodies, requiring the use of ‚bypassing agents', such as NovoSeven and FEIBA, to treat bleeds. An alternative approach is to inhibit anticoagulation.

Aims: We decided to target APC, in part due to the reduced bleeding severity in haemophilia patients with partial APC resistance (fV_{Leiden}). The endogenous inhibitors of APC are members of the serpin family, notably protein C inhibitor (PCI) and α_1-antitrypsin (α_1AT), however, both suffer from poor reactivity and selectivity towards APC.

Methods: By mutating residues in and around the reactive centre P1-P1´ bond of α_1AT we were able to dial in specificity for APC.

Results: The lead variant, SerpinPC, inhibits APC with a rate constant of 15,000 $M^{-1}s^{-1}$ without inhibiting thrombin, fXa or any other relevant coagulation protease. SerpinPC had no effect on PT or APTT in normal plasma, but rescued thrombin generation in the presence of soluble thrombomodulin. Importantly, SerpinPC was found to restore haemostasis in haemophilia mice in three different models, even at low doses.

Conclusions: Good subcutaneous bioavailability and the one-week half-life of a_1AT suggest that a once fortnightly subcutaneous injection might provide effective prophylaxis for all haemophilia sufferers.

OC 10.2 | Antithrombin Resistance and Heparosan Conjugation Synergistically Extend the *in vivo* Half-life of FVIIa

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Background: Long-acting bypassing agents allowing for safe and efficacious prophylactic treatment of haemophilia patients with inhibitors represent a clear medical need. Previous studies have shown that the half-life of FVIIa can be extended 5-7 fold through PEG attachment or other principles, however, at the expense of significant activity loss.

Aims: Here, a combined mutagenesis and polymer-conjugation approach was undertaken to increase the half-life of FVIIa beyond this limit while preserving activity.

Methods: FVIIa variants were screened for proteolytic activity in the presence of FX and phospholipid and for antithrombin (AT) reactivity with heparin as co-factor. Selected variants were conjugated to a linear 40-kDa heparosan polymer (HEPtune®, Caisson Biotech) through N-glycans on FVIIa. Pharmacokinetics was determined in Sprague-Dawley rats after IV administration and potency in FVIII inhibited human whole-blood using thrombelastography.

Results: Since inhibition by AT constitutes the major clearance pathway of FVIIa *in vivo*, a screen for substitutions conferring AT resistance was performed. From a library of 400 variants, a T293K substitution

was identified that reduced AT reactivity to less than 10% of FVIIa while preserving activity. Reflecting the *in vitro* AT resistance, minimal complex formation with AT was observed when tested in a rat PK model. Interestingly, while resulting in an only modest increase of half-life (≤2 fold) on its own, combination of T293K with a glyco-conjugated 40-kDa heparosan resulted in a half-life 15-fold longer than FVIIa. To compensate for the impairment of activity due to polymer conjugation additional mutational optimization was performed. Following screening of 300 variants, a variant was identified carrying substitution L288Y.

Conclusions: The L288Y T293K FVIIa variant conjugated with 40-kDa heparosan exhibited a half-life of 15.2 ± 0.8 hrs compared to 1.0 ± 0.1 hrs for FVIIa in rats, and potency on par with FVIIa in haemophilia A-like human whole blood.

OC 10.3 | Pharmacokinetic and Activity Levels Achieved with Daily Subcutaneously Administered CB 2679d/ISU304 in Hemophilia B Dogs

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Background: The rapid clearance of factor IX (FIX) necessitates frequent intravenous (IV) administrations to achieve effective prophylaxis for patients with hemophilia B. Subcutaneous (SQ) administration would be a preferred route for convenience and less pain, but has been limited by low bioavailability and potency of the marketed FIX products. CB 2679d is a rFIX with enhanced biological properties and was developed using a rational protein design approach. CB 2679d has resistance to inhibition by ATIII, increased affinity for FVIIIa, increased catalytic activity and a resultant 20-fold enhanced potency *in vitro* (clotting activity) and *in vivo* (tail clip model) and 8-fold increased duration of aPTT activity *in vivo* compared with wild-type rFIX dosed at the same mass.

Aims: Determine pharmacokinetics of daily SQ CB 2679d.

Methods: CB 2679d 300 IU/kg was injected SQ daily for 6 days in hemophilia B dogs and was sampled at 0, 6, 24, 30, 48, 54, 72, 78, 96, 102, 120, 126, 144, 168, 176, 192, 200, 219, 240, 248, 264, 272, 288, 312, 336 and 360 hours. rhFIX antigen in canine plasma was determined by ELISA using an Affinity Biologicals kit. FIX activity was measured in duplicate, using a single-stage aPTT-based FIX clotting assay. The assay was performed on an ACL-TOP instrument using Instrumentation Laboratories reagents.

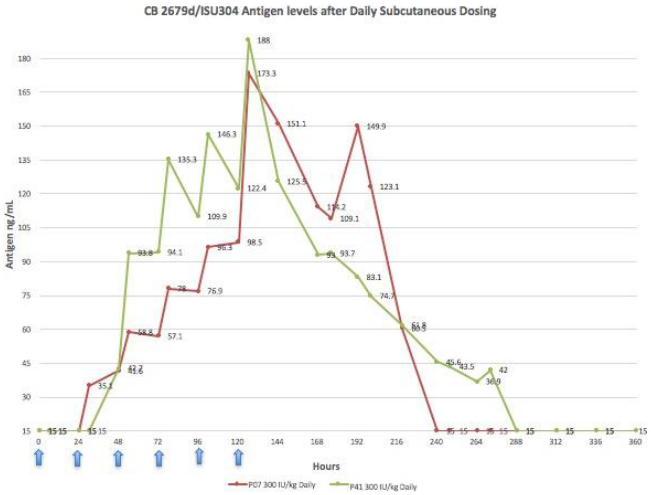

FIGURE 1 Blood Factor IX antigen levels after daily subcutaneous dosing of CB 2679d

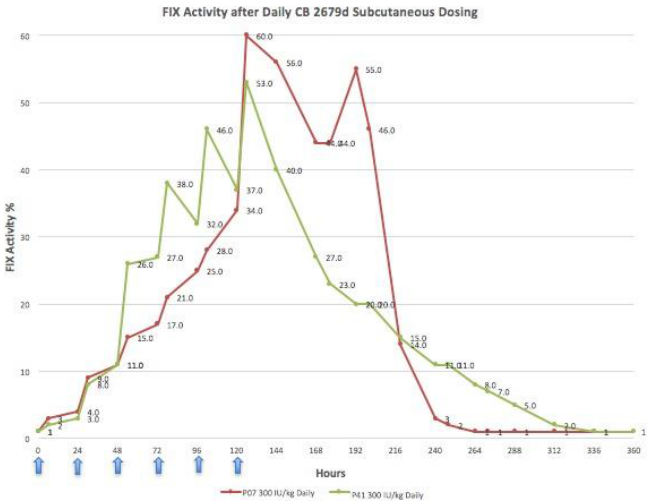

FIGURE 2 Blood Factor IX activity after daily subcutaneous dosiing of CB 2679d

Results: Daily SQ dosing of CB 2679d after 6 doses had peak rhFIX levels of 188 and 173.3 ng/mL at 126 hours. Trough activity levels 24 hours after 6 daily doses were 125.5 and 151.1 ng/mL respectively. Daily SQ dosing of CB 2679d after 6 doses had peak FIX activity levels of 60 and 53% at 126 hours. Trough activity levels 24 hours after 6 daily doses were 56 and 40% respectively. There were no emergent clinical adverse events or lab abnormalities recorded.

Conclusions: The progressive increase in FIX activity levels after daily SQ dosing of CB 2679d supports the initiation of the Phase 1 SQ dosing study in individuals with hemophilia B with the target of achieving normal FIX activity trough levels.

OC 10.4 | FcRn Mediated Recycling of Recombinant VWF D'D3-albumin Fusion Protein /rVIII-SingleChain Complex Is a Mechanism for FVIII Half-life Extension

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Background: Effective prophylactic treatment for severe haemophilia A requires intravenous administration of coagulation factor VIII (FVIII) 2-3 times a week due to the short in vivo half-life of FVIII products. FVIII circulates in complex with von Willebrand factor (VWF) and strategies to directly extend its half-life have had limited success as they do not overcome the VWF clearance mechanisms that also control FVIII clearance. A recombinant VWF D'D3 albumin fusion protein (rD'D3-FP) has been developed to extend the in vivo half-life of co-administered FVIII. The D'D3 domain of VWF interacts with FVIII, while the albumin moiety is able to engage the recycling pathway mediated by the neonatal Fc receptor, FcRn. In this study, we investigate the fate of internalised D'D3-FP/rVIII-SingleChain complex in cells expressing FcRn.

Aims: To determine whether rD'D3-FP has the potential to facilitate the recycling of associated rVIII-SingleChain via the FcRn mediated pathway.

Methods: The intracellular trafficking of rD'D3-FP and rD'D3-FP/ rVIII-SingleChain complex was examined in human 293 Freestyle™ cells stably expressing human FcRn alpha chain and beta 2 Microglobulin. Cells were pulsed for 10 minutes with fluorescent conjugates at pH 5.5 to facilitate FcRn mediated uptake via the albumin moiety of rD'D3-FP. Fresh media was then added and co-localisation with the early endosome (EEA1+), recycling endosome (Rab11+) and lysosome (CD63+) examined by immunofluorescence confocal microscopy after various chase periods.

Results: Following internalization, rD'D3-FP and the rD'D3-FP/ rVIII-SingleChain complex were first detected within the early endosome before entering the $Rab11^+$ recycling endosome and exiting the cells. Minimal co-localisation with the lysosomal compartment was observed.

Conclusions: We have demonstrated recycling of the rD'D3-FP/ rVIII-SingleChain complex, supporting a potential role for rD'D3-FP in further half-life extension of FVIII for the treatment of hemophilia A.

OC 10.5 | Half-life Extension of FVIII by Coadministration of a Recombinant D´D3 Albumin Fusion Protein

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Background: Recombinant D'D3-FP, the D'D3 domain of von Willebrand factor (VWF) fused to albumin, is a novel pharmaceutical compound in development intended to be used in combination with rVIII-SingleChain for treating hemophilia A patients. rD'D3-FP is designed to compete with endogenous VWF for binding to FVIII at respective molar excess, and, by its long half-life ($t_{1/2}$), to further improve the pharmacokinetic (PK) properties of rVIII-SingleChain in circulation.

Aims: The aim of the studies was to describe PK of rD'D3-FP in FVIII ko mice, rats and rabbits, and to investigate its impact on the PK of coadministered rVIII-SingleChain. Further, activated partial thrombin time (aPTT) and thrombin generation were monitored as pharmacodynamics (PD) readouts in FVIII ko mice over time.

Methods: rD'D3-FP was administered intravenously (i.v.) at a dose range of 0.15-30 mg/kg, alone or in combination with rVIII-SingleChain (100-1000 IU/kg). As appropriate, (co)administration of human plasma-derived (pd)VWF was used as control. Plasma PK of rD'D3-FP, VWF and FVIII (antigen and/or activity levels) was monitored for up to 72-168 h. Thrombin generation and aPTT were assessed in FVIII ko mice.

Results: Studies confirmed an extension of rD'D3-FP plasma exposure by 6-60 fold as compared to human pdVWF across species. This led to a dose-dependent prolongation of the terminal $t_{1/2}$ of rVIII-SingleChain by 1.5-3.0 fold in FVIII ko mice, 1.4-7.6 fold in rats and 1.2-3.8 fold in rabbits upon rD'D3-FP coadministration as compared to rVIII-SingleChain alone. The effect was even more pronounced when compared to rVIII-SingleChain co-administered with pd VWF, leading to improvements of 1.5-11.3 fold in rats and 2.0-8.2 fold in rabbits. In line, a prolongation of PD effects (correction of thrombin generation and aPTT) was shown in FVIII ko mice.

Conclusions: Studies in FVIII ko mice, rats and rabbits supported further improvement of rVIII-SingleChain PK upon coadministration with rD'D3-FP.

OC 13.1 | *SPK-9001*: Adeno-associated Virus Mediated Gene Transfer for Haemophilia B Achieved Durable Endogenous Prophylaxis at Levels of Activity Sufficient to Achieve Significant Mean Reduction in Annual Bleeding and Infusions Rates in Preliminary Data from an On-going Phase 1/2a Trial

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Background: Earlier AAV-8 mediated gene transfer at 2 x10^{12} vg/kg in haemophilia B demonstrated long-term mean FIX levels of ~5.1%. While clinical improvement was clear, these levels fall short of those obtainable with extended half-life FIX prophylaxis. Natural history data suggest levels of ~12% may eliminate spontaneous hemarthroses.

Aims: Sustained, endogenous FIX:C (≥12%) adequate to prevent spontaneous bleeding without prophylactic injections of exogenous FIX.

Methods: Investigational product, *SPK-9001*, utilizes a hepatotropic recombinant AAV capsid (Spark100) and a codon-optimised cassette encoding FIX Padua, a natural variant with 5-8x greater specific activity than wild-type FIX. Laboratory values, bleeding frequency, FIX consumption were monitored after vector infusion and compared to participants data for the year prior to vector administration.

Results: We infused 10 participants (ages 18-53 years, baseline FIX:C ≤2% and Spark100 NAb titer of ≤1:1) with 5 x10^{11}vg/kg of *SPK-9001*. In participants who have reached 12 weeks of follow-up, the mean steady-state FIX:C was ~29%. Asymptomatic increases in hepatic transaminases were observed in 2 participants resulting in a tapering course of prednisolone. Elevation of hepatic transaminases were resolved after the initiation of steroids. No participants reported study-related SAEs or development of FIX inhibitor. Discontinuation of prophylaxis occurred the day after vector infusion in all cases. Only 1 participant, with significant underlying hemophilic arthropathy, has required FIX injections for suspected bleeds. The cumulative FIX reduction for all participants amounts to >1.4M IUs.

Conclusions: These preliminary clinical data demonstrated high and consistent levels of sustained vector-derived FIX:C after gene transfer. Levels of FIX:C achieved by *SPK-9001* at 5x10^{11} vg/kg permitted termination of prophylaxis, prevention of bleeding, and markedly reduced factor use; while yielding the lowest (< 20%) reported incidence of capsid-specific T-cell immune response.

OC 13.2 | Updated Results from a Dose-escalation Study in Adults with Severe or Moderate-severe Hemophilia B Treated with AMT-060 (AAV5-hFIX) Gene Therapy: up to 1.5 Years Follow-up

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Background: Gene transfer for hemophilia offers the potential to convert the disease from a severe to mild phenotype with a single treatment. AMT-060 consists of an AAV5 vector containing a codon-optimized wildtype hFIX gene under control of a liver-specific promoter.

Aims: This phase 1/2 study investigates the safety and efficacy of AMT-060 at 2 dose levels in adults with moderate-severe or severe hemophilia B.

Methods: Multi-national, open-label, dose-escalating study in patients (pts) with factor IX (FIX) activity ≤2% of normal, and a severe bleeding phenotype (prophylactic exogenous FIX; or on-demand exogenous FIX, plus ≥4 bleeds/year or hemophilic arthropathy). Pts received either 5x10^{12} gc/kg (n=5) or 2x10^{13} gc/kg (n=5) of AMT-060 iv. Efficacy assessments include endogenous FIX activity (measured ≥10 days after use of exogenous FIX); reduction of exogenous FIX use; and annualized spontaneous bleeding rates. Safety assessments include treatment related adverse events, immunological and inflammatory biomarkers.

Results: There were no screening failures due to AAV5 antibodies. Mean FIX activity in the lower dose cohort was 5.2% (min-max, 3.0-6.8%; n=4; 1 patient remaining on prophylaxis excluded) during 1 year of follow-up, and 6.9% (min-max, 3.1-12.7%; n=5) in the higher dose cohort during 26 weeks follow-up. Eight of 9 pts on FIX prophylaxis discontinued use after AMT-060 infusion. Follow-up to up to 1.5 years will be presented, with annualized reduction of exogenous FIX use and spontaneous bleeding rates. Mild, temporary elevations in ALT were observed in 3 pts with higher mean FIX activity (6.3-12.7%; 1 in the lower and 2 in the higher dose cohort). Each received a tapering course of prednisolone. ALT elevations were not associated with changes in FIX activity or a capsid-specific T-cell response.

Conclusions: Patients continue to show sustained clinical benefit and endogenous FIX activity with no T-cell activation ≥1 year after a single infusion of AMT-060.

OC 13.3 | An Analysis of Bleeding Rates and Factor IX Consumption in the Phase I/II BAX 335 Gene Therapy Trial in Subjects with Hemophilia B

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Background: Adeno-associated virus (AAV)-based gene therapy is a promising technology to treat hemophilia B (HB). BAX 335 (AAV8.sc-TTR-FIXR338Lopt) contains the *F9* R338L gain-of-function transgene in an AAV8 capsid. Here we present an interim analysis of clinical endpoints from the multi-center phase I/II safety and efficacy study of BAX 335 (NCT01687608).

Aims: To describe clinical outcomes of subjects treated with BAX 335.

Methods: Adult males (18-75) with moderate-to-severe HB (FIX≤2%) were eligible. Subjects received BAX 335 in 3 ascending cohorts: 2E^{11} vector genomes (vg)/kg (C1), 1E^{12} vg/kg (C2), and 3E^{12} vg/kg (C3).

Standard of care treatment was allowed as needed during the study period, including exogenous FIX for bleeding episodes or prophylaxis. This study was approved by internal review boards; subjects provided informed consent prior to enrollment.

Results: 8 subjects received BAX 335 in 3 cohorts: C1 n=2, C2 n=4, and C3 n=2. 6/8 subjects were using prophylaxis prior to study enrollment. Median peak FIX levels were 3.5% (C1), 12.0% (C2), and 45.0% (C3). Only 2 subjects had sustained FIX expression at 1 year. Annualized bleeding rates (ABR) and FIX consumption (FC) were evaluated after 8 subjects reached 1 year of follow-up. The median ABR prior to gene therapy was 9.6 (0-24) and decreased to 6 (1-12) after 12 months. Median FC decreased by 43.4% (221250 IU to 99941 IU; p=0.009). Median number of infusions decreased from 49.5 (12-96) to 17.5 (1-55). Median time to first bleed was 48.5 days (d), with higher dose cohorts associated with longer times (C1=13.5 d, C2=48.5 d, C3=168.5 d) and higher peak FIX expression.

Conclusions: BAX 335 resulted in reduced FC and ABR related to the peak and duration of FIX expression in the majority of subjects. Even transient expression of FIX resulted in disease modification of HB. One subject continues to have sustained FIX=20% after 2.5 years of follow-up. A next-generation FIX gene therapy candidate is currently in development.

OC 13.4 | Immunogenicity and Efficacy of FIX-Padua in Distinct Canine Models of Severe Hemophilia B Following AAV Gene Therapy to Skeletal Muscle or Liver

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Background: Hemophilia B (HB) is an X-linked bleeding disorder caused by a deficiency in Factor IX (FIX). Ongoing gene therapy trials for HB using adeno-associated viral (AAV) vectors encoding FIX-Padua, a hyperactive FIX variant which exhibits ~8-fold increase in specific activity, offers a strategy to lower the AAV dose to minimize cellular immune responses while providing therapeutic levels.

Aims: Here we report new data AAV-cFIX-Padua in inhibitor-prone or with preexisting inhibitors to cFIX in HB dogs, respectively. These dogs have an underlying null mutation in the cF9 gene and typically a single injection of cFIX wild-type protein results in high titer inhibitors.

Methods: 3 adult dogs received intravascular delivery to the skeletal muscle or liver of $3x10^{12}$vg/kg of AAV-cFIX-Padua.

Results: Dog U04 achieved FIX activity levels of 45-60%; dog U05 reached a plateau of 75-100% circulating cFIX activity levels. Neither dog has developed inhibitors to cFIX or non-neutralizing (NNA) IgG1 and IgG2 against cFIX. Notably, immune tolerance has been maintained despite challenge with cFIX-wild type protein.

Table 1: Summary of AAV cFIX-Padua (1 to 3 x 10^{12} vg/kg)

HB Dog	Target	FIX (% of normal) Activity	FIX (% of normal) Antigen	Specific activity	Bleeds/Month Pre	Bleeds/Month Post
U01*	liver	81	10.9	8.6	7/49	0/42
U02	liver	30	4.4	8.5	0/32	0/60
U03*	liver	180	26	8.5	6/32	0/60
U06	liver	44	3.9	12	0/11	0/41
U04	muscle	53	7.7	7.3	8/13	0/31
U05	muscle	91	10.2	9.6	1/17	0/25
M55-CH	muscle	8	1.5	8.6	0/7	0/90
M59-CH	muscle	3.5	0.35	9.2	0/6	0/90
N07-CH	muscle	5.5	0.65	9.0	2/6	0/79
					24/173	0/518

- Dogs with pre-existing inhibitor to cFIX
- UO: null mutation model
- CH: missense mutation model

These are the first data showing complete normalization of HB phenotype by muscle gene therapy, a highly attractive strategy for those patients with underlying liver disease that precludes inclusion in liver-based strategies. In additional, dog U03 with pre-existing inhibitor to cFIX received AAV-FIX Padua liver gene therapy with rapid elimination of the inhibitor but with a long-lasting NNA IgG2 specific to cFIX for 400 days, when challenged with cFIX-WT protein, an anamestic response were noted with low inhibitor titers, IgG2 but high levels of NNA IgG1 that spontaneously resolved and cFIX reached 80%.

Conclusions: Taken together, these three dogs and 6 previous reported HB dogs (Table 1), expression of cFIX Padua exhibits ~8-fold specific activity and is both efficacious (no bleeding episodes in cumulative 518 months post gene therapy) without increased immunogenicity or evidence of thrombogenicity.

OC 13.6 | Development of SHP654, a Highly Efficient AAV8-based BDD-FVIII Gene Therapy Vector for Treatment of Hemophilia A

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Background: A recently initiated AAV5-based hemophilia A gene therapy clinical trial showed that by systemic administration of a very high vector dose (6x10E13 vg/kg) FVIII levels in the normal range can be achieved. While these data are promising they highlight the need for more efficient vectors to reduce constraints imposed by vector production and dose-dependent toxicities at high doses.

Aims: To develop a gene therapy candidate product that is built on AAV8, the prototypic liver-specific AAV serotype, and an expression cassette that confers strong and liver-specific expression of a B-domain deleted Refacto-type variant of FVIII.

Methods: The expression cassette was designed to harbor a strong yet compact liver-specific TTR promoter/enhancer, a CpG-depleted and codon-optimized BDD-FVIII coding sequence, and a short synthetic poly A element. To cope with the packaging size limit of AAV, non-essential DNA stretches were largely removed and a single-stranded genome architecture was chosen.

Results: Initial testing of several FVIII gene therapy vectors failed to identify a candidate expressing sufficient amounts of FVIII in FVIII ko mice. We therefore initiated a screening program for improved FVIII coding sequences using a combination of available codon-optimization algorithms and manual sequence editing. Altogether more than 50 codon-optimized BDD FVIII sequences were screened for FVIII expression. This approach resulted in identification of SHP654, a vector that expressed 74-fold higher levels of FVIII than the corresponding wild-type nucleotide sequence. SHP654 proved to be also producible with high yields. An improved formulation buffer allows higher strength formulations, prevents adsorption onto contact materials and ensures stability of the vector.

Conclusions: The features of SHP654 make it a promising drug candidate for FVIII gene therapy with the potential to effectively treat hemophilia A patients by doses in the 10^{12} vg/kg range.

OC 22.1 | CRISPR/Cas9-engineered VWF Knockout BOECs Have Increased Angiogenic Properties

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Background: Von Willebrand factor (VWF) is a pleiotropic protein that takes a central position in the maintenance of vascular homeostasis. VWF is synthesized by endothelial cells where it is stored in rod-shaped storage organelles called Weibel-Palade bodies (WPBs). Apart from its well established role in hemostasis, several lines of evidence have also implicated VWF in angiogenesis. This includes complications such as angiodysplasia that are often found in Von Willebrand disease (VWD) patients. However, studies in VWD patient-derived blood outgrowth endothelial cells (BOECs), which can be regarded as endothelial pathological deficiency models of VWD, have failed to unequivocally support these findings.

Aims: We used CRISPR/Cas9 genome editing to genetically ablate VWF expression in BOECs in order to study the implications of loss of VWF on angiogenic parameters.

Methods: Cord blood-derived BOECs (cBOECs) were transduced with gRNAs targeting VWF to generate $VWF^{-/-}$ cBOECs. True VWF null cBOECs were selected using a high throughput screen, validated by next generation sequencing and were phenotypically characterized, including analysis of the angiogenic potential in the absence of VWF.

Results: Clonal $VWF^{-/-}$ lines were obtained which contained CRISPR-induced frameshifting mutations around the gRNA target sites. $VWF^{-/-}$ cBOECs were entirely devoid of WPBs, which led to alternative routing of WPB cargo such as P-selectin, CD63, Ang-2, IL-6 and IL-8. Ang-2 was no longer retained in endothelial cells but constitutively secreted as evidenced by the increased levels of Ang-2 in unstimulated conditioned media. In scratch wound assays, spheroid assays and proliferation assays we found that $VWF^{-/-}$ cBOECs have increased angiogenic potential. Interestingly, we found that surface levels of $\alpha_V\beta$-3 and $\alpha5\beta$-1integrin were altered on $VWF^{-/-}$ cBOECs.

Conclusions: CRISPR targeted knockouts of VWF in BOECs further substantiate the role of VWF in angiogenesis and provide additional mechanistic detail how VWF controls vascular homeostasis.

OC 22.2 | Von Willebrand Factor Free-thiols Are Critical for Function under High Shear Stress

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Background: Von Willebrand Factor is a multimeric protein critical for platelet capture under high shear stress. Several studies have shown that Von Willebrand Factor contains a number of free thiols that may mediate its function. While this has been investigated using a plate and cone viscometer, no studies have addressed the role of VWF free-thiols under conditions of acute high shear stress.

Aims: To determine the effect of VWF free-thiols on function under physiological and pathological shear stress

Methods: VWF was purified from Haemate P by gel filtration or recombinantly expressed in HEK293T cells. Free-thiols were blocked with N-ethylmaleimide (NEM) or Maleimide-PEO2-biotin. Flow assays were performed using plasma free blood supplemented with VWF and perfused over collagen or VWF coated surfaces. Analysis of the VWF-collagen interaction was performed using atomic force microscopy (AFM).

Results: NEM blockade of VWF free-thiols reduced VWF mediated platelet capture to collagen in a shear dependent manner; with no effect at low shear and platelet capture virtually abolished at high shear (above $3000s^{-1}$). The effect was mediated by free-thiols in the C-terminal domains and was not due to an effect on platelet capture. Under extreme pathological shear and in flow chambers mimicking stenotic vessels, the formation of thick VWF fibres on collagen was inhibited by blocking VWF thiols. Interestingly some free-thiols in VWF were lost after collagen binding and AFM measurements demonstrated that the strength of the VWF-collagen bond was significantly reduced with NEM-VWF Moreover, NEM-VWF failed to effectively incorporate into long VWF fibres formed independently of collagen and platelets. Finally, molecular simulation studies on the VWF C-domains demonstrated alterations in free-thiol content following the application of force.

Conclusions: Free-thiols residues in VWF are essential for proper collagen binding under shear stress, possibly by mediating a conformational change in the VWF molecule.

OC 22.3 | Mouse VWF Propeptide Regulates Platelet Thrombus Formation *in vitro*

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Background: The VWF propeptide (VWFpp) critically mediates VWF multimerization and trafficking to Weibel-Palade bodies, from where mature VWF and VWFpp are co-secreted. While the hemostatic roles of mature VWF are well defined, an independent hemostatic function for the VWFpp remains to be determined.
Aims: To investigate a functional role for the VWFpp in mediating thrombus generation.
Methods: Mouse VWFpp and variants thereof were transiently expressed in HEK293T cells and purified via nickel affinity chromatography. Platelet thrombus generation was assessed using whole blood from ADAMTS13$^{-/-}$ or VWF/ADAMTS13$^{-/-}$ mice in a collagen-based flow chamber system. Platelet thrombi were visualized using a Quorum WaveFX-X1 spinning disk confocal microscope. The VWFpp glycosylation profile was investigated by liquid chromatography tandem mass spectrometry.
Results: In the absence of VWF, VWFpp alone was incapable of supporting platelet thrombus formation on a collagen-coated surface (surface coverage VWFpp vs negative control; 3.6 ± 0.8% vs 2.0 ± 0.3%, p=0.99). Reconstitution of mouse VWF was required to restore the ability to form a platelet thrombus (17.2 ± 3.4%). Notably, subsequent addition of VWFpp significantly enhanced thrombus formation compared with VWF alone (28.4 ± 4.1%, p=0.0169). Similarly, addition of exogenous VWFpp to whole blood of ADAMTS13$^{-/-}$ mice promoted more extensive thrombus growth compared with whole blood alone (21.2 ± 5.0% vs 7.9 ± 2.6%). To investigate factors regulating this activity, the VWFpp glycosylation profile was evaluated. LC-MSMS identified 20 discrete N-linked glycan structures occupying 3 sites; N99, N156 and N666. Importantly, removal of the glycans at N156 and N666 via site directed mutagenesis significantly delayed initial thrombus formation in both ADAMTS13$^{-/-}$ and VWF/ADAMTS13$^{-/-}$ models.
Conclusions: Mouse VWFpp enhances platelet thrombus formation *in vitro*, a process that is regulated, at least in part, by the VWFpp N-linked glycans.

OC 22.4 | Protein Kinase C Signaling Dysfunction in von Willebrand Disease Type 2B Platelets

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Background: von Willebrand disease (VWD)-type 2B is characterized by gain-of-function mutations in von Willebrand factor (VWF) enhancing its binding affinity for the platelet receptor GPIbα. VWD-type 2B patients display a bleeding tendency associated with loss of high molecular weight VWF multimers and thrombocytopenia of varying degrees. We recently demonstrated that a marked defect in agonist-induced activation of the small GTPase, Rap1 and integrin αIIbβ3 in VWD-type 2B platelets, also contributes to the bleeding tendency. Two pathways contribute to Rap1 activation in platelets: rapid activation mediated by the calcium-sensing guanine nucleotide exchange factor, CalDAG-GEFI (CDG1), and sustained activation dependent on signaling by protein kinase C (PKC) and the ADP receptor, P2Y12.
Aims: Here, we investigated the molecular mechanisms underlying impaired platelet Rap1 signaling in VWD-type 2B.
Methods: To investigate which Rap1 signaling pathway is affected, we induced VWD-type 2B in *wild-type*, *Caldaggef1*$^{-/-}$ and *Adam17*$^{fl/fl}$*PF4-Cre*$^{+}$ mice by hydrodynamic injection of VWF/p.V1316M.
Results: In flow cytometry studies using JON/A-PE, which irreversibly binds to the activated form of integrin αIIbβ3, we demonstrate that platelet dysfunction in VWD-type 2B affects PKC-mediated, but not CDG1-mediated activation of Rap1. Consistently, downstream of PKC, we observed impaired granule release and decreased PKC substrate phosphorylation in response to agonist stimulation. Interestingly, the defect in PKC signaling was caused by a significant increase in PKC substrate phosphorylation in circulating VWD-type 2B platelets, suggesting that the VWF-GPIbα interaction leads to PKC pre-activation and subsequent desensitization of this pathway. This conclusion is supported by the fact that VWD-type 2B mice also exhibited marked shedding of platelet GPIbα, a cellular response known to be triggered by PKC signaling.
Conclusions: Our studies identify altered PKC signaling as the underlying cause of platelet hypofunction in VWD-type 2B.

OC 22.5 | Silent von Willebrand Factor Variant c.4146G>T (p.Leu1382=) Causes Type 1 von Willebrand Disease via Disruption of an Exonic Splice Enhancer Motif

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Background: Type 1 von Willebrand disease (VWD1) is caused by mutations resulting in quantitative deficiency of von Willebrand factor (VWF). Mutation screening of a patient diagnosed with mild VWD1 highlighted a silent variant (c.4146G>T; p.Leu1382=) in exon 28 which tracked with disease phenotype within the family.
Aims: To investigate whether the c.4146G>T variant caused the mild VWD1 phenotype and to determine the disease mechanism involved.
Methods: The influence of the variant on protein expression was analysed via transfection or co-transfection of reference (R; c.4146G)

and non-reference (NR; c.4146T) bacterial expression plasmids in HEK293T cells, and measuring VWF:Ag levels using ELISA. RNA was also isolated from expression plasmids and quantified using duplex TaqMan® gene expression analysis. Half-life of expressed mRNA was determined at 0, 2, 3 and 4 hours (h) post-treatment with actinomycin D. *In silico* analyses were performed to predict variant effect on RNA splicing motifs. Influence on exonic splice enhancer (ESE) motifs was determined using a plasmid-derived ESE-dependent splicing assay.

Results: A significant reduction in VWF secretion was observed for the NR allele in both the homozygous and heterozygous states when compared to R allele alone (~39% and ~24% respectively). A similar reduction in RNA quantity following NR allele expression and a significant reduction in mRNA half-life (R: t½=2:38h; NR: t½=1:44h, ~40% decrease) was also observed. *In silico* analyses predicted disruption of an existing ESE motif in exon 28, which was confirmed *in vitro*.

Conclusions: Silent variant c.4146G>T causes mild VWD1 by disrupting an ESE motif in VWF exon 28 resulting in reduced mRNA half-life of the ‚mutant' allele, subsequently leading to reduced RNA and protein expression. This represents a novel disease causing mechanism not previously reported in VWD.

OC 24.2 | Non-neutralizing FVIII Antibodies after Successful ITI Influence FVIII Pharmacokinetics

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Background: Inhibitor development is still the most severe complication in modern hemophilia A treatment and the eradication of inhibitors during immune tolerance induction (ITI) is a great challenge. After successful ITI lower factor VIII (FVIII) levels are observed in some patients.

Aims: The analysis aimed to collect clinical and pharmacological data and to correlate the data to the presence and type of FVIII antibodies.

Methods: A total of 51 patients with severe hemophilia A were included in this analysis. 11 patients had undergone a successful ITI in the past. Data on treatment FVIII trough levels, incremental recovery, Bethesda Units were collected and plasma was analyzed for FVIII specific antibodies, which were further characterized.

Results: All patients received prophylactic treatment with recombinant or plasma-derived FVIII. Incremental recoveries ranged from 0,7 to 3,3. As expected, recoveries increased with age. Bethesda titers of all patients were negative. Antibodies to FVIII were detected in 11 patients with 5 patients showing specific competition. All 11 patients had a history of inhibitors and a successful ITI. These antibodies included the IgG subclasses 1 or 4. Cognate epitopes on FVIII were located in both, the heavy and the light chain of FVIII including the A2, C1 and / or C2 domain. The presence of antibodies correlated with a lower recovery (0,7 to 2,3) in this cohort.

Non-neutralizing antibodies against FVIII could be detected only in patients with a history of successful ITI and not in patients who never developed an inhibitor. Of note, antibody negative, post ITI patients were older and time since ITI success was longer compared to antibody positive patients.

Conclusions: Non-neutralizing antibodies seem to influence pharmacokinetic parameters of FVIII, mainly in patients with history of FVIII inhibitor and ITI.

OC 24.3 | Intracranial Haemorrhage in Neonates with Haemophilia - Vaginal or Caesarean Section?

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Background: In the neonatal period, haemophilia patients have a high risk of intracranial haemorrhage (ICH) compared to the normal population. The optimal mode of delivery - vaginal or Caesarean section (CS) - for a haemophilia carrier is still debated. Knowledge of a family history of haemophilia could influence the mode of delivery and the rate of ICH.

Aims: To determine the incidence of ICH in newborns with haemophilia and the relationship with mode of delivery and knowledge of family history of haemophilia.

Methods: All children included in the PedNet Registry by 1 January 2015 with severe and moderate haemophilia A and B were enrolled (n=994). The outcomes ICH, mode of delivery and family history of haemophilia were recorded. The PedNet Registry is approved by ethical boards in all 31 participating centres and includes all consecutive children with haemophilia born after Jan 1, 2000.

Results: Twenty ICH (2.0%) occurred in 994 neonates. There was no statistical difference in the rate of ICH regarding the mode of delivery: The incidence of ICH after all vaginal deliveries was 2.3% (15/631) and 1.4% (8/560) for non-instrumental vaginal delivery compared to 1.7% (5/295) after CS with no statistical difference. Neonates born by instrumental delivery were more likely to have a diagnosis of ICH compared to a non-instrumental vaginal delivery ($P< 0.001$; RR 6.9) or after a CS ($P< 0.001$; RR 5.8). There was no significant difference between the group with and without a known family history (2.5% 8/322 vs 2.4% 7/292 ; p=0.37) regarding incidence of ICH. CS was more likely to be performed in the group with a known family history (169/495, 34%) than in the sporadic group (123/480, 26%, P=0.004).

Conclusions: Vaginal instrumental delivery was found to be a risk factor for ICH but otherwise the mode of delivery - vaginal or CS - had no impact on the rate of ICH. The knowledge of family history changed the mode of delivery but not the occurrence of ICH.

OC 24.4 | Thai Pediatric Bleeding Assessment Tool (BAT) for the Screening of Mild Bleeding Disorders

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Background: Mild bleeding disorders might be underdiagnosed due to difficulty in reporting subjective hemorrhagic symptoms in a consistent format. BATs include the Pediatric Bleeding Questionnaire (PBQ) which was developed for children with bleeding disorders and the International Society on Thrombosis and Haemostasis (ISTH)-the -BAT that has been studied mainly in adults. In Thailand, currently there is no standardized bleeding questionnaire.

Aims: To develop a Thai pediatric-BAT for screening mild bleeding disorders in children.

Methods: This was a multicenter case control study approved by local research ethics boards. A Thai pediatric-BAT was first developed by translating the PBQ and ISTH-BAT into Thai language. After validation, the questionnaire was administered to consented patients diagnosed with mild bleeding disorders and normal subjects; bleeding scores were calculated using the PBQ and ISTH-BAT scoring keys. The Receiver-operator curve (ROC) analysis, sensitivity, specificity and likelihood ratios were used to determine the cutoff value of bleeding scores in patients and controls.

Results: Of a total of 203 subjects from 3 medical centers in Thailand, 67 were patients and 136 were normal controls. The median (range) age in patients and controls were 5.4yrs (0.01-16.2) and 4.1yrs (0.1-18.0) years, respectively. The median PBQ and ISTH-BAT scores in VWD, platelet disorders, mild hemophilia and controls were 5, 6, 4.5 and 0, respectively. The cutoff value of 2 had similar sensitivity and specificity of 91.2% (PBQ score) and 94.1% (ISTH-BAT score). The likelihood ratio using the PBQ score for a positive bleeding score (≥ 2) was 12.38 (95% CI 6.79-22.6) while for the ISTH-BAT score was 14 (95% CI 14-26.4).

Conclusions: The Thai pediatric-BAT with bleeding score ≥ 2 using either the PBQ or ISTH-BAT scoring key can be used as a tool for screening mild bleeding disorders in children to guide the need for further investigation. Further prospective study in children with suspected bleeding disorders is required.

OC 24.5 | Tranexamic Acid in Adolescent Girls with Heavy Menstrual Bleeding

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Background: Heavy menstrual bleeding (HMB) occurs in up to 40 % of adolescent girls, significantly impacting their daily activities. Identifying new treatment strategies for HMB is particularly important for adolescents for whom hormonal contraception may not be preferable.

Aims: To perform an open label, multi-institutional, single arm, efficacy study of tranexamic acid (TA) in decreasing menstrual blood flow (MBL) and increasing health-related quality of life (HRQoL) in girls, age 10-19 years, with HMB.

Methods: IRB approval was obtained from each participating site. Informed consent was obtained from each subject. Girls with baseline hemoglobin < 8 grams/dL or taking hormonal contraception were excluded. Patients were treated with oral TA 1.3 grams thrice a day during the first five days of menses and monitored over the course of 4 menstrual cycles (1 baseline; 3 treatment cycles). The assessment of MBL and HRQoL was done using the Menorrhagia Impact

TABLE 1 Study Schema

	STUDY ENTRY	MENSES 1 (BASELINE)	MENSES 2	MENSES 3	MENSES 4	STUDY EXIT
History and Physical examination	X					X
Menorrhagia Impact Score (MIQ) and Pictorial Blood Assessment Chart (PBAC)		X	X	X	X	
Complete Blood Count	X					X
Ferritin	X					X
Creatinine	X					
Blood Urea Nitrogen/Liver Function Tests	X					X
Urine Pregnancy test	X					
Study related phone call		X	X	X	X	

TABLE 2 Results

OUTCOME VARIABLE	BASELINE	ON TREATMENT WITH TRANEXAMIC ACID	p VALUE (difference of mean; 95% CI)
PBAC score	255.1 (n = 24)	154.6 (n = 24)	< 0.001 (100.5; 46.30 to 154.74)
Total MIQ score. Range: 4 - 22	9.56	7.15	< 0.001 (2.41; 1.33 to 3.49)
MIQ 1 score: perceived blood loss. Range: 1 - 4	3.00	1.90	< 0.001 (1.09; 0.76 to 1.43)
MIQ 2 score: limitation on school attendance. Range: 1 - 5	1.64	1.40	0.47 (0.24; -0.43 to 0.91)
MIQ 3 score: limitation on physical activities. Range: 1 - 5	2.08	1.40	0.002 (0.68; 0.28 to 1.08)
MIQ 4 score: limitation on social activities. Range: 1 - 5	1.96	1.33	0.01 (0.63; 0.171 to 1.084)
MIQ 5 score: perceived change in menstrual blood loss from last cycle. Range: 0 - 2	0.68	0.86	0.20 (-0.18; -0.45 to 0.1)
MIQ 6: meaningful change for the patient. Range: 0 - 1	0.20	0.65	<0.001 (-0.46; -0.68 to -0.23)
(a)Mean hemoglobin (grams/dL) (b)Mean ferritin (nanograms/dL)	(a) 12.67 Range: 10.4 - 15.3 (b) 25.94 Range: 4 - 82	(a) 12.84 (n = 22) Range: 10.6 - 14.7 (b) 26.34 (n = 22) Range: 5 - 59	(a) 0.94 (0.01; -0.23 to 0.25) (b) 0.62 (2.13; -6.58 to 10.84)

score (MIQ), Pictorial Blood Assessment Chart (PBAC), hemoglobin and ferritin. MIQ is a validated tool to assess the influence of HMB on HRQoL. In previous studies, ≥ 1 point increase in score has been shown to be indicative of clinically significant improvement. A paired t-test was used to analyze the data.

Results: 30 patients were enrolled in the study. Of these, 25 (83.33%) completed baseline and at least 2 of 3 treatment cycles and were deemed evaluable. Mean age was 14.7 years (range 11-18). 8 (32%) had an identified bleeding disorder. The total MIQ score improved from an average of 9.56 pre-treatment to 7.15 on treatment (n = 25, $p < 0.001$). Overall, there was a significant decrease in PBAC scores when using TA (n = 24; $p < 0.001$). There was no significant improvement noted in hemoglobin or ferritin concentrations during the time frame of the study.

Conclusions: Use of tranexamic acid in adolescent females with HMB leads to clinically meaningful reduction in MBL.

OC 23.1 | A Novel Platform for Immune Tolerance Induction in Hemophilia A Mice

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Background: Hemophilia A (HA) is caused by factor VIII (FVIII) deficiency. FVIII is mainly produced in the liver by sinusoidal endothelial cells (LSEC). Several attempts have been carried out to cure HA, but the immune response to FVIII is still the main issue to overcome.

Aims: To investigate the role of LSEC, the natural site of FVIII synthesis, in tolerance induction after HA gene transfer (GT) using LV-expressing FVIII driven by the endothelial-specific VE-cadherin (VEC) promoter ± miRNA target sequences (miRTs).

Methods: We prepared LV containing BDD-hFVIII, RH-hFVIII or codon optimized hFVIII (co-hFVIII) cDNAs under the control of VEC promoter alone or in combination with miRT142.3p (silencing in hemopoietic cells) and miRT122 (silencing in hepatocytes) and injected HA mice naïve or previously immunized with FVIII. To study the role of LSEC in Tregs induction we depleted Tregs before and after GT.

Results: C57Bl/6 HA mice injected with LV.VEC.FVIII±122-142 reached long-term phenotypic correction up to 1y with FVIII activity up to 12% using more active forms of FVIII. LV.VEC.FVIII±122-142 injection in FVIII-immunized mice resulted in FVIII activity and reversion of inhibitor titers. Tregs depletion before GT resulted in inhibitors formation and low FVIII levels. Tregs depletion after GT lead to inhibitor formation and decreased FVIII activity followed by recover of FVIII activity and reduction of inhibitor titers. Finally, injection of LV.VEC.FVIII in two additional HA mouse strains, B6/129 and BALB/c, resulted in long-term FVIII activity and absence of inhibitors as well.

Conclusions: We first report FVIII expression on its natural site of production with therapeutic levels without inhibitor formation. In FVIII-immunized HA mice inhibitors were eradicated in a Tregs-dependent mechanism. Transgenes expression on the natural sites of synthesis provides both efficacy and safety for long-term correction of hemophilia and likely to others genetic disease.

OC 23.2 | Cellular and transcriptional immune profiling of early factor VIII responses in hemophilia A mice

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Background: Developing factor VIII (FVIII) inhibitory antibodies is the most serious complication in hemophilia A treatment, representing a significant health and economic burden. A better understanding of the innate immune responses leading to this outcome may provide insight into inhibitor prevention.

Aims: To identify early mediators of FVIII immunity and to outline the reactionary immune profiles in the spleen and liver.

Methods: C57Bl/6 *F8* E16 knockout mice were infused with 5 µg (1700 IU/kg) of recombinant FVIII. Spleens were frozen at various time points post-infusion and stained for FVIII and cellular markers. Splenic and liver RNA expression analysis was performed 3 hours post-infusion of 0.6 µg (200 IU/kg) FVIII by nCounter technology using a 561 gene immunology panel.

Results: FVIII localization did not change over 2.5 hours. We observed significantly higher co-localization of FVIII with MARCO+ cells compared to Siglec1 and SIGNR1 (Pearson's R: 0.72 +/- 0.06 versus 0.58 +/- 0.07 and 0.5 +/- 0.09; $p < 0.0001$). FVIII exhibited little co-localization with CD11c+ dendritic cells and the macrophage mannose receptor, CD206 (0.22 +/- 0.1, 0.146 +/- 0.03, respectively). Splenic mRNA immune profiling identified 5 immune transcripts that were elevated or reduced by at least 1.5-fold. We observed significant upregulation in Tnfaip6 and Lif (3.6- and 2.5-fold; p=0.02, 0.04) and downregulation in Cxcr4, Cish, and IL17r (2.2-, 2.2-, 1.6-fold; p=0.002, 0.02, 0.03). In contrast, the tolerogenic liver immune profile showed increases in Ikbkap and Traf2 expression (4.2-, 1.9-fold; p=0.03, p=0.003) and a decrease in Casp1, Alas1, Fcgr1 and Ccl5 (2.6-, 2.4-, 3.6-, 3.7-fold; p=0.04, 0.0004, 0.002, 0.04).

Conclusions: FVIII has the greatest affinity for marginal zone macrophages (MARCO+) in the spleen upon infusion. Targeting genes that are implicated in immunity in the spleen and tolerance in the liver may lead to therapeutic interventions to prevent FVIII inhibitors.

OC 23.3 | Interleukin-6 Inhibition Reduces Factor VIII Inhibitor Formation in Factor VIII-deficient Mice

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Background: The biggest issue facing the hemophilia A community in industrialized nations today is risk of inhibitor development. Thirty percent of patients with severe Factor VIII (FVIII) deficiency develop inhibitors. Immune tolerance therapy for the treatment of inhibitors is resource intensive, takes up to 33 months, and is only 70% effective. In addition, most inhibitors develop in the first 50 exposure days. There is growing evidence that interleukin 6 (IL-6) is involved in the pathogenesis of inhibitor development. For instance, in mice induced to develop high inhibitor levels, increased effector T-cell responses were identified in the presence of elevated expression of IL-6. Several IL-6 inhibitors are clinically available and there is low morbidity associated with their administration.

Aims: The aim is to determine if IL-6 blockade prevents inhibitor formation.

Methods: Inbred Exon 16-deleted FVIII-deficient mice were injected with weekly doses of Factor VIII (100 U/kg) for 7 weeks, then euthanized at 9 weeks of study. A similarly-treated cohort was supplemented with anti-IL-6 antibody on weeks 1, 4, and 7. Plasma obtained from both groups at weeks 3, 5, 7, and 9 was evaluated for the presence of FVIII inhibitors via speed of clot formation using Pathromtin activation on a CoaScreener.

Results: At the 9 week timepoint, 4/10 (40%) of mice receiving FVIII alone had clot time delayed ≥4 seconds versus untreated controls. In contrast, 1/10 (10%) of mice receiving supplemental IL-6 experienced the same delay. At 7 weeks, 9/11 (82%) of mice receiving FVIII alone had clot times delayed ≥2 seconds versus controls. In contrast, 3/11 (27%) of mice receiving supplemental IL-6 experienced the same delay. A 4 second delay in this assay corresponds with an inhibitor Bethesda Unit >2.

Conclusions: IL-6 inhibition reduces the development of FVIII inhibitors in FVIII-deficient mice. These data suggest that the administration of anti-IL-6 therapy may significantly reduce the incidence of inhibitor formation in hemophilia A.

OC 23.4 | Inhibition of the bruton's tyrosine kinase alters the anti-FVIII memory B-cell response in mice with severe hemophilia A

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Background: Thirty percent of severe hemophilia A patients develop anti-factor VIII (FVIII) inhibitory antibodies after FVIII replacement therapy. Immune tolerance induction (ITI) upon daily injection of large amounts of FVIII is to date the only treatment to eradicate FVIII inhibitors. It is efficient in only 60-80% of the patients. Recently, the success of ITI was associated with a lower inhibitor peak following initiation of the treatment, suggesting that strategies dedicated to prevent the recall humoral response may improve the rate of ITI success.

Aims: We evaluated whether blocking B-cell receptor (BCR) signalisation by the inhibition of the Bruton's tyrosine kinase (BTK) reduces the anti-FVIII immune response in a mouse model of severe hemophilia A.

Methods: A new highly selective inhibitor of BTK, (R)-5-amino-1-(1-cyanopiperidin-3-yl)-3-(4-[2,4-difluorophenoxyl] phenyl)-1H pyrazole-4-carboxamide (PF-06250112), was administered to FVIII-deficient mice by gavage to inhibit BCR signaling. A preventive 4-weeks treatment of naive mice was first evaluated. We also assessed the efficiency of BTK inhibition on ongoing anti-FVIII immune responses in FVIII-sensitized mice and on the *in vivo* differentiation of adoptively-transferred memory B cells.

Results: We first confirmed the efficacy of PF-06250112 in preventing *in vitro* and *in vivo* BCR-mediated B cell activation. We then

observed that inhibition of BTK during the initiation phase of the anti-FVIII immune response does not prevent the development of anti-FVIII antibodies in mice. In contrast, the anti-FVIII memory B-cell response was drastically reduced in PF-06250112-treated mice as compared to control mice (1.0±0.3 versus 124.0±115.7 arbitrary units anti-FVIII IgG, P=0.010).

Conclusions: We propose that the inhibition of BTK using PF-06250112 at the time of initiation of ITI may prevent the reactivation of FVIII-specific memory B cells and increase the chances for ITI success.

OC 23.5 | Targeted Depletion of Gut Microbes Increases Factor VIII Inhibitor Development in Hemophilia A Mice

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Background: To date no studies have investigated the role of the gut microbiome in the anti-FVIII immune response. Ligation of the aryl hydrocarbon receptor (AhR) activates Tregs and down regulates Th17 cells. Specific ampicillin sensitive gut microbes convert dietary tryptophan into AhR ligands.

Aims: To assess if targeted modification of the gut microbiome, to deplete AhR ligands, in immature FVIII KO mice influences inhibitor incidence following administration of recombinant FVIII.

Methods: A minimally immunogenic regimen of FVIII in C57Bl FVIII KO mice was devised (0.5 IU recombinant FVIII twice weekly for 2 weeks). Immediately following weaning, 10 mice were transferred into a flexible film isolator for the study duration. Mice were gavaged twice daily with 500mcg ampicillin for 7 days. These microbiome manipulated mice (MMM) were fed autoclaved chow and sterile water during the remaining 6 weeks of the study. The MMM and controls (housed under regular conditions with no antibiotic exposure) were administered the FVIII regimen described. Mice were sampled weekly from the start of FVIII injections until euthanasia 4 weeks later.

Results: One of the MMM died following anesthesia during sampling (all mice otherwise appeared healthy throughout). 7 out of 9 surviving MMM developed anti-FVIII IgG antibodies detectable by ELISA at the penultimate week and study endpoint (median titre 1:320, range 0-5120), whereas only one of the control mice had detectable IgG at these times. Bethesda assay of plasma samples obtained at the end of the study demonstrated a mean inhibitor titre of 1.4 BU in MMM and 0.3 BU in control mice. Similarly, IgM had been detectable by the end of the study in 9 out of 10 MMM, but only in 1 of the 10 control mice.

Conclusions: Targeted depletion of tryptophan converting bacteria results in increased incidence of inhibitory antibodies in this hemophilia mouse model and highlights a role for the gut microbiome in modulating the immune response to therapeutic FVIII.

OC 31.1 | The Common VWF Variants p.T789A/p.Y795= Modify VWF Expression and Half-life in a Murine Model

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Background: The common *VWF* single nucleotide variants rs1063856 (c.2365A>G; p.T789A) and rs1063857 (c.2385T>C; p.Y795=) are in strong linkage disequilibrium (>99%) and associate with increased VWF:Ag and risk for thrombosis. Our previous studies suggest the non-reference (NR) allele increases VWF synthesis by enhancing mRNA half-life.

Aims: To characterize the influence of the VWF p.T789A/p.Y795= variants on VWF expression, half-life, and FVIII levels in a mouse model.

Methods: The human reference (R) allele is poorly conserved across species. The murine VWF (mVWF) wild-type (WT) cDNA contains the NR allele and was "humanized" by site-directed mutagenesis (c.2365G>A/c.2367T>C, c.2385C>T). *In vivo* expression was assessed through hydrodynamic injections in VWF/FVIII double knockout (DKO) mice. The half-life of plasma-derived mVWF generated in VWF/FVIII DKO mice and recombinant human (hVWF) was measured. rs1063856/rs1063857 genotype and VWFpp/VWF:Ag ratio was assessed in a cohort of 369 healthy individuals.

Results: In VWF KO mice, p.A789T (16.7%, p=0.059) and p.A789T/p.Y795= (25%, p=0.032) had decreased expression compared with WT mVWF, while p.Y795= expressed alone did not (94%, p=0.9). In VWF KO mice, infusion with p.A789T/p.Y795= mVWF (FVIII-free) had a shorter half-life than WT (2.53 vs 5.98 h, p< 0.0001) and impaired stabilization of endogenous FVIII:C (AUC=80% decrease, p< 0.001). In contrast, when recombinant p.T789A/p.Y795= hVWF was co-infused with recombinant hFVIII into VWF/FVIII DKO mice, VWF (38.1 vs 39.7 min, p=0.66) and FVIII (39.95 vs 35.56 min, p=0.5) half-life was not different from WT. In healthy human subjects, the NR allele was associated with decreased VWFpp/VWF:Ag ratio (NR/NR: 1.16, n=35; NR/R: 1.23, N=159, p=0.09; R/R: 1.29, N=175, p=0.01).

Conclusions: The p.T789A/p.Y795= variants associate with increased VWF levels in mice as well as humans. This effect may be related to both clearance and biosynthetic-related mechanisms.

OC 31.2 | Macrophage Scavenger SR-AI Mediates von Willebrand Factor Clearance

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Background: Previously, we identified macrophage-LRP1 to mediate VWF clearance in a flow-dependent manner. However, its contribution to VWF clearance is moderate at best, suggesting that also other macrophage-receptors contribute to VWF elimination.

Aims: To identify alternative macrophage-receptors involved in VWF clearance.

Methods: Cell- and solid-phase binding studies using recVWF variants were performed to analyze VWF-receptor interactions. Scavenger Receptor-AI (SR-AI)-deficient mice were used to determine VWF propeptide (VWFpp)/VWF:Ag ratios (a measure for VWF clearance).

Results: Although we confirmed that only active VWF binds to LRP1, we nevertheless observed efficient binding of inactive VWF to monocyte- and THP1-derived macrophages, suggesting that other VWF-receptors exist. In search for alternative receptors, we focused on SR-AI, a macrophage-specific receptor. In solid-phase binding assays, VWF bound efficiently to SR-AI (half-max binding 6±2 nM). Binding to SR-AI was calcium-dependent and inhibited by 72±4% in the combined presence of two MoAbs against the VWF A1- & D4- domains. Recombinant VWF D'-D3-, A1- and D4-domain fragments displayed dose-dependent binding to SR-AI, indicating the presence of multiple binding sites.

In cell-binding assays using macrophages and SR-AI-transfected HEK293-cells, marked co-localization of VWF and SR-AI was detected, and cellular binding was significantly reduced by the anti-A1/D4-domain MoAb combination. VWFpp/VWF:Ag ratios following expression of human VWF in wt-, SR-AI- and LRP1-deficient mice, were significantly lower in SR-A1-deficient mice compared to wt-mice & LRP1-deficient mice (0.7±0.2 vs 1.3±0.1 & 1.1±0.1; p< 0.0001). Interestingly, VWF-clearance mutants VWF/p.R1205H (Vicenza) and VWF/p.S2179F but not the type 2B mutant VWF/p.V1316M had increased binding to SR-AI.

Conclusions: We have identified SR-AI as a novel macrophage-specific receptor for VWF. Enhanced binding of VWF mutants to SR-AI may contribute to the increased clearance of these mutants.

OC 31.3 | Real-time Intravital Imaging and Quantification of VWF Clearance by Endothelial Cells and Macrophages in the Murine Liver

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Background: Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), and the LSEC receptor stabilin-2 (Stab2) contribute to VWF clearance. *STAB2/VWF* DKO mice have 2.2-fold increased half-life of human VWF compared to VWF KO controls.

Aims: To characterize the role of KCs, LSECs, and Stab2 in VWF clearance using intravital microscopy, IHC, and *in vivo* clearance studies.

Methods: Fluorescent polystyrene microspheres (50 nm) were coated with human plasma-derived VWF (pdVWF), glycine (which does not bind LSECs), or BSA (an LSEC ligand). Coating was confirmed using IF on Stab2 expressing cells and by solid phase assay. In *VWF* KO mice, LSECs (CD31) and KCs (F4/80) were labelled using fluorescent antibodies. The liver was exteriorized by midline laparotomy, and microspheres were injected via jugular catheter. Association of fluorescent microspheres with LSECs and KCs was visualized in real time by spinning disc confocal microscopy. IHC was performed on livers from VWF KO mice infused with human pdVWF. VWF half-life was measured in *STAB2/VWF* DKO mice.

Results: Microspheres were observed binding to LSECs and KCs within 5 minutes of infusion. Clearance of both VWF (68.74%, p< 0.0001) and BSA (64%, p< 0.0001)-coated microspheres was mediated predominantly by LSECs as compared to uncoated (17%) and glycine-coated microspheres (20.9%). Using IHC, infused VWF was confirmed to associate with LSECS (CD31 and Stab2), and KCs (F4/80) in the liver.

Clodronate liposome-induced depletion of macrophages (including KCs) increased VWF half-life in *STAB2/VWF* DKO mice 1.5-fold (235.4 min) compared to liposome controls (156.2 min, p=0.018). Cyclophosphamide-induced LSEC cytotoxicity increased VWF half-life 1.3-fold in *STAB2/VWF* DKO mice (206.1 min, p= 0.042) compared to controls (158.8 min).

Conclusions: VWF interactions with LSECs and KCs can be observed in real time with intravital microscopy. IHC and LSEC and KC depletion studies confirm the role of both cell types in VWF clearance.

OC 31.4 | A Novel Macrophage-mediated Pathway Regulates Enhanced Clearance of Hyposialylated von Willebrand Factor *In vivo*

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Background: von Willebrand Factor (VWF) is a plasma sialoglycoprotein which plays a critical role in haemostasis. While the biosynthesis and function of VWF are well characterized, the mechanisms underlying VWF clearance remain poorly understood. However, increased clearance is important in the etiology of von Willebrand disease.

Aims: To define the mechanisms involved in the enhanced *in vivo* clearance of hyposialylated VWF.

Methods: The asialoglycoprotein receptor (ASGPR) plays a key role in clearing hyposialylated VWF. To investigate if other receptors and/or cell types may also be important, $VWF^{-/-}$ and $Asgr1^{-/-}$ mice were crossed creating a novel dual $VWF^{-/-}/Asgr1^{-/-}$ knockout model. Human VWF (pdVWF) was modified using specific neuraminidases creating two glycoforms; α2-3NeuVWF and α2-3,6,8,9NeuVWF. *In vivo* clearance of these glycoforms was studied in $VWF^{-/-}/Asgr1^{-/-}$ mice.

Results: Although previous studies described a critical role for ASGPR in regulating clearance of hyposialylated VWF, we observed markedly enhanced clearance of both α2-3NeuVWF and α2-3,6,8,9NeuVWF in $VWF^{-/-}/Asgr1^{-/-}$ mice (T½= 8.2±0.6 and 3.2±0.4 vs. 50.6±2 mins for pdVWF, respectively). Importantly, the enhanced clearance of hyposialylated VWF was not significantly attenuated in the presence or absence of ASGPR. In contrast however, clearance of hyposialylated VWF in $VWF^{-/-}/Asgr1^{-/-}$ mice was reduced in the presence of asialoorsomucoid (p< 0.05). Furthermore, immunohistochemistry demonstrated localization of asialo-VWF within hepatic macrophages. Finally, in vivo macrophage depletion with liposomal clodronate also significantly attenuated the enhanced clearance of hyposialylated VWF in $VWF^{-/-}/ASGPR1^{-/-}$mice (p< 0.05).

Conclusions: Collectively, these findings demonstrate that a novel macrophage-dependent pathway is a critical modulator of the enhanced clearance of hyposialylated VWF. Given that quantitative variations in N- and O-linked sialylation has been described in specific patient cohorts, these findings are of direct clinical importance.

OC 31.5 | Genetic Variability and Glycans Modulate the Interaction between VWF and the Sinusoidal Endothelial Clearance Receptor Stabilin-2

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Background: Stabilin-2 (Stab2) binds, internalizes and clears human VWF. Stab2 contains an X-link carbohydrate binding domain and repeating fasciclin and EGF-like domains. Common single nucleotide variants (SNVs) in *STAB2* associate with VWF (rs4981022, -3.6%) or FVIII (rs12229292, 3.1%) in normal individuals. The rare *STAB2* SNV p.E2377K associates with higher VWF-FVIII levels (33.7%, 26.8%).

Aims: To characterize the genetic and biochemical mechanisms that regulate VWF-Stab2 interactions.

Methods: Human VWF half-life was measured in *VWF/STAB2* DKO mice. Endocytosis of VWF by Stab2 transfected HEK-293 cells was observed by immunofluorescence. *STAB2* variant pathogenicity was analyzed with Alamut software. SNVs in *STAB2* were genotyped in 165 type 1 VWD index cases.

Results: Stab2 expressing cells demonstrated enhanced uptake of de-N-glycosylated VWF compared to plasma derived VWF (210%, p< 0.0001). The half-life of de-N-glycosylated VWF was 3-fold longer in *STAB2/VWF* DKO mice (24.1 min) than VWF KO mice (8.1 min, p< 0.0001). VWF internalization was decreased by preincubation of Stab2-expressing cells with the polysaccharide ligands hyaluronic acid (-81.1%, p=0.0002), unfractionated heparin (-95.7%, p< 0.0001), mannan (-93.6%, p< 0.0001) and dermatan sulfate (-75.7%, p=0.0002).

In silico analysis suggests the Stab2 p.E2377K SNV may be damaging to protein function. In HEK 293 cells p.E2377K Stab2 demonstrated decreased expression (1.2 vs 12.5% positive, 48% decreased MFI) and overall VWF binding (28%, p< 0.0001) compared to wild-type.

In type 1 VWD patients, the *STAB2* SNV rs12229292 associated with elevated VWF:Ag (β:3.8%; CI:0.8, 6.8; p=0.014) and FVIII:C (β:1.9%; CI:-3.8, 7.6; p=0.51). rs4981022 associated with lower VWF:Ag (β:-2.8%; CI:-5.5, -0.1; p=0.041) and FVIII:C (β:-1.4%; CI:-6.5, 3.6; p=0.58).

Conclusions: Binding of VWF to Stab2 is not N-linked glycan-dependent and can be inhibited by Stab2 polysaccharide ligands. SNVs in *STAB2* modify its interaction with VWF and contribute to altered levels of plasma VWF in VWD.

OC 38.1 | Correcting the Bleeding Phenotype in Hemophilia A Using Lentivirally FVIII-corrected Endothelial Cells Differentiated from Hemophilic Induced Pluripotent Stem Cells (iPSCs)

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Background: Hemophilia A (HA) is caused by factor VIII (FVIII) gene mutations. Somatic cells can be reprogrammed to generate autologous, disease-free iPSCs and differentiated into cells relevant for gene and cell therapy.

Aims: To generate patient-specific iPSCs after cell reprogramming of CD34+ cells from peripheral blood and differentiation into FVIII-secreting endothelial cells (EC) after genetic correction by lentiviral vectors (LV).

Methods: CD34+ cells isolated from healthy and HA donors and reprogrammed with a Cre-Lox LV carrying OCT4-SOX2-KLF4 and miRNA302/367. iPSCs were characterized for stem cell markers, telomeres length and karyotype analysis. Germ layers markers expression and differentiation potential assessed on embryoid bodies (EB). iPSCs were differentiated in EC and characterized by FACS and RT-PCR. HA EC were transduced with LV carrying GFP and/or FVIII under the control of EC-specific promoter (VEC) and transplanted in NOD/SCID-gNull (NSG) HA mice. Cell engraftment and proliferation analyzed by immunofluorescence.

Results: iPSCs were differentiated into endothelial cells (EC) with an optimized protocol, acquired endothelial-like morphology, expressed ECs markers and were able to form tubules when cultured in matrigel. EC transplanted intraportally in NSG mice, engrafted and proliferated in the livers up to 12 weeks and confirmed by FACS analysis to be GFP and CD31+ representing the 30% of liver non-parenchymal cells. Moreover, transplanted cells formed vessels-like structure in the host liver. Finally, we transplanted HA-IPSC-derived ECs corrected by

LV-VEC-FVIII in NSG-HA mice that showed a reduced bleeding time and a stable 5% FVIII activity after 12 weeks.

Conclusions: These data will be instrumental to assess engraftment, proliferation and the FVIII expression from differentiated EC, gene corrected and reprogramming factor-free iPSCs to confirm the suitability of this approach for HA gene-cell-therapy.

OC 38.2 | Inhibitory Nanobodies against Antithrombin Correct Thrombin Generation and Reduce Bleeding in FVIII-deficient Mice

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Background: Inhibition of natural anticoagulant factors (antithrombin (AT), TFPI, ...) defines a novel strategy for improving hemostasis in hemophilia patients. Targeting these anticoagulants is associated with improved clinical phenotype in these patients, both with and without inhibitors.

Aims: Assessing the use of anti-AT nanobodies as a new strategy to rebalance the hemostatic system by improving thrombin generation in hemophilia A.

Methods: Llama-derived nanobodies against AT were generated. Engineered bi- and multivalent variants where tested for inhibition of AT activity, thrombin generation (TGT) in factor VIII (FVIII)-deficient plasma and hemostatic activity in a tail vein-transection bleeding model.

Results: Among the isolated nanobodies, several showed reactivity to AT of several species including murine AT, which facilitates in vivo analysis. Three variants (KB-AT-002/003, KB-AT-113, KB-AT-1123) revealed a high affinity for AT (apparent Kd < 1 nM) and proved their effectiveness in blocking the activity of the AT (inhibition 50-100%). In a thrombin generation assay using FVIII-deficient plasma, normalization of the endogenous thrombin potential was observed in the presence of each of these nanobodies. In addition, KB-AT-113 and -1123 were able to normalize other TGT-parameters as well, such as peak-height, lag-time and time-to-peak, in a manner similar to FVIII at 1 U/ml. In vivo, the intravenous injection of KB-AT-113 in FVIII-deficient mice significantly reduced blood loss compared to vehicle-treated mice.

Conclusions: Inhibitory anti-AT nanobodies efficiently correct the hemostatic deficit in the absence of FVIII in vitro and in vivo. The particular characteristics of nanobodies (subcutaneous application, high stability at room temperature and ease of production in microorganisms) make them promising tools for low-cost treatment of hemophilia. Their small size may also facilitate gene therapeutic approaches.

OC 38.3 | Exploring Chaperone-like Compounds as Innovative Therapeutic Strategy for Hemophilia B

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Background: Missense mutations impairing protein folding are a frequent cause of human disease. Small molecules with chaperone activity and able to assist protein folding could rescue protein biosynthesis, with therapeutic implications. This could be relevant in coagulation factor disorders that would benefit even from a tiny increase of functional protein levels (>2%).

Aims: To characterize intracellular processing of a panel of FIX missense variants causing severe Hemophilia B (HB) and to challenge them with chaperone-like drugs.

Methods: Transient and stable expression of recombinant FIX (rFIX) variants in mammalian cells to create HB cellular models followed by evaluation of secreted/intracellular protein levels (ELISA, Western Blotting) and intracellular trafficking (Immunofluorescence) upon treatment.

Results: Transient expression studies indicated that the selected model mutations Y115C, Y161C, R294Q, Y305C, F424L and Y450C severely impair FIX secretion (0.2-0.8% of wild-type), which recapitulates finding in HB patients. Co-localization studies on stable cells showed that missense variants scarsely co-localized with the Golgi apparatus, which could underlie an altered trafficking due to mis-folding. The observation that cell treatment with the proteasome inhibitor MG-132 increased secreted rFIX levels for some variants supports partial intracellular degradation of mis-folded proteins.

These findings prompted us to challenge a first model mutation with Na-PBA, a chaperone-like compound succesfully exploited for other proteins. Intriguingly, these pilot studies on the rFIX-305C variant showed that treatment with 0.1-1 mM NaPBA induced a 2-fold increase in secreted rFIX levels in a dose-dependent manner.

Conclusions: Our data, besides contributing to dissect the molecular basis of Hemophilia B, provide a preliminary but intriguing experimental evidence for the rescue of HB-causing missense variants with pharmacological chaperones.

OC 38.4 | Development of Nanobodies Fused to Albumin Binding Peptide: A Tool to Increase Plasmatic Levels of Endogenous Proteins

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Background: Many hemorrhagic disorders are characterized by partial quantitative deficiencies in plasmatic levels of coagulation factors. Type 1 von Willebrand disease (VWD-type 1), a typical example of these quantitative disorders, is characterized by reduced levels of circulating von Willebrand factor (VWF), and a corresponding bleeding tendency. VWF mediates platelet aggregation/adhesion to damaged endothelium and carries factor VIII (FVIII). Current treatment options include replacement therapy by intravenous injection of VWF-concentrates, or by intravenous injection of Desmopressin (DDAVP), provoking the release of intracellular VWF and increasing FVIII levels.

Aims: Our aim is to develop a new therapeutic approach to increase the plasmatic level of VWF by prolonging its half-life in plasma.

Methods: A series of non-inhibitory nanobodies against human/murine VWF was generated. Nanobodies were fused to an albumin-binding peptide (ABP).

Results: A bivalent construct of such nanobody fused to ABP (KB-VWF-013bv_ABP) was constructed. KB-VWF-013bv_ABP bound efficiently to VWF and albumin *in vitro*. Half-life of human VWF was prolonged about 7-fold when it was pre-incubated with KB-VWF-013bv_ABP prior to intravenous (IV) injection. IV-injection of this bivalent construct into wild-type C57B6 mice resulted in increased levels of endogenous VWF (15±7 fold at 3 days and 8±5 fold at 7 days after injection). FVIII levels increased concomittantly (7±3 fold and 6±3 fold, respectively). Subcutaneous injection resulted in increased levels of VWF at day 3 and 7 (4±1 fold in both cases).

Conclusions: These results clearly show that KB-VWF-013bv_ABP is able to bridge VWF and albumin, leading to an important increase of VWF half-life, thereby increasing endogenous plasma levels. Fusion of the same ABP to nanobodies targeting other relevant proteins (eg factor IX) could be used to increase the endogenous level of coagulation factors in other hemorrhagic disorders characterized by quantitative deficiencies.

OC 38.5 | Development of a Gene Therapy Strategy for von Willebrand Disease Based on Dual Adeno-associated Virus Vectors

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder in humans, caused by quantitative or qualitative defects in von Willebrand factor (VWF). Current therapies have still limitations mostly related to their short-term efficacy. In this context, VWD represents a potential target for gene therapy approaches, as a single treatment could potentially results in a long-term correction of the disease.

Aims: Develop an innovative gene therapy strategy for VWD based on dual overlapping adeno-associated virus vectors (AAV).

Methods: In our approach the large 8.4 kb VWF coding sequence is split in two AAV vectors, thus permitting to overcome the AAV size limitation (5 kb). In this system each AAV delivers one half of the VWF cDNA with an overlapping region, which mediates the reconstitution of the entire genome by homologous recombination. We injected each vector in the tail vein of VWF KO mice, at a dose of 2E+13 vg/kg, and assessed the circulating VWF levels by ELISA assay 4 and 6 weeks post-administration.

Results: We generated a dual AAV8 vector expressing murine VWF under the control of the liver specific human alpha-1 antitripsin promoter (hAAT). The two halves of the transgene expression cassette (5‘ and 3‘ cassette) contained in the dual AAV share a homologous region of 400 base pairs. Delivery of the two vectors resulted in detectable levels of VWF expressed by the liver (up to 3% of normal) at 6 weeks post-administration. Conversely, circulating murine VWF levels were undetectable in control mice injected with the 5‘ or the 3‘-cassette alone.

Conclusions: Results obtained in VWF KO mice demonstrate that, taking advantage of the ability of the dual AAV vector system to reconstitute a full-length cDNA in hepatocytes, we were able to express the large gene of VWF from the liver. Future experiments will be aimed at optimizing the dual AAV in order to increase expression levels and to subsequently evaluate biochemically the effect of VWF transgene expression on the hemorrhagic phenotype.

OC 37.1 | Influence of Phospholipid Configuration of APPT Reagents in the Diagnosis of Mild Haemophilia A with Discrepancy

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Background: Mild haemophilia A (MHA) is defined by a level of FVIII:C between 5-40 IU/dL. In some cases, discrepancies between chronometric and chromogenic assays have been described inducing difficulties to define the severity of their disease.

Aims: Evaluation of the impact of APTT reagents with different phospholipids configuration on FVIII:C results in MHA patients with discrepancy.

Methods: 29 patients with a MHA and previously diagnosed with discrepant FVII:C results were studied. The discrepancy is defined by a APTT FVIII:C / chromogenic FVIII:C ratio < 0.5 or >1.5. Among those patients 18 have the same mutation of the F8 gene: p.Phe2146Ser.

Five APTT reagents (Ck prest®-Stago, Actin FS®- Siemens, APTT-SP®, SynthASil SF®, SynthAFax®-Werfen) were used and BIOPHEN FVIII:C Hyphen as chromogenic assay. Measurements were run on an ACL TOP device with the same calibrator (Siemens©).

Results: In patients with standard discrepancy (APTT /chromogenic ratio >1.5) this discrepancy is confirmed with all APTT reagents. For patients with APTT /chromogenic ratio < 0.5) no difference (p>0.05) was found between APTT reagents except for patients with the p.Phe2146Ser mutation. For those patients, when using APTT-SP®, SynthASil SF® or SynthAFax® there was no discrepancy between chronometric and chromogenic assays. In contrary, when using Ck prest® or Actin FS, we still found the initially described discrepancy (p=0,0002).

Conclusions: In this study we observed discrepancies in several mutations between APTT assays and chromogenic assay. In patients with p.Phe2146Ser, the discrepancy seems to be more linked to the composition of phospholipids in APTT reagents than to the method (chronometric or chromogenic) used for FVIII:C measurement. This mutation modifies the C1 domain of the FVIII protein which is known to interact with phospholipids. Further studies are needed to define how this mutation interacts with phospholipids and which the most relevant test is.

OC 37.2 | Desmopressin in Hemophilia: The Need for a Clinical Response Definition and Individualized Test Regimen Based on FVIII: C Values One Hour after Infusion

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Background: Due to inter-individual variation in desmopressin response, non-severe hemophilia A (HA) patients require testing prior to therapeutic treatment. However, adequate response or frequency of blood sampling are not defined in international guidelines. Consequently, various definitions and blood sampling protocols are used. Interestingly, sustainability of desmopressin response is not incorporated.

Aims: To study desmopressin response rates in a cohort of non-severe HA patients using currently accepted desmopressin response definitions. This, in order to formulate a response definition which includes information on sustainability. This study aims to launch a standardized blood sampling protocol which is widely applicable.

Methods: Currently used desmopressin response definitions in non-severe HA were derived from a literature search. Actual response rate according to the varying definitions was analyzed in 105 non-severe HA patients in the Erasmus MC hemophilia treatment center. In this study, sustained response was defined as FVIII:C ≥ 0.30 IU/ml three and six hours after desmopressin infusion.

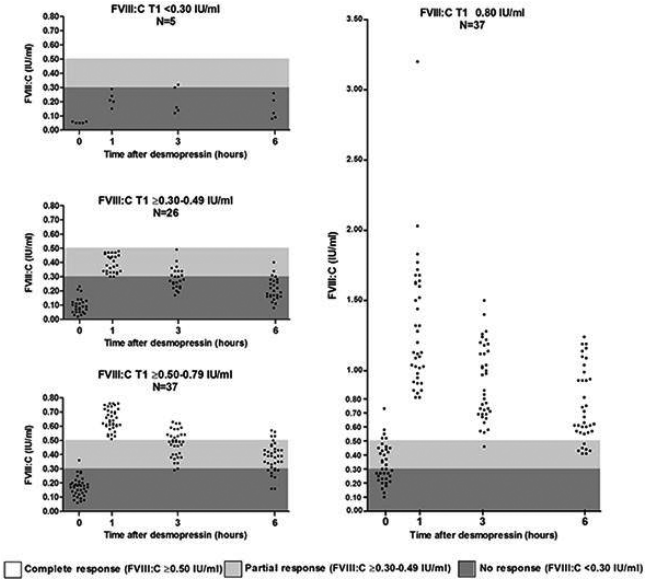

FIGURE 1 FVIII:C before and after desmopressin test in several subgroups based on FVIII:C levels measured one hour (T1) after desmopressin

Results: Five response definitions were evaluated, three of which included only FVIII:C cut-off levels and two also incorporating FVIII:C-fold increase of baseline value. Strikingly, FVIII:C-fold increase showed no association with response sustainability. FVIII:C 1 hour after infusion (< 0.30, ≥0.30-0.49, ≥0.50-0.79 or ≥0.80 IU/ml) was however indicative of response after 6 hours: 100% of patients with FVIII:C ≥0.80 IU/ml after 1 hour had a sustained response after 6 hours (Figure).

Conclusions: We suggest a desmopressin response definition based on clinically relevant FVIII:C levels, e.g. 0.30 and 0.50 IU/ml, and an individualized blood sampling regimen. Patients with FVIII:C < 0.30 IU/ml after 1 hour (non-responders) or ≥0.80 IU/ml will not require subsequent blood sampling. However, patients with FVIII:C ≥0.30-0.79 IU/ml after 1 hour should undergo blood sampling after 6 hours to determine response sustainability.

OC 37.3 | The Role of Vascular Remodeling and Clotting Factor Thresholds in the Context of Hemophilic Joint Bleeding: Results from a Prospective Imaging Study in Patients with Hemophilia and Advanced Arthropathy

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Background: Raising clotting factor (CF) troughs to prevent hemophilic joint bleeding is gaining popularity. Ideal levels and the role of individual pharmacokinetic (PK) parameters are unknown.

Aims: We studied PK, joint- and patient-specific parameters on hemophilic joint bleeding.

Methods: Thirty-one adults with hemophilia and ≥ 1 arthropathic joint (defined by abnormal radiographic Pettersson Score [PS]) were studied prospectively for 2 yrs with Musculoskeletal Ultrasound of major joints (n≈600 joint exams) during pain-free intervals and during painful events for bleed status. Microvascular perfusion was determined by Power Doppler (PD). CF infusion logs and plasma levels allowed PK calculations (1/2 life; area under curve [AUC]; time spent below CF activity thresholds) during and between bleeds. PK modeling estimated AUC and time spent below 5, 10, 15 or 20 units/dL for the 15 days prior to each bleed, and for random periods in the same patients and those without bleeding events.

Results: Of 23 reported painful events, 15 were associated with bleeding. Bleeds were not preceded by lower AUC, time spent below particular CF levels, or lower CF consumption compared to random control periods before and after bleeding. PK parameters were comparable in patients having painful events without bleeding or patients with no painful events. At baseline, only PS were higher in patients with future bleeds (p = 0.02), while age, weight, vascular PD signal and clinical joint scores were similar compared to patients without future bleeds. During painful events, only bleeding joints had increased vascular PD signal and flow intensity that were 2-3 times greater compared to non-bleeding joints (both p = 0.03).

Conclusions: Joint bleeds were not explained by fluctuations below individual CF thresholds, but linked to pronounced vascular remodeling. This suggests that raising CF troughs may not entirely control bleeding and that vascular remodeling and leak may contribute.

OC 37.4 | Development and Validation of the "Joint Activity and Damage Exam" (JADE) for Quantitation of Structural Abnormalities by Musculoskeletal Ultrasound (MSKUS) in Hemophilic Joints: Intra- and Inter-rater Reliability

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Background: Several semi-quantitative scales using musculoskeletal ultrasound (MSKUS) to assess hemophilic arthropathy have been proposed. However, a quantitative method to measure tissues, osteochondral surfaces and vascular perfusion is missing.

Aims: To develop, standardize and validate a quantitative protocol to measure tissue abnormalities with MSKUS in hemophilic arthropathy.

Methods: Thirty-one adults with hemophilia were studied prospectively for 2 yrs with MSKUS at regular intervals (n≈600 joint exams; ≈6000 images). Based on the spectrum of pathologies, a quantitative algorithm to measure osteochondral surface defects, cartilage thickness, soft tissue expansion, microvascular perfusion abnormalities (Power Doppler [PD]) and to detect effusions was developed for ankles, elbows, and knees (Joint Activity and Damage Exam [JADE]). To study feasibility and intra-/inter-rater reliability, 8 hemophilia providers experienced in MSKUS performed anatomical landmark recognition and measurements on 45 knee MSKUS images, with repetition 1 month later. Similar assessments were performed by 23 *in*experienced MSKUS providers post training. A radiologist was the adjudicator. Intra-class correlation coefficients and Fleiss' Kappa were calculated with AgreeStat 2015.6. Study procedures complied with the UCSD Human Research Protection Program.

Results: Recognition of anatomical landmarks was nearly 100% for all providers. Measurements of structures were feasible to as little as 1/10th of a millimeter. Intra-/inter-rater reliability of cartilage thickness, osteochondral surface defects, soft tissue expansion, effusions and PD signals ranged from substantial to nearly perfect (Correlation Coefficient and Fleiss' Kappa 0.63-0.94; Table 1/2).

Conclusions: JADE is the first quantitative MSKUS algorithm of precise measurements in hemophilic joints. High intra-/inter-rater reliability should enable easy quantification of progression of hemophilic arthropathy, which will benefit clinical practice and multi-center research.

TABLE 1 Intra-Class Correlation Coefficient for Knee Measurements

	Experienced Providers				Inexperienced Providers
Measurement	Inter-Rater Reliability First Assessment	Inter-Rater Reliability Second Assessment	Intra-Rater Reliability	Inter-Rater Reliability with Adjudicator	Inter-Rater Reliability
Cartilage Thickness Point 1	0.79	0.84	0.80	0.78	0.73
Cartilage Thickness Point 2	0.92	0.92	0.90	0.93	0.88
Osteochondral Interface	0.70	0.83	0.85	0.73	0.70
Soft Tissue Expansion	0.89	0.84	0.84	0.89	0.63

TABLE 2 Fleiss' Kappa for Knee Measurements

	Experienced Providers				Inexperienced Providers
	Inter-Rater Reliability First Assessment	Inter-Rater Reliability Second Assessment	Intra-Rater Reliability	Inter-Rater Reliability with Adjudicator	Inter-Rater Reliability
Power Doppler	0.91	0.94	*	0.92	**
Anechoic vs. Mixed Echogenicity	0.88	0.90	*	0.70	**
Simple vs. Complex Effusion	0.88	0.90	*	0.69	**

* There is no intra-rater reliability calculation formula for ordinal data **PD and Effusion MSKUS images were not provided to inexperienced providers

OC 37.5 | Evaluation of the Hemostatic Effect of the Combination of Factor Concentrate with Tranexamic Acid (TXA) in vitro Using Global Assays

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Background: Prophylaxis is the gold standard for the treatment of severe hemophilia(sH). However, there is a need for low cost,safe,and well tolerated therapies that have the potential to enhance prophylaxis. Such therapies may reduce the frequency of factor infusions,thereby improving adherence and reducing the cost of prophylaxis. The addition of TXA to factor concentrates may be one strategy for optimizing prophylaxis.

Aims: We aimed to study whether the clot stability in patients with sHA is improved with the combination of TXA and low FVIII plasma concentration(achieved in prophylactic setting).

Methods: Following informed consent, the whole blood(WB) and plasma(PL) obtained from 12 adult patients with sHA were studied in vitro after spiking the samples to achieve a final concentration(FC) of 0,3,10 and 30IU/dL of FVIII with or without adding TXA at FC of 0.1mg/ml. WB was assessed with ROTEM in the presence of t-PA in 6 patients. Thrombin generation(TG) was measured in PL of 6 patients. The clots obtained from TG reaction, were analyzed using electron microscopy. Diameters of randomly selected 100 fibrin fibers were measured in each clot. Results obtained with FVIII alone were compared to those with FVIII+TXA.

Results: A dose dependent improvement of TG and fibrin clot structure was observed after adding FVIII alone(p=0.024). The addition of TXA had no effect on TG capacity. However,in the presence of TXA better fibrin clot structure was observed. Fibrin fiber diameters were significantly decreased with TXA+FVIII compared to FVIII(p=0.007), suggesting a strong fibrin network as thin fibrin fibers are characteristic of a robust fibrin mesh. In the presence of TXA,ROTEM analysis was not suitable to evaluate the resistance of clots against fibrinolysis, the test being too sensitive to the effect of TXA. In all patients,ROTEM was fully normalized after addition of TXA only with no exogenous FVIII.

Conclusions: Our results suggest a potential benefit of TXA when used in combination with FVIII in prophylactic setting.

OC 36.1 | Results from Italian Registry of Activated Prothrombin Complex Concentrate in Acquired Hemophilia A: The FAIR Study

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Background: Bypassing agents are the first line therapy in patients with acquired hemophilia A (AHA). Activated prothrombin complex concentrate (aPCC) proved to be effective and safe but its use is not fully standardized.

Aims: To evaluate dosage, days of treatment, efficacy and safety of aPCC in an Italian population of AHA patients with acute bleeding.

Methods: The FAIR Registry is a retrospective-prospective study started in Dec 2012. All patients (pts) with AHA treated with aPCC and diagnosed within the previous ten years if retrospective pts and up to the end of Dec 2015 if prospective ones were initially enrolled.

Results: Statistical analyses were performed on 31 retrospective and 25 prospective pts, 50% males, mean age 69.9±15.1 years. 51.8% were idiopathic AHAs. 101 total bleeds were reported, 65.3% of which in the retrospective group. 84.1% were spontaneous, 71.3% involving muscles or skin. aPCC as first line therapy was used in 82.2% of cases, median dose 72.6±26.6 IU/kg, median frequency: 12 hours (IQR 0-84)

and median number of doses: 9 (IQR 1-104). Treatment was continued for a median of 8 days (IQR 1-48). aPCC was considered effective in the 96.4% and was associated with antifibrinolytics in 39.6% of cases, being higher in the prospective group (p< 0.05). Low-dose aPCC as short-term prophylaxis to prevent recurrences was started after the first episode in 26.8% of pts, in a mean dose of 54.2±23.0 IU/kg, continued up to a mean of 20.5±17.6 days, more frequent in prospective pts (p< 0.05). Anamnestic response was reported in 6/101 of treatments, 2 in the prospective group, median inhibitor titer was 9.3 BU (IQR 0.6-41.8) and increased after a median of 6 days (IQR 2-19) from first treatment start. No thrombotic events were reported. 8 pts died, one uncontrolled bleeding was AHA related.

Conclusions: aPCC proved to be effective and safe in the treatment of bleeds in AHA. Low-dose aPCC as secondary short-term prophylaxis seems to prevent bleeding recurrences.

OC 36.2 | Low Dose of aPCC as Short-term Prophylaxis after the End of Initial Treatment of Acquired Hemophilia a to Reduce Bleeding Recurrences: Data from the FAIR Registry

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Background: Bypassing agents are the first line therapy in patients with acquired haemophilia A (AHA). Activated prothrombin complex concentrate (aPCC) proved to be effective as initial treatment, but 20% of patients (pts) had recurrences. aPCC as short-term prophylaxis to reduce subsequent bleeds is not still clear.

Aims: To evaluate whether a short-term prophylaxis with low dose of aPCC can reduce bleeding recurrences after initial AHA treatment, maintaining safety.

Methods: The FAIR Registry is a retrospective-prospective study that collected data on all pts with AHA treated with aPCC in 12 Italian Hemophilia Centers. All statistical analyses were carried out in the 56 pts included in the registry, 31 retrospective (from 2003 to 2012) and 25 prospective (from 2013 to 2015).

Results: 31 retrospective and 25 prospective pts were evaluated, 51.8% were idiopathic AHAs. 101 bleeds requiring treatment were reported, 84.1% spontaneous, 71.3% involving muscles or skin. Major bleeds were 38,6%. aPCC was used as first line therapy in 82.2% events and administered in a median dose of 72.6 ± 26.6 IU/kg. Time of treatment was 8 days (IQR 1-48), without significant differences between the two groups. aPCC was considered effective in 96,4% of cases. Low-dose aPCC as short-term prophylaxis was started after first episode in 15/56 pts, 60% of whom prospective, in a mean dose of 54.2±23.0 IU/kg, higher (61.4±23.4 IU/kg) in the prospective group than in the retrospective one (44.3±19.7 IU/kg) and it was continued up to a mean of 20.5±17.6 days, similar in both groups. A total of 32 bleeding recurrences were reported, 87.5% in the retrospective group. Only 9.4% occurred during short-term prophylaxis (p< 0.05). In our Registry no thromboembolic events were found.

Conclusions: Initial AHA treatment with aPCC proved to be highly effective, but a consecutive low dose as short-term prophylaxis seems to demonstrate a significant reduction in bleeding recurrences maintaining safety profile.

OC 36.3 | Blood Pump Hemocompatibility: in vitro and in vivo Evaluation of von Willebrand Factor Degradation

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Background: Approximately 40% of heart failure patients who receive an implantable rotary blood pump (RBP) suffer from gastrointestinal bleeding complications, which arise from the degradation of high molecular weight (HMW) von Willebrand Factor (VWF). While standardized test methods exist to address the simple task of pre-clinical hemolysis testing, no accepted standards or regulatory guidance exist for assessing VWF compatibility.

Aims: In this study, we investigated (i)how various experimental factors influence VWF compatibility in RBPs and (ii)methods for evaluating prototype RBP VWF compatibility during in vivo testing.

Methods: .Human blood bank plasma was circulated through bench-top flow loops with a HeartWare HVAD and Levacor (LEV), Thoratec-SJM HeartMate II (HMII) and CentriMag (CM), and Vadovations prototype blood pump (V-O VAD) while a control loop was rocked at 1Hz. The pumps were operated to generate 4L/min against 75mmHg. Samples were collected at pre-test, 0, 30 and 60 min, then hourly for 6h and analyzed for VWF collagen binding activity (VWF:CB) and VWF antigen level (VWF:Ag) using commercially available kits. Healthy ovines were also implanted with the V-O pump for up to 90 days, and plasma samples were analyzed for VWF:CB using a modified commercially available kit. VWF multimer profiles of in vitro and in vivo plasma samples were visualized using gel electrophoresis and near-IR in-gel scanning and analyzed using standard densitometry techniques.

Results: VWF:CB/Ag remained near baseline levels after 6h in the LEV and V-O pumps. In the HVAD, HMII and CM pumps, VWF:CB/Ag at 6h was decreased by 33%, 40% and 41%, respectively. These three pumps also degraded HMW VWF while the LEV and V-O pumps did

not. The V-O pump also preserved VWF:CB and HMW VWF in ovines out to 90 days.

Conclusions: RBP prototypes can be tested both in vitro and in vivo for VWF compatibility. Standardizing these test methods would improve the pre-clinical device design process and potentially enhance patient quality of life.

OC 36.4 | Relationship of Markers of Inflammation, Infection, and Endothelial Function to Mortality and Severity of Coagulopathy in Patients with Sepsis-associated DIC

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Background: Sepsis-associated disseminated intravascular coagulation (DIC) is a complex clinical scenario involving derangement of many processes, including hemostasis. Assessment of markers of these aspects of disease, including inflammation, endothelial function, and endogenous anticoagulants may provide insight into DIC pathophysiology and lead to improved methods for assessment of patient and response to treatment.

Aims: To measure biomarkers representative of multiple systems involved in DIC development in a cohort of patients with sepsis and DIC and determine the association of these markers with DIC score and mortality.

Methods: Plasma samples were collected from 102 patients with sepsis and suspected DIC at ICU admission. CD40L, PAI-1, nucleosomes, PCT, endocan, MP-TF, TFPI, and F1.2 were measured using commercially available ELISA kits. Protein C activity was measured using a clot-based assay. IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL-1α, IL-1β, MCP-1, and EGF were measured using biochip technology.

Results: Significant differences in levels of Protein C (p=0.0093), PCT (p=0.0005), IL-6 (p=0.0199), IL-8 (p=0.0149), PAI-1 (p=0.0153), and endocan (p=0.0252) were observed between survivors and non-survivors. Significant variation of Protein C (p=0.0017), nucleosomes (p=0.05), PCT (p< 0.0001), IL-6 (p=0.001), IL-8 (p=0.0025), IL-10 (p=0.0115), TNFα (p=0.0209), IL-1β (p=0.0324), MCP-1 (p=0.0209), and EGF (p=0.0002) were observed based on DIC score.

Conclusions: Markers from multiple systems perturbed in DIC were associated with mortality, suggesting that while these systems are not routinely evaluated in the normal course of patient care, dysfunction of these systems contributes significantly to mortality. In addition, several inflammatory cytokines also showed an association with DIC score. This suggests that while inflammation contributes to DIC, other processes play a large role in patient outcome.

OC 36.5 | Angiopoietin-2 Levels in the Risk Stratification and Mortality Outcome Prediction of Sepsis Associated Coagulopathy

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Background: Angiopoietin-2 (Ang-2) is a 70 kDa glycoprotein which plays multiple roles in angiogenesis, inflammation, and vascular development. Ang-2 is upregulated in vascular stress, and circulates at high levels in certain pathologic conditions such as sepsis. Ang-2 upregulation may be related to the severity of sepsis associated coagulopathy (SAC), and therefore it may have a predictive role in the clinical outcome of this syndrome.

Aims: The aim of this study is to measure Ang-2 levels in a defined cohort of patients with sepsis and suspected disseminated intravascular coagulation (DIC), to compare the circulating levels of this biomarker to controls, and to demonstrate its predictive value for the clinical outcome and severity of SAC.

Methods: Plasma samples were collected from 102 patients with sepsis and suspected DIC at ICU admission and immediately frozen at -80°C for batch analysis. Control plasma represented 50 normal samples which were commercially obtained from George King (Orland Park, KS). Ang-2 levels were quantified using a sandwich ELISA method (R&D Systems, Minneapolis, MN, USA). This method employs a monoclonal antibody specific for human Ang-2. DIC scores in all 102 patients were evaluated using the ISTH scoring algorithm.

Results: Table 1 shows that in comparison to normal levels, the samples collected from all patients showed significantly higher levels of Ang-2 for all groups (p=0.0005). Table 2 shows that the Ang-2 levels were also significantly different between the survivors and non-survivors for 28-day mortality outcome by the Mann-Whitney test (p=0.001).

TABLE 1 Ang-2 Levels in Patients with Sepsis and Suspected DIC Broken Down by ISTH DIC Score

Category	Normals	+ Sepsis - DIC	+ Sepsis + Non-Overt DIC	+ Sepsis + Overt DIC
ISTH DIC Score	N/A	0-2	3-4	≥ 5
n	50	20	57	24
Ang-2 Mean ± SEM (ng/mL)	1.87 ± 0.15	8.34 ± 2.50	11.53 ± 1.40	29.44 ± 6.27
Ang-2 Std. Deviation (ng/mL)	1.07	11.19	10.54	30.73

TABLE 2 Significant Difference in Ang-2 level between Survivors and Non-Survivors

	Survivors	Non-Survivors
n	86	15
Ang-2 Mean ± SEM (ng/mL)	12.54 ± 1.54	30.17 ± 8.62
Ang-2 Std. Deviation (ng/mL)	14.28	33.39

Conclusions: This study demonstrates that Ang-2 levels are significantly upregulated in SAC, and this biomarker can be used to risk stratify the severity of this disease. Ang-2 level can also be used to differentiate non-overt and overt DIC. Furthermore, Ang-2 level at the time of initial diagnosis provides a predictive biomarker for mortality outcome.

OC 47.1 | Pharmacodynamic Data and Coagulation Biomarkers in Persons with Hemophilia A (PwHA) with Inhibitors: Results from the HAVEN 1 Emicizumab (ACE910) Phase 3 Study

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Background: Emicizumab is a novel, subcutaneously administered bispecific humanized monoclonal antibody in development for prophylactic treatment of PwHA with and without FVIII inhibitors. Emicizumab bridges FIXa and FX, replacing the function of missing FVIII, with resultant downstream thrombin generation and coagulation.

Aims: To evaluate the effects of emicizumab in HAVEN 1 as determined by laboratory assays.

Methods: Frozen plasma samples from persons receiving emicizumab in HAVEN 1 (NCT02622321) were analyzed at central laboratories. Emicizumab concentration was measured by immunoassay. Pharmacodynamic (PD) effects were assessed using a FVIII chromogenic activity assay containing human factors (Hyphen Biophen FVIII:C) as well as by FXIa-triggered thrombin generation (TG). In addition, aPTT, PT, Clauss fibrinogen and antigen levels of FIX, FX, vWF, D-dimer and prothrombin fragment 1.2 [PF1.2] were determined. Data are presented for n=103 patients with exposure to emicizumab.

Results: Mean chromogenic FVIII activity increased from < 1% at baseline to approximately 30% during maintenance dosing (Wk 5 onwards); reported activity was strongly correlated with emicizumab concentration [**Fig 1**]. TG peak height (PH) also correlated with emicizumab levels, reaching 109.8 ± 34.6 nM (mean±SD) at Wk 25. In contrast, elevated baseline aPTT was normalized (< 40 sec) after the first dose of emicizumab and showed no further concentration-response [**Fig 2**]. FIX:Ag and FX:Ag (binding targets of emicizumab), vWF:Ag, and PT did not change significantly over time. D-dimer and PF1.2 were largely within normal limits and were not correlated to emicizumab levels.

Conclusions: Emicizumab had a concentration-dependent PD effect on TG and FVIII activity (Hyphen chromogenic assay). aPTT was normalized at subtherapeutic emicizumab levels. FIX and FX levels, and a panel of coagulation assays including D-dimer, were unaffected by emicizumab, in alignment with the overall safety profile of the molecule.

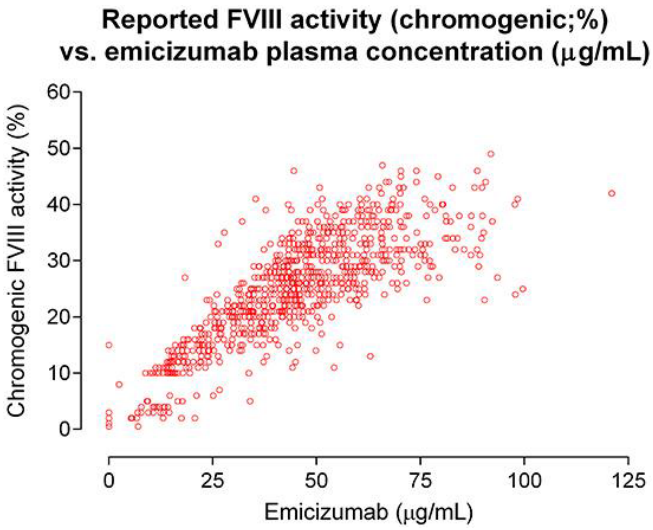

FIGURE 1 Reported FVIII activity (chromogenic;%) vs. emicizumab plasma concentration (ug/ml)

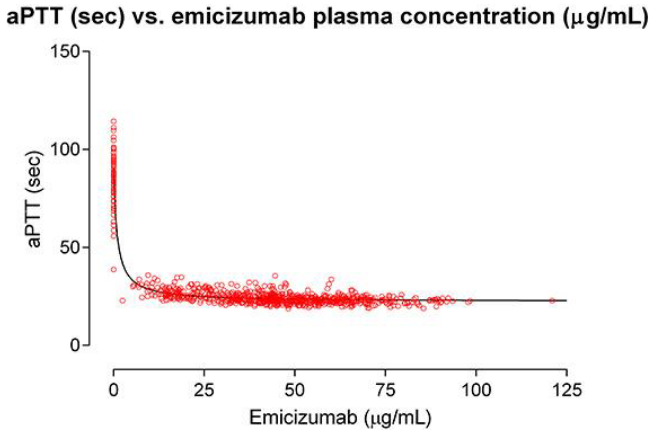

FIGURE 2 aPTT (sec) vs. emicizumab plasma concentration (ug/ml)

OC 47.2 | Synergistic Interplay of A Sequence Identical Analog of ACE910, a Bispecific Antibody, and a Bypassing Reagent and its Components

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Background: Investigational non-factor products such as ACE910, an antibody to FIX(a) and FX(a), may offer new treatment options for hemophilia patients with inhibitors. The unregulated mechanism of action of ACE910, however, raises questions regarding clinical safety and efficacy.

Aims: To detect the source of excessive in vitro coagulation with a sequence identical analog of ACE910 (SIA) combined with bypassing agent FEIBA.

Methods: SIA was analyzed in thrombin generation (TG) experiments using platelet poor plasma (PPP) from hemophilia A inhibitor patients and hemophilia A plasma reconstituted with platelets from 16 healthy donors (PRP). A normal TG range was established in healthy donor plasma. Therapeutic doses of SIA (20-600nM) were tested alone and with FEIBA (0.05-1U/mL) or rFVIIa (0.88-5.25µg/mL). To measure FEIBA components' contribution to the synergistic effect with SIA, PPP was spiked with purified plasma proteins. Clot formation was analyzed in FVIII-inhibited blood by ROTEM and T-TAS.

Results: Normal peak thrombin range was 47-144nM (PPP) and 88-231nM (PRP). rFVIIa and FEIBA had a synergistic effect on TG in combination with SIA in PPP and PRP. Combined with rFVIIa (0.88µg/mL) or FEIBA (0.5U/mL), SIA (600nM) induced a ~2- and ~16-fold increase over SIA alone. SIA+rFVIIa did not reach the normal range, while SIA+FEIBA far exceeded it. Clot formation in FVIII-inhibited whole blood confirmed the synergistic effect of SIA+FEIBA. Adding individual FEIBA components to PPP showed that FIX was, with a half-maximal effect, the main driver for enhanced TG, followed by FIXa.

Conclusions: Excessive thrombin generation and faster clot formation occurred when combining SIA at presumed clinical concentrations (ACE910 study NCT02622321) with FEIBA. In vitro, this effect is mainly mediated by FEIBA component FIX. ACE910 binds to FIX and FIXa, and displays its pro-coagulant effect via an unregulated mechanism. Therefore, careful judgment is required in treating breakthrough bleeds with FEIBA.

OC 47.3 | Challenges in Quantifying FVIIIa-mimetic Bispecific Antibody Activity Relative to FVIII for Hemophilia A Treatment

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Background: A new generation of potential hemophilia A therapies seeks to improve dosing regimens and enable prophylactic treatment of patients with and without inhibitors. With a variety of mechanisms of action across these therapies, benchmarking their activity against Factor VIII (FVIII) is important, but challenging. Here, we focus on activated FVIII (FVIIIa)-mimetic bispecific antibodies (bsAbs).

Aims: Using a number of activity assays, we compared an emicizumab biosimilar (Emi-bsim) and a Bioverativ bsAb, BS-026125, to recombinant FVIII (rFVIII).

Methods: Activity of Emi-bsim, BS-027125, their respective bivalent homodimers, and rFVIII was measured by factor Xa (FXa) generation assay, thrombin generation assay (TGA) triggered with factor XIa, and activated partial thromboplastin time (aPTT) triggered with Actin FSL.

Results: Although Emi-bsim was highly active across all assays, the concentration at which it achieved peak activity was disparate between them. BS-027125 was highly active in aPTT, but relatively less active in TGA. Similar to Emi-bsim, BS-027125 showed inconsistencies between concentration and peak activity across assays. For both bsAbs, lag time and peak height in TGA correlated with different levels of rFVIII activity. Interestingly, both Emi-bsim bivalent homodimers exhibited significant activity in several assays, while only the BS-027125 FIXa bivalent homodimer retained moderate activity in the FXa generation assay. As expected, rFVIII loses all activity in the absence of phospholipids (PL) in FXa generation assay. In contrast, Emi-bsim has very significant activity in the absence of PL, while BS-027125 shows minimal PL-independent activity.

Conclusions: Overall, the bsAbs demonstrate little consistency between the three assays tested here, which makes benchmarking against FVIII activity difficult. This suggests a need for a deeper understanding of the mechanisms and hemostatic potential of these FVIIIa-mimetic bsAbs.

OC 47.4 | Mode of Enhancement in the Global Hemostatic Potentials with Concomitant Use of Bypassing Agents and Emicizumab in Hemophilia A Patients with Inhibitor Evaluated by ROTEM

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Background: Emicizumab is a novel therapeutic bispecific antibody in development for treatment of patients with hemophilia A (PWHA) with and without FVIII inhibitors. The additive hemostatic effect on concomitant use of emicizumab and the bypassing agents (BPAs) for breakthrough bleeding among PWHA with inhibitor has been demonstrated in vitro by clot waveform analysis(#1388 ASH2016).

Aims: In the present study, we aimed to elucidate the global hemostatic function during the concomitant therapy with BPAs and emicizumab by $CaCl_2$-triggered ROTEM.

Methods: The hemostatic potentials were evaluated in the citrated whole blood samples obtained from mild or severe PWHAs (N=6)

added *in vitro* with an anti-FVIII polyclonal antibody (10BU/ml) and supplemented with the various plasma concentration of APCC (0-0.8 U/ml) or rFVIIa (0, 2.2 and 6.6 μg/ml) in the presence of emicizumab (0, 10, 30 and 100 μg/ml). The parameters, CT, CFT, MCF and alpha angle (α), for each sample were compared to the normal controls (938±127s, 330±86s, 43±5mm and 41±7deg as average±SD, respectively).

Results: The presence of emicizumab dose-dependently shortened CT and CFT from 5258±669s and 1807±303s to 1112±147s and 427±137s respectively. The spike of APCC to the samples without emicizumab facilitated a dose-dependent enhancement in the ROTEM pattern represented by shortening of CT+CFT, maximally ~90% reduction compared to its absence. Interestingly, APCC also demonstrated ~90% reduction of CT+CFT on top of the shortening caused by emicizumab, suggestive of the additive effect(Fig.1A). Addition of rFVIIa to both samples with and without emicizumab resulted in a dose-dependent reduction of CT+CFT but showed milder additive shortening on top of that by emicizumab than the addition of APCC(Fig.1B).

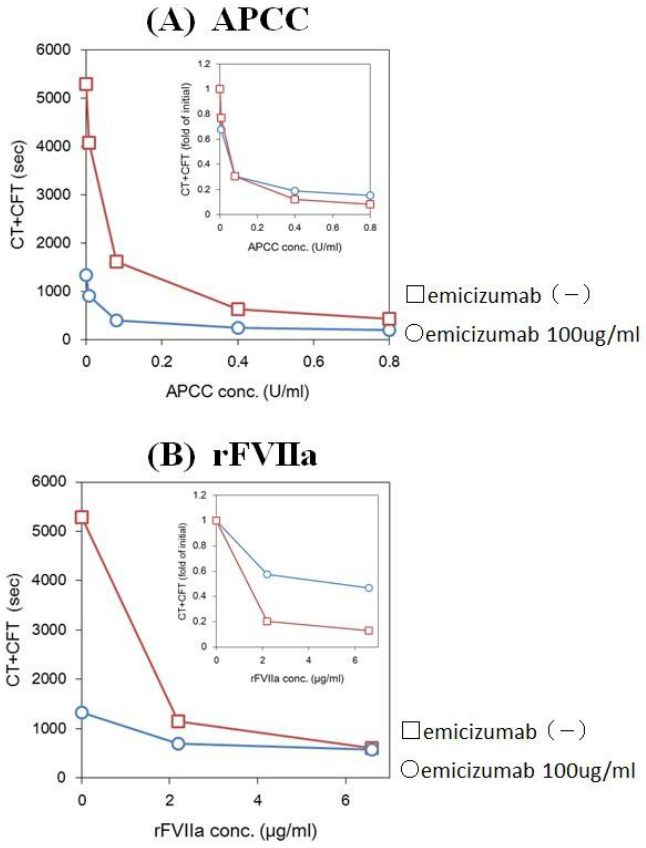

FIGURE 1 Effect on CT+CFT of APCC or rFVIIa with/without emicizumab

Conclusions: In conclusion, the spiking experiments by ROTEM clarified the different mode of enhancing effect on the hemostatic function between APCC and rFVIIa in combination with emicizumab, and would be useful to predict and adjust their enhancing effect.

OC 47.5 | Identification of FIXa- and FX-specific Antibodies for the Generation of Bispecific Antibodies with FVIIIa-like Activity

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Background: Recently, there has been an increased focus on developing factor VIII (FVIII)-independent therapies for the treatment of Hemophilia A, particularly for inhibitor patients. One approach is to replace FVIII with a bispecific antibody (bsAb) capable of mimicking the cofactor function of FVIII.

Aims: To identify a bsAb specific for factor IXa (FIXa), not FIX, and factor X (FX), not FXa, to replicate the cofactor function of activated FVIII (FVIIIa).

Methods: We leveraged the Adimab in vitro yeast presentation platform with FACS-based selection to identify panels of antibodies specific for FIXa and FX. A variety of FIX and FX constructs were used for positive and negative selections, as well as for specificity screening after individual clones were isolated. Based on desired specificity profiles, select antibodies were reformatted into bsAbs and screened for the ability to promote activation of FX in the presence of FIXa, calcium, and phospholipids. BsAbs with detectable activity in the FXa generation assay were further tested in a one-stage clotting assay to identify a lead.

Results: We identified >200 unique antibodies specific for FIXa and >250 unique antibodies specific for FX. These antibodies, when formatted into bsAbs, mimic the specificity of FVIIIa for FIXa and FX and some promote FXa generation. A further subset of bsAbs maintained significant activity in a one-stage clotting assay. Of those, BS-027025 had the highest FVIIIa-like activity. Affinity maturation of the anti-FIXa arm led to a bsAb (BS-027125) which achieved >90% FVIIIa-like activity as determined by one-stage clotting.

Conclusions: The Adimab platform offers a diversity and experimental flexibility not afforded by standard immunization methods and allowed for the unprecedented identification of hundreds of antibodies able to differentiate between the zymogen and active forms of FIX and FX. Furthermore, this specificity-based approach successfully led to the generation of bsAbs capable of replicating the cofactor function of FVIIIa.

OC 49.1 | VKA-related Major Bleeding in VTE Patients is Associated with a High Mortality Rate and is Rarely Treated with Prohemostatic Agents: Results from the RIETE Registry

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Background: Major Bleeding (MB) is the most feared complication in patients receiving anticoagulant therapy for venous thromboembolism (VTE). However real-world data on the management and outcomes of MB is scarce.

Aims: We assessed the management and outcomes of VTE patients presenting with MB under therapy with vitamin K antagonists (VKA) for VTE.

Methods: We used prospectively collected data from consecutive patients enrolled in the RIETE database (Registro Informatizado Enfermedad Trombo Embólica, NCT02832245). Bleeding events were classified as ‚major' if they were overt and required a transfusion of two units or more of blood, or were retroperitoneal, spinal or intracranial, or when they were fatal.

Results: From January 2013 to December 2016, 15480 VTE patients receiving long-term therapy with VKA were recruited. During the course of therapy, 263 (1.7%) had MB: 85 gastrointestinal, 72 intracranial, 45 hematoma, 16 genitourinary, and 45 other sites. Overall, at the time of the MB, 22% of patients had an INR < 2.0, 27% 2-3, 35% >3, and in 16% no INR was available. Prohemostatic agents (PCC, aPCC or recombinant FVIIa) were used in 11% of patients with MB (33% in those with intracranial hemorrhage (ICH)), and vitamin K in 105 (40%). During the first 48 hours after MB, 41 (16%) patients died, 38 (14%) of fatal bleeding and 26 (10%) with ICH. Fourteen (5.3%) had a recurrent VTE, and no patient had a rebleeding. From Day 3 to Day 30, 13 additional patients died, 4 had VTE recurrence and one re-bleeding. 105 of 222 (47%) patients who survived restarted anticoagulant therapy.

Conclusions: In clinical practice, VKA-related major bleeding is rarely treated with prohemostatic agents, despite the high rate of fatal bleeding. All fatal bleeds occurred within the first 48 hours after bleeding. After a major bleeding, less than half of the patients restart anticoagulation.

TABLE 1 Outcomes after major bleeding, according to bleeding site

		Intra-Cranial	Gastro-Intestinal	Hematoma	Genito-Urinary	Other
Patients, N		72	85	45	16	45
Day 2	Death	26 (36%)	7 (8.2%)	0	1 (6.3%)	7 (16%)
	Fatal Bleeding	26 (36%)	6 (7.1%)	0	0	6 (13%)
	Rebleeding	0	0	0	0	0
	Recurrent VTE	5 (6.9%)	5 (5.9%)	3 (6.7%)	1 (6.2%)	0
Day 30	Death	29 (40%)	14 (16%)	1 (2.2%)	2 (13%)	8 (18%)
	Fatal Bleeding	26 (36%)	6 (7.1%)	0	0	6 (13%)
	Rebleeding	0	0	0	0	1 (2.2%)
	Recurrent VTE	6 (8.3%)	7 (8.2%)	3 (6.7%)	1 (6.2%)	1 (2.2%)

OC 49.2 | Hypofibrinolysis on ROTEM Early after Traumatic Injury Represents a Heterogenous Group with Significant Variation in Both Fibrinolytic Activity and Mortality

L.S. Gall[1], K. Brohi[1], C. Gaarder[2], J.C. Goslings[3], N.P. Juffermans[4], M. Maegele[5], P.I. Johansson[6], R.A. Davenport[1], on behalf of the International Trauma Research Network (INTRN)

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Background: An early hypofibrinolytic phenotype after major trauma, with increased mortality has recently been described using thromboelastography. The biomarker profile and clinical significance of this diagnosis for antifibrinolytic therapy in traumatic haemorrhage is unclear.

Aims: To quantify the mediators of fibrinolytic activation and clinical characteristics of trauma patients with hypo- vs hyperfibrinolysis.

Methods: Prospective study of adult patients with severe injury and evidence of bleeding at five European trauma centres. No patients received tranexamic acid. Blood was drawn on admission for ROTEM and fibrinolytic marker analyses. Maximal Lysis (ML) was normal if 5-15% (ML_{NORMAL}) with hypo- and hyperfibrinolysis defined as < 5% (ML_{LOW}) and >15% (ML_{HIGH}). Groups were further subdivided using a D-dimer (DD) threshold of 30,000ng/ml into DD_{LOW} and DD_{HIGH}.

Results: In 928 patients analysed, DD in the ML_{LOW} group varied greatly with a ten-fold difference between patients who died vs survived (103,200 vs 13,670ng/ml, p< 0.0001). We found no difference in mortality within the DD_{LOW} group (ML_{LOW} vs ML_{HIGH}, 3.8 vs 10.5%, p=0.11) or compared to patients with ML_{NORMAL} (7.1%, ns). Regardless of ML, mortality was increased in the DD_{HIGH} groups (Figure 1). Plasmin-Antiplasmin (PAP) levels in the DD_{HIGH} groups were elevated in both hypo- and hyperfibrinolysis (ML_{LOW} vs ML_{HIGH}: PAP 12,196

vs 24,185µg/L, ns) and significantly higher than ML_{NORMAL} (PAP: 2583µg/L, p< 0.05). In the DD_{HIGH} groups, patients with ML_{LOW} were less shocked, had lower tPA (12.0 vs 41.8ng/ml, p< 0.05) and higher α2-antiplasmin levels than the ML_{HIGH} patients.

Conclusions: Patients with DD_{LOW}, regardless of ML have a low mortality rate. ROTEM hypofibrinolysis is a heterogeneous group with significant variation in biomarker-defined fibrinolytic activity. Patients with DD_{HIGH}, irrespective of ML, share an early hyperfibrinolytic biomarker profile.

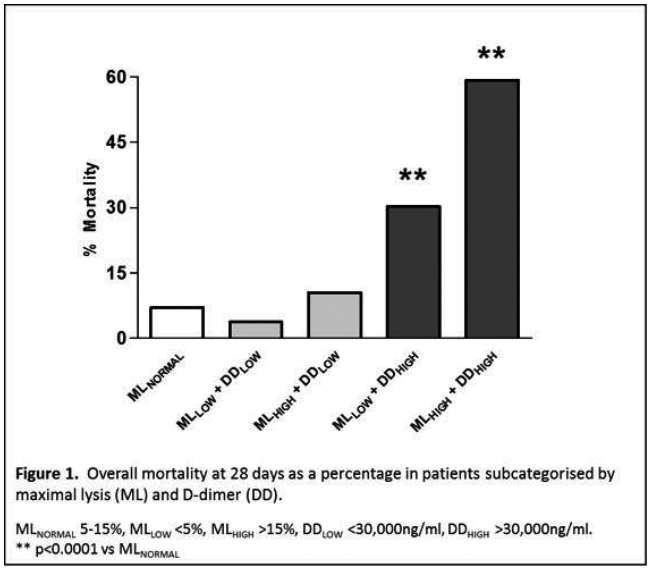

Figure 1. Overall mortality at 28 days as a percentage in patients subcategorised by maximal lysis (ML) and D-dimer (DD).

ML_{NORMAL} 5-15%, ML_{LOW} <5%, ML_{HIGH} >15%, DD_{LOW} <30,000ng/ml, DD_{HIGH} >30,000ng/ml.
** p<0.0001 vs ML_{NORMAL}

FIGURE 1 Overall mortality at 28 days as a percentage in patients subcategorised by maximal lysis (ML) and D-dimer (DD)

OC 49.3 | Data-driven ROTEM and TEG Algorithms for the Management of Trauma Haemorrhage

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Background: Trauma induced coagulopathy (TIC) may exacerbate bleeding and is associated with high morbidity and mortality. Current resuscitation strategies are often non-specific and do not correct this coagulopathy.

Aims: We aimed to develop pragmatic data-driven algorithms for management of TIC during trauma haemorrhage for viscoelastic haemostatic assays (VHAs).

Methods: Admission data from conventional coagulation tests (CCT), rotational thrombelastometry (ROTEM) and thrombelastography (TEG) were collected prospectively at 6 European trauma centres during 2008-2013. To identify significant VHA parameters for the detection of TIC (defined as INR >1.2), hypofibrinogenaemia (< 2.0g/L) and thrombocytopenia (< 100 $x10^9$/L), univariate regression models were constructed and area under the curves (AUCs) were calculated.

Results: 287 adult trauma patients (ROTEM in 2019 and TEG in 968) were enrolled. FIBTEM clot amplitude at 5 minutes (CA5) had the largest AUC and 10mm was found to detect hypofibrinogenaemia with 70% sensitivity. The corresponding value for Functional Fibrinogen (FF) TEG Maximum Amplitude (MA) was 19mm. Thrombocytopenia was detected with 74% sensitivity using the calculated threshold EXTEM-FIBTEM CA5 30mm, corresponding rTEG-FF TEG MA was 46mm. TIC was identified by EXTEM CA5 41mm with 73% sensitivity and rTEG MA 64mm with 80% sensitivity. For hyperfibrinolysis, the relationship between viscoelastic lysis parameters and clinical outcomes resulted in threshold values of 85% for EXTEM Li30 and 10% for rTEG Ly30.

Based on these analyses we constructed algorithms for ROTEM, TEG and CCTs to be used in addition to baseline ratio driven transfusion and tranexamic acid (TXA).

Conclusions: A systematic approach to define threshold parameters for ROTEM and TEG has been described. These parameters were incorporated into algorithms to support data-driven adjustments of resuscitation with available therapeutics, in order to optimize damage control resuscitation practice.

OC 49.4 | Management of Hemorrhage Using Self-propelling Particles to Deliver Thrombin or Tranexamic Acid

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Background: Bleeding is a major cause of morbidity and mortality in multiple clinical situations, including trauma and surgery. Bleeding is especially problematic when it originates from non-compressible anatomic locations or when the source of bleeding is not easily visualized. Topical hemostatic agents have limited efficacy because they cannot penetrate to reach damaged vasculature to form stable clots which can resist fibrinolysis or mechanical rupture. Self-propelling particles loaded with existing hemostatic agents could overcome these limitations by increasing their penetration to form robust and stable clots.

Aims: To demonstrate the safety and efficacy of a self-propelling particle-based hemostatic agent *in vitro* and *in vivo* using preclinical models of bleeding and fibrinolysis.

Methods: Self-propelling calcium carbonate particles were combined with tranexamic acid and thrombin. These formulations were tested in *in vitro* models of bleeding and fibrinolysis, in two mouse models of hemorrhage, and one pig model of severe junctional hemorrhage.

Results: By propelling tranexamic acid and thrombin, their ability to form clots and inhibit fibrinolysis were significantly increased. The formulation significantly decreased bleeding in both mouse models, and significantly increased survival in the pig model of severe hemorrhage. In these models, the formulation was superior to typical hemostatic interventions, such as topical thrombin or kaolin. No adverse effects were observed.

Conclusions: Self-propelling particles and dressings are effective at stopping bleeding *in vivo* and may be useful for delivering a variety of clinically approved hemostatic agents for managing bleeding. These particles present an attractive new route of administration for hemostatic agents, which could expedite their administrations and increase their ease of use.

OC 49.5 | A Chimeric Factor VIIa Molecule Shows Reduced Thrombogenicity in Animal Models

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Background: We have engineered a Chimeric factor VIIa molecule (ChFVIIa) that has the Gla and EGF1 of factor IX and the EGF2 and catalytic domain of factor VIIa (FVIIa). In a mouse model of hemophilia, the mouse ChFVIIa was as effective as mFVIIa in stopping bleeding (Blood. 2014;123:1764-1766). ChFVIIa does not bind to tissue factor (TF) so we have postulated that ChFVIIa will have reduced thrombogenicity.

Aims: The goal of these studies was to compare FVIIa and ChFVIIa in three animal models of thrombosis.

Methods:

1. Wild type mice were injected with with human TF and mChFVIIa or FVIIa. Mice were sacrificed and their lungs examined for thrombi using fluorescently labeled fibrinogen.
2. Hemophilia B mice were administered AAV vectors carrying mFVIIa or mChFVIIa sequences. Hemostasis was assessed by tail bleeding and survival was monitored.
3. Rabbits were injected with 50 µg/kg human FVIIa or ChFVIIa. Stasis was created in both jugular veins and maintained for 30 minutes. The veins were opened to detect thrombi.

Results:

1. Mice that were injected with human TF and FVIIa showed strong lung fluorescence indicative of significant fibrin accumulation in the lungs. By contrast, mice injected with TF and mChFVIIa had no lung fluorescence above background.
2. Over six months, mice expressing mChFVIIa had an 0.85 probability of survival whereas for mice expressing mFVIIa the value was 0.55. Littermate animals expressing mChFVIIa had equivalent (or shorter) tail bleeding times compared to mice expressing mFVIIa indicating similar levels of hemostatic function.
3. For the jugular vein stasis model in rabbits, proteins were assayed for clotting activity and factor Xa generation. In animals given equivalent amounts of activity, 6 out of 6 rabbits that received FVIIa formed a visible thrombus. By contrast, no rabbits given the same dose of ChFVIIa formed a visible thrombus.

Conclusions: These studies provide evidence that the reduced TF binding of ChFVIIa can translate into reduced risk of thrombosis in some vascular settings.

OC 50.1 | Modulation of F8 Secretion by Autophagy Related Proteins

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Background: The F8 protein interacts with several intracellular proteins that control and facilitate its secretion. Known interaction proteins include Calnexin, Calreticulin and BIP in ER, LMAN1 and MCFD2 in the ERGIC compartment and recently we identified GABARAP as a potential interacting partner in the GOLGI. The later belongs to the Atg8 like molecules that play a key function in the autophagy process. This suggested that an autophagy or non-typical autophagy related function of Atg8-like proteins could play a role in fine-tuning/modulating the secretion of F8.

Aims: In this study we explored such contribution of key components of autophagy machinery on the F8 secretion; namely we investigated the effect of GABARAP, GAPARAPL1 and ATG7. In addition known influential proteins on the secretion, LMAN1, MCFD2, Calnexin, Calreticulin were also included.

Methods: We used siRNA as well as CRISPR/Cas9 mediated knockout (KO) in order to study the individual effect/contribution of each protein to the secretion of F8 in a stable F8 secreting HEK cell.

Results: Our results show the expected decrease in F8 secretion in the KOs of LMAN1 and MCFD2 (60% and 70% reduction, respectively, compared to mock), while the KOs of other proteins showed effects going in opposite directions. KOs of GABARAP and Calreticulin are showing a clear decrease in F8 activity (both up to 25-30% reduction), while KOs of GABARAP-L1, ATG7 and Calnexin show a clear increase of F8

secretion: GABARAP-L1 KO increases F8 activity up to 70% compared to mock. Moreover KOs of ATG7 or Calnexin increase F8 activity up to 90% compared to mock in our stable F8 secreting cell system. Confocal microscopy depicts GABARAP as bulky pericentriolar fraction and as small vesicular structures in the cytoplasm, both co-localizing with F8.

Conclusions: Based on these results we postulate that GABARAP and autophagy related proteins modulate the secretion of F8 through both typical autophagy and non-autophagy related pathways.

OC 50.2 | Clustered F8 Missense Mutations Cause Hemophilia A Phenotypic Heterogeneity by Combination of Altered Splicing, Protein Secretion and Activity

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Background: Pleiotropic effects of mutations, scarcely investigated in hemophilia A (HA), hamper the elucidation of genotype-phenotype relationships. Missense mutations, frequent in HA, might impair FVIII mRNA processing, protein biosynthesis, activity and/or half-life.

Aims: To quantitatively evaluate the pleiotropic effects of the *F8* p.Arg2016Trp/c.6046C>T (exon 19, A3 domain) mutation, one of the most prevalent FVIII amino acid substitutions (>60 cases).

Methods: CHO cells were transduced with lentiviral expression vectors for recombinant (r)FVIII variants to evaluate secreted FVIII antigen (FVIII:Ag) and activity (FVIII:C) by ELISA and chromogenic assays. FVIII mRNA splicing was evaluated by RT-PCR on RNA from: 1) patients' leukocytes and 2) HepG2 cells transfected with *F8* minigene variants.

Results: The rFVIII-2016W displayed reduced secretion (FVIII:Ag 11.0±0.4% of wt) and activity (FVIII:C 6.0±2.9%). *F8* mRNA studies demonstrated that the c.6046C>T change also decreases correct splicing to 70±5%, predicted to lower further FVIII:C (4.2±2%), consistent with FVIII:C levels observed in patients with the mutation (1-5%). Through an antisense U7snRNA targeting the mutated exon 19 region we identified a splicing enhancer, potentially affected by other HA-missense mutations. Strikingly, the c.6037G>A (p.Gly2013Arg) reduced exon inclusion to 41±3% and the c.6053A>G (p.Glu2018Gly) to 28±2% of wt, similarly (26±2%) to the c.6113A>G, (p.Asn2038Ser), a variant affecting the 5'splice splice. At protein level, the p.Gly2013Arg similarly reduced FVIII:Ag (7.0±0.9%) and FVIII:C (8.4±0.8%), while the p.Glu2018Gly produced a dysfunctional molecule (FVIII:Ag, 69.0±18.1%; FVIII:Cc, 19.4±2.3%). However, the rFVIII2038Ser displayed normal FVIII:Ag and FVIII:C.

Conclusions: The integrated approach of analysing mRNA and protein levels highlights the combination of both pathogenic mechanisms triggered by clustered mutations, which accounts for a gradient of residual FVIII:C recapitulating the HA coagulation phenotypes.

OC 50.3 | Vascular Remodeling in Hemophilic Arthropathy is Exacerbated by Defective TAFIa-mediated Inactivation of SDF1α (CXCL12)

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Background: Vascular remodeling after hemophilic joint bleeding promotes rebleeding, but mechanisms are unknown. In hemophilia, activation of the procarboxypeptidase thrombin-activatable fibrinolysis inhibitor (TAFI) is impaired and TAFI was recently shown to inhibit vascular remodeling. SDF1α, an elevated angiogenic mediator in hemophilic arthropathy, contains a C-terminal (CT) Lys required for binding to CXCR4, and may be negatively regulated by activated TAFI (TAFIa).

Aims: To determine the contribution of defective inactivation of SDF1α by TAFIa on vascular remodeling in hemophilic joints.

Methods: TAFIa-mediated cleavage of SDF1α was determined by W-blot and transwell migration assays. Hemarthrosis was induced by a knee puncture in $FVIII^{-/-}$ mice and in WT and $TAFI^{-/-}$ mice treated with a transient FVIII inhibitor. AMD3100 (CXCR4 antagonist) treatment started at 2 days post injury. Vascular remodeling was assessed by histology and Power Doppler (PD) at week 4 post injury.

Results: TAFIa cleaved the CT-Lys of SDF1α and attenuated SDF1α-induced cell migration. After joint injury, SDF1α primarily localized with $αSMA^{+}$ remodeled vessels. The active vs. total SDF1α ratio was increased in $TAFI^{-/-}$ and $FVIII^{-/-}$ compared to WT mice. $FVIII^{-/-}$ and $TAFI^{-/-}$ mice were treated with AMD3100 to determine the contribution of the SDF1α/CXCR4 pathway. AMD3100 did not promote joint tissue hemorrhaging. PD signals in the joints were increased (3.5-fold; $p< 0.001$) in untreated $FVIII^{-/-}$ and $TAFI^{-/-}$ mice, but not in AMD3100-treated mice. Vessel diameters and perivascular αSMA expression were increased untreated but not in AMD3100-treated $FVIII^{-/-}$ and $TAFI^{-/-}$ mice ($p< 0.01$), indicating reduced vascular remodeling after hemarthrosis.

Conclusions: Due to the TAFI activation defect in hemophilia, active SDF1α accumulated on synovial vessels after hemarthrosis. Excessive CXCR4-mediated recruitment/proliferation of αSMA-expressing perivascular cells to the injured joint led to aberrant vascular remodeling, which was prevented by CXCR4 antagonism.

OC 50.4 | 90-bp Insertion of Deep Intron between E18 and E19 (E18ins90bpE19) May Be a Hot-spot Mutation for Mild Hemophilia A Patients without Found DNA Mutation

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Background: Identifying genetic mutations in hemophiliacs is important for disease diagnosis and genetic counseling. There are 2~7% of Hemophilia A (HA) patients whose mutations cannot be found by DNA analysis and it increases up to 10~18% in mild HA patients.

Aims: We aimed to identify the genetic defects by mRNA analysis in mild-type HA patients without DNA mutation.

Methods: From our 49 mild-type HA patients of 35 unrelated families from two hemophilia centers, who had undergone genetic tests from 2008 to 2016, we collected those patients without found DNA mutation and performed mRNA analysis. Total cellular RNA was reverse-transcribed to cDNA. F8 cDNA was amplified as eight fragments using nested PCR.

Results: There were 10 mild-type HA patients from 6 unrelated families without found mutation, which accounted for 17.1% (6/35). The median age was 18 years old, ranging from 2 to 73. The average FVIII:C level was 12.3%, ranging from 6.5% to 28.9%. Nine of the 10 (90%) patients were confirmed to have F8 gene mutation. Eight patients from 5 unrelated families were remarkably found to have both presence of normal 585 bp and aberrant 675 bp fragments. Sequencing of the aberrant 675 bp fragment showed that there were two separate insertion of 35 bp and 55 bp from intron 18. It confirmed that there was 90 bp of intron 18 insertion between exon 18 and exon 19 (E18ins90bpE19) in mRNA, which was reported as a result of c.5999-277G>A in deep intron. Another one patient was found to have exon 19 spliced out and the last one patient remained nonconclusive.

Conclusions: Our study demonstrates that mRNA analysis was an effective assay for mild-type HA patients who was wild type by DNA analysis and the undetected rate can be reduced from 17.1% (6/35) to 2.9% (1/35). The E18ins90bpE19 (90-bp insertion of deep intron between E18 and E19) may be a hot spot for mild-type hemophilia without found DNA mutation.

OC 50.5 | Imbalance between Wound Healing and Inflammation in Hemophilia

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Background: Macrophages are master regulators of inflammation and wound healing. As such they play an important role in hemophilia, which is associated with delayed tissue regeneration and bleeding-induced joint inflammation.

Aims: Determine if macrophage function is deregulated in hemophilia and if this affects the balance between tissue regeneration and inflammation.

Methods: Monocytes from hemophilia patients and healthy individuals were treated with M-CSF or GM-CSF and probed for morphological features of macrophage differentiation by phase contrast microscopy. TNFα, CD163 and CD206 were measured by fluorescence microscopy, CD14/CD16 and Tie2 by flow cytometry. Wound infiltration of macrophages was determined by probing for invasive podosomes in clotted plasma. Phagocytosis was assessed by measuring the uptake of fluorescent latex beads and red blood cells.

Results: Morphological analysis of hemophilia macrophages revealed a defect in cell polarization and filopodia formation. The deficit was most pronounced in response to M-CSF, which induced TNFα/CD163 expression in monocytes from healthy donors but failed to do so in hemophilia monocytes. In contrast, expression of TNFα and CD206 in response to GM-CSF was largely maintained. Using flow cytometry, we detected a significant reduction of Tie2 on hemophilia monocytes. This phenotypical change was associated with impaired regenerative macrophage functions such as clot infiltration and red blood cell phagocytosis in hemophilia. CD14 and CD16 on hemophilia monocytes was not affected.

Conclusions: Our data indicate that M-CSF-mediated regenerative macrophage functions such as clot invasion and red blood cell phagocytosis are deregulated in hemophilia and that these deficits correlate with reduced Tie2 expression on hemophilia monocytes. Given the central role of red blood cells in promoting hemophilic arthropathy, we speculate that hemophilia macrophages take on a so far unappreciated role in joint inflammation.

OC 61.1 | Mutational Repertoire in the SIPPET Cohort and Prediction of FVIII Inhibitor Risk

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Background: *F8* mutation type is the main predictor of inhibitor development in patients with hemophilia A. Mutations expected to allow a residual synthesis of FVIII are likely to play a protective role towards alloantibody formation by inducing immune tolerance. According to the expected full or partial impairment of FVIII synthesis, *F8* mutations are commonly classified as null and non-null; however there is no consensus in the definition of two groups.

Aims: The association existing between mutation type and inhibitor risk was explored in a cohort of 231 patients with severe hemophilia A, enrolled in the SIPPET trial.

Methods: Mutational scanning of the *F8* gene was accomplished by long-range PCR, direct sequencing and MLPA. The functional effects of missense and splicing variants were predicted by multiple web-based tools. FVIII antigen levels were measured in patient plasma using the Asserachrom ELISA kit. Kaplan Meier and Cox regression survival analyses were performed to assess the risk of inhibitor development.

Results: The genetic defects found in the analyzed patients, consisting of inversions of intron 22 (n=110) and intron 1 (n=6), large deletions (n=16), nonsense (n=38), frameshift (n=28), missense (n=19) and splicing (n=14) mutations, of which 35 previously unreported, were reclassified in null and non-null according to in-silico analyses and FVIII antigen levels.

A 2-fold increase in inhibitor risk development for "in-silico null" mutations compared to "in-silico non-null" mutations [hazard ratio 2.08, 95% confidence interval (CI), 0.84 to 5.17] and a 3-fold increase in inhibitor risk development for "antigen negative" mutations compared to "antigen positive" mutations [hazard ratio 3.09, 95% CI, 0.76 to 12.6] were found.

Conclusions: Our findings confirm an association between the synthesis of minute amounts of FVIII and inhibitor protection and underline the importance to investigate *F8* mutations with further in-silico analyses in order to predict the risk of inhibitor development.

OC 61.2 | Improved Immunogenicity Prediction Using Composite Variables that Incorporate the Known Patient, Product and Therapy Related Risk Factors for Inhibitor Development with Immunologic Parameters from the HLA-class-II (HLAcII)-factor (F)VIII Peptidome

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Background: T-cells are critical for inhibitor development in HA. We hypothesize that combining known variables of inhibitor risk with parameters from individual HLAcII/FVIII peptidomes will enhance predictive power. Dendritic cells (DCs) express HLAcII repertoires & present peptides to T-cells *in vivo*. We used DC protein presentation assays (PPAs) to measure therapeutic FVIII proteins (tFVIIIs) for these parameters.

Aims: Reassess known patient, product & therapy-related variables of inhibitor risk in combination with measured peptidomic parameters.

Methods: Used DCs in 2 PPAs. In PPA 1, DCs from donors 1-12 were incubated with 5 r-tFVIIIs: 1 full-length (FL), 3 B-domain deleted (BDD), 1 BD-truncated (BDT). In PPA 2, DCs from donors 13-24 were incubated with 1 of 5 tFVIIIs: BDT-rFVIII±VWF, FL-rFVIII±VWF, pdFVIII+VWF. After lysis, HLAcII/peptide complexes were purified as DR, DQ & DP bound peptides with monoclonal anti-DR, DQ & DP. Eluted peptidomes were LC-MS/MS sequenced. Peptides were ID'd & compared to the reference human proteome and unique sequences of BDD/T &*F8* ns-SNPs. All individually sequenced peptides (IPSs) from each donor were assembled as groups of overlapping peptides (GOPs) along FVIII (Fig.1).

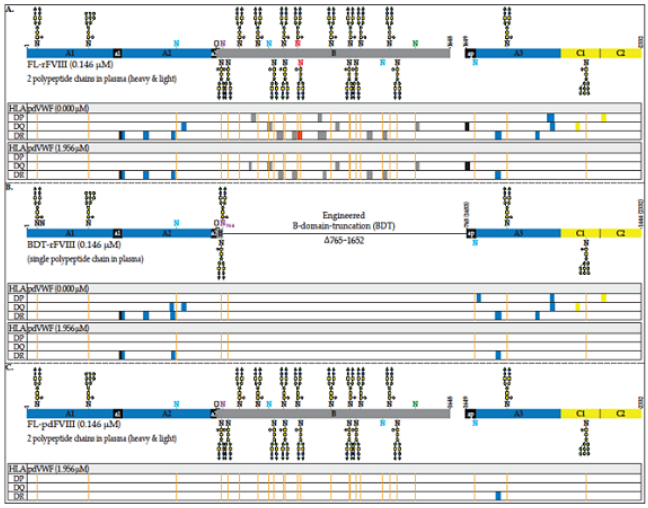

FIGURE 1 Representative Individual HLAcII-Petidomes for Various FVIII Therapeuitics

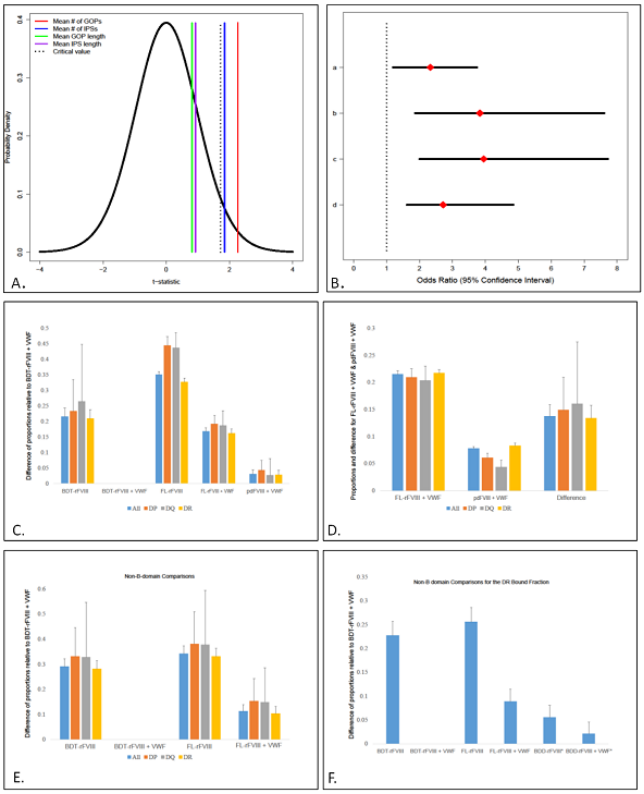

FIGURE 2 A-F Reassessing the Known Patient, Product & Treament Related Variables of Inhibitor Risk in Combination with Parameters of the FVIII Peptidome

Results: The number of GOPs & IPSs from FL-rFVIII were greater in PPA 2 vs 1, likely due to its higher concentration (Fig.2A). We found a protective effect of glycosylation with non-glycosylated sites at significantly greater risk of being in the bound fraction of potential epitopes (Fig.2B, a-c). We examined inhibitor development in patients with F8 missense mutations and found a greater risk when in the bound fraction (Fig.2B, d). We found that BDT-rFVIII+VWF yielded significantly less bound peptides in comparisons with all other tFVIIIs, and that FL-rFVIII yielded significantly more peptides than pdFVIII (Fig.2C-F).

Conclusions: Concentration, glycosylation & VWF co-administration significantly influenced the number of GOPs & IPSs, which both significantly affected the inhibitor risk of F8 missense mutations.

OC 61.3 | Immune Monitoring by Epigenetic Cell Counting - Application in the Hemophilia Inhibitor PUP Study (HIPS)

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Background: The Hemophilia Inhibitor PUP Study (HIPS) is a prospective multicenter observational study with the primary objective to elucidate immune system changes in severe hemophilia A patients during their first 50 exposure days (EDs) to FVIII (clinicaltrials.gov NCT01652027). Immune monitoring in a prospective multi-center study in PUPs has been challenging because of the lack of suitable technology applicable to small volumes of frozen blood samples.

Aims: Here, we asked if key immune regulatory cells such as FoxP3$^+$ T cells (Tregs), pro-inflammatory Th17 cells or granulysin+ NK cells, can be monitored in the circulation of patients by longitudinal epigenetic cell counting (www.epiontis.com).

Methods: Quantitative PCR-based methylation assays were used to quantify Tregs, Th17 cells, granulysin$^+$ NK cells and CD3$^+$ T cells, prior to the first dose of FVIII and after ED 1, 5, 10, 20, 30, 40 and 50. A cohort of 20 healthy adults was included for comparison. Comprehensive antibody analytics was done as described in Hofbauer 2015.

Results: Analysis of samples for ten HIPS subjects (age range: 0.4 - 13 months at ED1) was completed. Blood concentrations of Tregs prior to first FVIII exposure were in the range of healthy adults in most patients and remained fairly stable throughout the study. TH17 cell counts prior to first FVIII exposure were very low when compared to healthy adults and remained at low levels.

Granulysin$^+$ NK cell counts prior to first FVIII exposure were below the range of healthy adults but underwent considerable variation

during the study in most patients. Correlation analyses to the presence of FVIII-specific antibodies are ongoing.

Conclusions: Epigenetic cell counting using frozen blood samples provided robust results for monitoring immune cell populations during the first 50 ED of PUPs to FVIII. Normal levels of FoxP3^{+} Tregs and very low levels of TH17 cells indicate an early window of opportunities for immune tolerance induction.

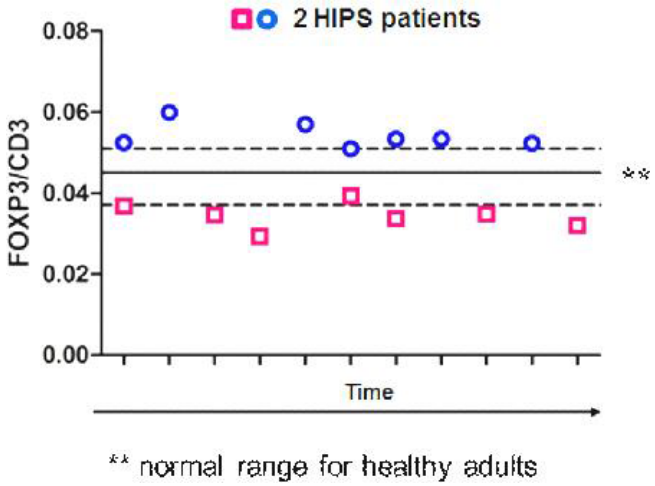

FIGURE 1 Epigenetic counting of FoxP3+ Tregs

OC 61.4 | Comparative Profiling of HLA-DR and HLA-DQ Associated FVIII Peptides Presented by Monocyte-derived Dendritic Cells

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Background: The development of anti-factor VIII (FVIII) antibodies represents a major complication in the treatment of hemophilia A patients. The initiation of the anti-FVIII immune response involves the internalization and subsequent presentation of FVIII-derived peptides on MHC class II on the surface of professional antigen-presenting cells. Previously, we identified the FVIII-derived peptides that are presented on HLA-DR. Both HLA haplotypes DRB1*15 and DQB1*06:02 have been associated with higher incidence of inhibitor in hemophilia A patients, suggesting that HLA-DQ participates in the development of anti-FVIII antibodies.

Aims: To identify the repertoire of FVIII-derived peptides presented on HLA-DQ.

Methods: Monocyte-derived dendritic cells from 11 HLA-typed healthy donors were pulsed with recombinant full-length FVIII. HLA molecules were purified using monoclonal antibodies directed against HLA-DR (L243) or HLA-DQ (SPV-L3) and FVIII-derived peptides were identified by mass-spectrometry.

Results: The number of FVIII peptides eluted from HLA-DR was 3.85 fold higher compared to HLA-DQ (33.6±15.6 vs. 8.7±8.5, mean±SD). This was consistent with a higher number of total peptides eluted from HLA-DR compared to HLA-DQ and a higher expression level of HLA-DR compared to HLA-DQ. We found that FVIII peptide repertoire eluted from HLA-DQ overlapped with HLA-DR. Four FVIII derived peptides were exclusively identified on HLA-DQ. With the exception of one donor, no B domain derived peptides were found on HLA-DQ while several B domain-derived peptides were presented by HLA-DR.

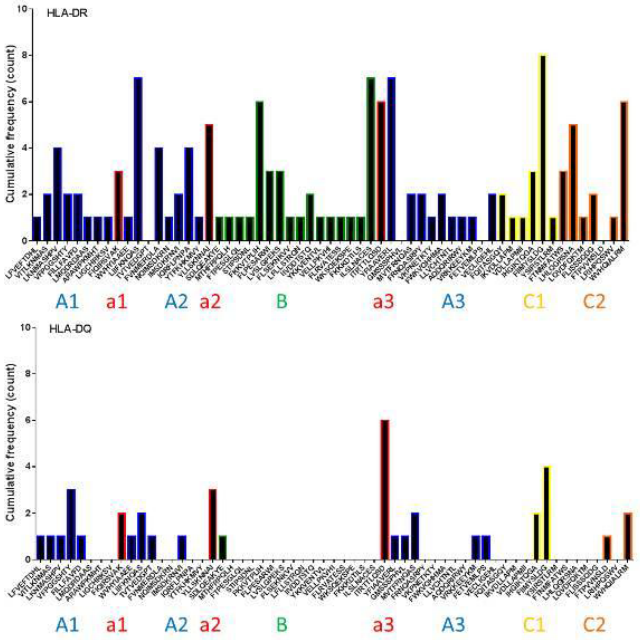

FIGURE 1 Comparative profiling of FVIII presentation on HLA-DR and HLA-DQ

Conclusions: HLA-DQ presents a smaller set of FVIII-derived peptides that overlap with HLA-DR. This suggests that HLA-DQ increases the amount of peptides that are presented to T cells. Since T-cell activation requires the recognition of a unique peptide-HLA combination by their TCR, it does not exclude that presentation of FVIII-derived peptides by HLA-DQ activates a distinct repertoire of FVIII-specific T-cells.

OC 61.5 | Differential Effects of von Willebrand Factor on Processing and Presentation of Factor VIII-derived Peptides

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Background: Neutralizing antibodies against factor VIII (FVIII) remain the major challenge in hemophilia A care. Previous studies proposed von Willebrand factor (VWF) to affect FVIII immunogenicity by reducing the uptake of FVIII by antigen presenting cells (APCs) (Dasgupta S 2007). Sorvillo 2016 showed VWF to bind to human dendritic cells and modulate patterns of FVIII-derived peptides presented on MHC-class II, when FVIII was complexed with VWF.

Aims: Here, we asked if VWF modulates presentation of FVIII-derived peptides and subsequent stimulation of peptide-specific T-cells.

Methods: FVIII-specific $CD4^+$ T cell hybridoma libraries were generated using humanized HLA-DRB1*1501 (human MHC-class II) hemophilic mice (Steinitz 2012). APCs derived from the mice or from HLA-DRB1*1501 homozygous human subjects were challenged with FVIII (with or without VWF) and subsequently co-incubated with the hybridoma libraries. Stimulation of T-cells was assessed by IL-2 release into cell culture supernatants. $CD4^+$ T cell hybridoma clones, specific for 10 different FVIII peptides presented by HLA-DRB1*1501, were tested.

Results: The presence of VWF during incubation of APCs with FVIII reduced the subsequent activation of 4/10 T cell hybridoma clones. The reduction was almost complete in one of the clones and about 50% in the other 3 clones. Activation of the remaining 6 T cell hybridoma clones was not affected. The presence of an irrelevant control protein instead of VWF did not have any effect.

Conclusions: Our data suggest that VWF has distinct effects on the presentation of some FVIII peptides but not on others. We hypothesize that VWF may alter the sorting of FVIII into endolysosomal compartments of APCs, thereby modifying the patterns of FVIII peptides generated and presented on MHC-class II as well as the subsequent activation of T cells. It remains to be shown if this alteration would have any effect on the induction of anti-FVIII antibodies or the induction of immune tolerance.

OC 62.1 | Platelet-targeted Hyperfunctional FIX Gene Therapy of Hemophilia B Mice

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Background: While platelet gene therapy can restore hemostasis and induce immune tolerance in hemophilia B mice, the levels of platelet-FIX (plt-F9) in transduced recipients were only around 3% of normal whole blood even when a lethal 11Gy total body irradiation (TBI) was employed.

Aims: To improve the efficacy of plt-F9 gene therapy, it is desirable to optimize our vector.

Methods: A novel lentiviral vector, 2bCoF9R338L harboring a codon-optimized FIX Padua cassette was used to introduce plt-F9 expression in $F9^{null}$ mice by transduction and transplantation of hematopoietic stem cells under a sub-lethal 6.6Gy TBI.

Results: Both antigen and activity levels of plt-F9 from 2bCoF9R338L-transduced recipients were significantly higher than those from normal 2bF9-transduced animals. There are an approximately 5.8-fold higher antigen and a 28-fold higher activity level in the 2bCoF9R338L group compared to in the 2bF9 group. Using a 6-hour tail bleeding test, we showed that the hemophilic phenotype was fully rescued in the treated animals. All 2bCoF9R338L-transduced recipients' tail bleeding clotted within 6 hours and the remaining hemoglobin level was of 69.3±8.8%, which were not significantly different from those of the wild type controls. In contrast, none of the $F9^{null}$ control mice clotted within 6 hours and the remaining hemoglobin level (40.5±1.9%) was significantly lower than in the 2bCoF9R338L group. Importantly, none of the 2bCoF9R338L-transduced $F9^{null}$ mice developed anti-F9 inhibitors even after extensive rhF9 immunization in the presence of adjuvant. In contrast, all $F9^{null}$ control mice developed anti-F9 inhibitors when the same immunization protocol was employed. Of note, anaphylaxis can occur in these $F9^{null}$ mice with rhF9 infusion if the immune system was primed by FIX.

Conclusions: Platelet-targeted codon-optimized gain-of-function FIX gene therapy is a promising approach for gene therapy of hemophilia B.

OC 62.2 | SHP648: A High Performing Next Generation FIX Gene Therapy Vector Based on AAV8

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Background: In the BAX 335 hemophilia B gene therapy trial using an AAV8-based vector and expressing the Padua FIX variant, high levels of expression were seen, however, sudden drops in activity were observed in some patients. A root cause analysis pointed to exaggerated immunogenicity as the potential cause for the drop of FIX expression.

Aims: To develop a next generation FIX gene therapy vector with a reduced immunogenic risk and an improved expression efficiency.

Methods: Vector improvement was initiated with the replacement of the FIX Padua coding sequence by codon-optimized and CpG-depleted sequences.

Results: Screening of these constructs identified a sequence that induced FIX Padua expression approximately two-fold in FIX knock-out mice compared to the BAX 335 vector. Next, we showed that a series of single-stranded (ss) vectors showed FIX expression levels that were similar compared to the corresponding self-complementary (sc) vectors. Finally, the strength of the promoter was increased by inserting the recently described liver-specific regulatory element CRM8 (Chuah et al., 2014, Nair et al., 2014) upstream

of the TTR promoter. Testing the resulting vectors in an in-vitro biopotency assay using human liver cells showed a robust 5-fold increase in FIX expression by the CRM8 element. Consistent with the in vitro results, the ss version including the CRM8 element further improved expression in FIX knock-out mice approximately two-to three-fold.

Conclusions: SHP648 represents a next generation ss AAV8-based FIX vector with enhanced performance and reduced immunogenic risk. These features increase the safety profile and are expected to allow for lower dosing.

OC 62.3 | Preventive Immune Suppression Using CTLA-4 IgGs Enables Re-administration in AAV8 Gene Therapy

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Background: Gene therapy using adeno associated virus serotype 8 (AAV8) is considered one of the most promising technologies to treat monogenic diseases such as hemophilia A and B. A major drawback of AAV8 gene therapy is that it can be only applied once due to formation of anti-AAV8 immunity. Re-administration might be required to boost suboptimal expression levels of the target transgene. We developed a strategy using immune suppression to enable re-administration of AAV8-human-FIX (AAV8-huFIX) gene therapy.

Aims: To boost human FIX (huFIX) plasma levels using repeated dosing with an AAV8-huFIX gene therapy vector in the presence of an immune suppressive regimen.

Methods: AAV8-huFIX gene therapy vector was applied to C57BL/6 mice in the presence or absence of immune suppressive regimens. Anti-AAV8 immunity after AAV8 gene therapy was assessed by analyzing neutralizing antibodies (Nabs) and binding antibodies (Babs) to AAV8 using an in vitro and ELISA assay, respectively. AAV8-specificT cell responses were analyzed by IFNg ELISPOT and FACS. HuFIX plasma levels were determined by FIX antigen ELISA.

Results: Abatacept (CTLA-4 IgG fusion protein), a marketed drug that blocks the CD80/CD86-CD28 interaction, was tested alone and in combination with the calcineurin inhibitor Tacrolimus. Both products induced highly efficient inhibition of anti-AAV8 Nabs and AAV8-specific T cell responses after AAV8 gene therapy: Neither anti-AAV8 Nabs nor Babs were detectable even after two doses of AAV8-huFIX treatment, neither were T cell responses. Furthermore, immunosuppression did not affect huFIX expression in mice. Boosting of huFIX plasma levels from about 2 to 20 µg/ml was achieved by first applying a low dose of AAV8-huFIX followed by a second higher dose of AAV8-huFIX in the presence of abatacept. No toxic side effects were observed.

Conclusions: Abatacept can enable re-administration of AAV8-huFIX to boost suboptimal FIX levels in patients.

OC 62.4 | Modulation of AAV Vector Dosing and Avoidance of Capsid Immune Responses via Repeated Co-administration of Vector with Tolerogenic Rapamycin Nanoparticles

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Background: Gene therapy for Hemophilia B (HB) using adeno-associated viral vectors (AAV) showed promising results in preclinical and clinical trials. However, Immune responses to AAV represent an important issue since anti-capsid neutralizing antibodies develop at high titers post-vector administration, impeding effective AAV re-dosing. Additionally, capsid-specific T cell responses can drive clearance of AAV transduced cells, resulting in short-lived transgene expression.

Aims: Assess the efficacy of a novel strategy to mitigate immunogenicity of AAV vectors based on the co-administration of the vector with PLGA-nanoparticles containing rapamycin (SVP-Rapa).

Methods: Hemostatically normal and HB mice were first injected with an AAV8 vectors expressing luciferase together with SVP-Rapa or empty nanoparticles (SVP-empty), then re-administered few weeks later with an AAV8 expressing human FIX (hFIX) together with SVP-Rapa or SVP-empty. Anti-AAV8 antibodies levels, capsid-specific T cells, and hFIX antigen levels in plasma were measured. Specific immunoassays were used to further understand the mechanisms of action of SVP-Rapa. The safety and efficacy of the approach was also tested in non-human primates (NHP).

Results: Co-administration of AAV with SVP-Rapa inhibits the formation of anti-AAV antibodies, allowing successful vector re-injection in both mice and NHP. Modulation of AAV vector immunogenicity appeared to be antigen-specific, as treated animals showed normal immune responses to subsequent challenges with unrelated antigens. Additionally, depletion experiments suggested a role of regulatory T cells as mediators of this mechanism. We also demonstrated that SVP-Rapa efficiently control memory responses and CD8+ T cell responses to the AAV capsid. Results were also confirmed in HB mice, were vector dose modulation via repeated administrations was successfully implemented.

Conclusions: These results validated the use of SVP-Rapa to mitigate AAV vector immunogenicity allowing for efficient vector re-administration.

OC 62.5 | CRISPR/Cas9-mediated Genome Editing via Postnatal Injection of AAV Vector Cures Hemophilia B in Mice

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Background: Hemophilia B, a bleeding disorder caused by mutations in *F9*, is considered an appropriate target for genome editing technology.

Aims: Here, we considered the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 system for the treatment of hemophilia B.

Methods: We used an adeno-associated virus 8 (AAV8) vector to deliver gene-editing tools *in vivo*. Genomic mutations were detected using T7 endonuclease I assay or next generation sequencer. Phenotypic corrections were assessed by plasma coagulation factor IX (FIX) activity, thrombin generation, intravital microscopy, and bleeding time.

Results: The administration of AAV8 vector harboring *Staphylococcus aureus* Cas9 (SaCas9) and single guide RNA (sgRNA) to wild-type adult mice induced a double-strand break (DSB) at the target site of *F9* in hepatocytes, sufficiently developing hemophilia B. The SaCas9/sgRNA-expressing AAV8 vector together with the repairing homologous sequence significantly, but marginally, increased in FIX levels in hemophilia B mice. Alternatively, the SaCas9/sgRNA-expressing AAV8 vector targeting the intron 1 together with the *F9* cDNA more efficiently restores hemostasis via both processes of non-homologous end-joining and homology-directed repair following DSB. Notably, these therapies also cure neonate mice with hemophilia, which cannot be achieved with conventional gene therapy with AAV vector. The phenotypic correction after the neonatal injection was sustained life-long although the AAV genome quickly diluted out. Finally, the ongoing hemophilia therapy targeting antithrombin gene with antisense oligonucleotide might be replaced by that with SaCas9/sgRNA-expressing AAV8 vector.

Conclusions: Our results suggest that CRISPR/Cas9-mediated genome editing using an AAV8 vector provides a flexible approach to induce DSBs at target genes in hepatocytes and could become an attractive strategy for hemophilia gene therapy.

OC 75.1 | Pharmacological Intervention and Improved Platelet-factor VIII Functionality Enhanced Efficacy of in vivo Platelet-targeted Gene Therapy of Murine Hemophilia A via Intraosseous Delivery

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Background: Factor VIII (FVIII) expressed and stored in platelets is protected from neutralization by anti-FVIII antibodies following gene therapy of hemophilia A (HemA). Partial phenotypic correction was achieved in HemA mice following intraosseous (IO) infusion of lentiviral vectors (LVs) carrying a human FVIII transgene (F8/N6) driven by the megakaryocyte-specific Gp1bα promoter.

Aims: We aimed at developing a protocol for effective gene therapy of murine HemA by improving LV transduction efficiency using pharmacological agents to suppress the innate and adaptive immune responses and increasing platelet-FVIII functionality using new hFVIII variants with higher secretion efficiency and function.

Methods: C57BL6 mice or HemA mice were pretreated with dexamethasone (Dex) (IP, 5 mg/kg at -24h, -4h, 4h and 24h) and anti-CD8α monoclonal antibody (mAb) (IP, 4 mg/kg on day -1, 4, 11, 16 and 21), or Dex only. IO infusion of MND-GFP-LVs driven by a ubiquitous MND promoter or G-F8/N6-LVs and G-F8X10K12-LV driven by Gp1bα promoter was performed on day 0. GFP or FVIII expression was evaluated overtime.

Results: For MND-GFP-LV treated mice, higher numbers of GFP+HSCs were observed in drugs + LVs treated mice compared with LV-only treated mice (48.3% vs 44.4% on day 7; and 10.7% vs 2.6% on day 160). Furthermore, compared with G-F8/N6-LV, G-F8X10K12-LV + Drugs produced a 75% increase of platelet-FVIII antigen levels by FVIII-specific ELISA, less blood loss in tail clipping assay, and relatively higher peak height thrombin and total thrombin in thrombin generation assay. However, inhibitory antibodies were detected in G-F8X10K12-LV treated mice.

Conclusions: Administration of Dex and anti-CD8α mAb improved lentiviral transduction efficiency and persistence of transduced cells, leading to over 10% GFP+HSCs in treated mice up to 160 days. IO delivery of G-F8X10K12-LV combined with the drug treatment improved phenotype correction in HemA mice, indicating that this strategy can be used as a potential novel treatment for hemophilia.

OC 75.2 | Persistent Expression of FVIII Following Intravenous Administration of Lentiviral Vectors in Neonatal Hemophilia A Mouse and Dog Models

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Background: AAV based gene therapy for Factor VIII (FVIII) and Factor IX has demonstrated promising clinical benefit when tested in a sub population of hemophiliac subjects; however, the high frequency of preexisting anti-AAV antibodies (abs), small cargo capacity and non-integrating nature of AAV may prevent the use of AAV approach to achieve lifelong cure for all hemophilia patients. Lentiviral vector (LV) technology may circumvent these problems with its large capacity, ability to sustain transgene expression via integration, extremely low incidence of preexisting anti-LV abs, and encouraging efficacy and safety profile demonstrated in pre-clinical and clinical studies.

Aims: Evaluate the potential use of LV-FVIII for the treatment of hemophilia A (HemA).

Methods: Codon optimized Human FVIII (cohFVIII) variants were cloned into a liver specific LV expression system and LV-FVIII mediated FVIII expression was assessed in HemA mice/dogs post Intravenous (IV) delivery of LV at 1.5E10 or 1.3E9 transducing units/kg dose respectively.

Results: Significant improvement on FVIII expression was observed in HemA mice for all cohFVIII variants, with the lead candidate driving 100-fold higher FVIII expression compared to unmodified FVIII; Inclusion of XTEN, a non-structured poly-peptide in B-domain region of FVIII further increased FVIII level by 5-fold, resulted in 30-50IU/mL long-term FVIII expression, represent 30 to 50-fold of normal FVIII level. Furthermore, anti-FVIII abs. were only detected in mice with supra physiological level of FVIII expression, but no cytotoxic T lymphocyte response was observed against LV transduced cells in anti-FVIII abs. positive mice. LV-cohFVIIIXTEN was then evaluated in HemA dog neonates, 30-100% FVIII activity was achieved post IV delivery of LV with no adverse event observed.

Conclusions: LV-FVIII treatment had achieved curable level of persisting FVIII expression in HemA mice and dogs, may potentially be used for the treatment of HemA.

OC 75.3 | Vector Dose-dependent Delayed CD8+ T Cell-mediated Clearance of AAV Encoded Antigen in the Liver

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Background: Hepatic AAV gene transfer should be ideal for the treatment of hemophilia. However, $CD8^+$ T cell responses against the viral capsid may target transduced hepatocytes even months after gene transfer. This is reminiscent of similarly delayed responses against hepatitis viruses.

Aims: To develop a model for $CD8^+$ T cell activation in the liver, we performed hepatic gene transfer with an AAV8 vector expressing ovalbumin (OVA).

Methods: Three different doses (low: $1x10^8$ vg, medium: $1x10^9$ vg, and high: $1x10^{10}$ vg) of AAV8-OVA were injected IV into in C57BL/6 mice.

Results: Dose dependent OVA expression resulted in distinctly different immune responses. Only the mid dose showed circulating OVA-specific $CD8^+$ T cells, which emerged at 2 weeks in 40-50% of mice and reached a high frequency (5-35%) that was maintained for >3 months. Nonetheless, OVA expression lasted for >2 months, indicating an inability to eliminate the liver-expressed antigen. This was attributed to expression of inhibitory molecules. Loss of OVA expression at ~2.5 months correlated with downregulation of PD-1 and up-regulation of IFN-g expression. PD-$1^{-/-}$ mice also developed circulating OVA-specific $CD8^+$ T cells. However, none of the PD-$1^{-/-}$ mice had systemic OVA expression at 2 or 4 weeks, indicating rapid target clearance. Significantly increased numbers of $CD8^+$ T cells were also observed in liver sections of mice that lost expression (e.g. in low-dose transduced mice) despite absence of circulating OVA-specific $CD8^+$ T cells, suggesting that a localized immune response was responsible for the loss of expression in these animals. At the high dose, OVA expression was sustained without $CD8^+$ T cell response.

Conclusions: At limited vector doses, $CD8^+$ T cell responses that are not functionally competent may develop. Upon down regulation of negative checkpoint receptors, a functional response emerges. These responses may not always be detectable in circulation, are not dependent on systemic antigen delivery, and can even occur in TLR9-deficient mice.

OC 75.4 | Prophylactic Platelet Factor (F) VIII Infusions Established from Induced Pluripotent Stem Cell (iPSC)-derived Megakaryocytes in Hemophilia A

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Background: Ectopically expressed FVIII in megakaryocytes (MKs) and platelets (pFVIII) is stored in a-granules and released at sites of vascular injury by activated platelets (Plts), restoring hemostasis in $FVIII^{null}$ mice, even in the presence of neutralizing inhibitors. Expressing FVIII in Plts, however, has limitations that make pFVIII gene therapy through bone marrow transplantation in severe hemophilia A patients with intractable inhibitors problematic.

Aims: We propose an alternative strategy based on infusing iMKs derived from iPSCs and expressing either human B-domain-deleted (BD) FVIII or a R1645H BDFVIII variant with greater specific activity.

Methods: Plts isolated from *P38*, transgenic mice expressed pFVIII, and iMKs that expressed pFVIII after transfection with *Cxcl4* promoter-driven pFVIII lentiviruses were utilized. In vitro assays of efficacy were done using rotational thromboelastography (ROTEM) and in vivo studies were done using both the tail clip assay and a $FeCl_3$ carotid artery injury model.

Results: pFVIII-iMKs were successfully established and did not show any injury while expressing pFVIII. Both *P38* Plts infused into *F8*-/- murine whole blood

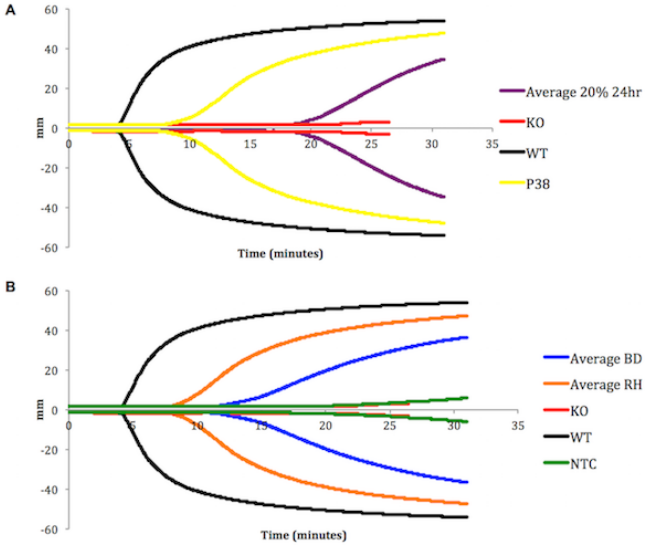

FIGURE 1 In vitro efficacy of P38 platelets and pFVIII-iMKs in mouse FVIII KO whole blood by ROTEM

(Fig. 1A) and pFVIII-iMKs (Fig. 1B) demonstrated hemostatic efficacy by ROTEM that was additive with recombinant FVIIa (not shown). *P38* Plts infused into *F8-/-* mice were hemostatically effective for up to 72 hrs post-infusion in the tail clip assay

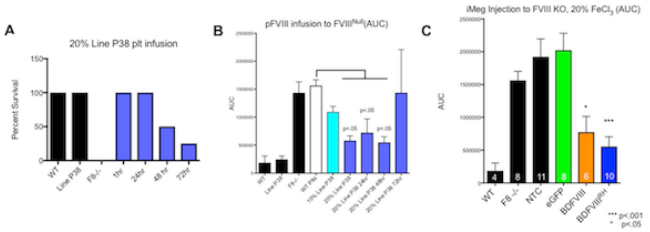

FIGURE 2 In vivo efficacy of FVIII expressing P38 and iMegs-derived Plts in mice tail clip and FeCl3 carotid artery injury models

(Fig. 2A) and up to 48 hrs in the $FeCl_3$ injury model (Fig. 2B). Infused pFVIII-iMKs release Plts and these were hemostatically effective as well in clodronate liposome-pretreated *F8-/-* mice (Fig. 2C).

Conclusions: Plt-delivered FVIII may be a useful, long-lasting prophylactic agent in lieu of present-day bypassing agents and may be used additively in complement with them. Such Plts may be derived from iPSCs established from the effected patient or from "universal" iPSCs modified to minimize its antigenicity when differentiated into iMKs.

OC 75.5 | Targeted FVIII Expression under the Control of its Native Promoter for Hemophilia A Gene and Cell Therapy

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Background: Despite the development of blood products and recombinant FVIII has improved the patients quality of life, replacement therapy do not represent yet a definitive cure for Hemophilia patients.Thus other therapeutic approaches, such as gene and cell therapy, are required.

Aims: We studied the activity of F8 promoter (pF8) sequence to drive transgene expression in a Lentiviral Vector construct to verify the feasibility of expressing FVIII under its natural promoter for gene therapy approaches.

Methods: In silico analysis of Transcriptional Factors (TF) consensus sequences predicted the presence of several cell-specific TF validated by a luciferase specific assay . We injected LV.pF8.hFVIII in hemophilic mice and aPTT assay measured FVIII activity. We transplanted LV-pF8.FVIII transduced human cord-blood CD34+ cells in busulfan-treated NOD/SCID gamma-null HA-mice (NSGHA).

Results: pF8 study showed its ability to drive transgene expression in hepatic endothelium, splenic macrophages and, generally, in myeloid cells by *in vivo* and *ex-vivo* LV delivery. We injected LV.pF8.hFVIII in hemophilic mice and aPTT assay demonstrated FVIII activity in therapeutic range (up to 12% of normal FVIII activity) without antibodies formation up to 1 year and consistent blood loss reduction. Using more active forms of FVIII cDNA we improved FVIII activity levels in HA mice. Moreover LV pF8.hFVIII injection in FVIII-immunized HA mice resulted in therapeutic FVIII activity and reversion of inhibitor titers. Transplantation of LV-pF8.FVIII transduced human cord-blood CD34+ cells in busulfan-treated NSGHA mice showed therapeutic levels of FVIII activity up to 10% of normal with a 30% of cell chimerism.

Conclusions: Our results demonstrate that pF8 is differentially active in cell-subpopulations of several organs contributing to identify the FVIII producing cells and targeting transgene expression in these cells by LV produced FVIII in therapeutic range in HA mice without immune response in two different hemophilic mouse strains.

MANAGEMENT OF THROMBOEMBOLISM

OC 12.1 | Prevalence of Major Medical Illnesses Associated with Venous Thromboembolism Risk in US Hospitals

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Background: Major medical illnesses contribute greatly to population-attributable risk for venous thromboembolism (VTE). Recent annual numbers of episodes of each medical illness have not been described.

Aims: To estimate the annual number and diagnoses of medical illnesses in US acute-care hospital discharges at risk of VTE.

Methods: The American College of Chest Physicians (ACCP) 2012 guideline described major medical illnesses that place patients at risk of VTE (respiratory failure, chronic obstructive pulmonary disease, heart failure, pneumonia, other infections, stroke, rheumatologic disorders). These were examined in Y2014 US hospital discharges using the first three diagnosis codes derived from the Clinical Classification System in the National Inpatient Sample (a database of acute-care hospital discharges from the US Agency for Health Care Quality and Research).

Results: In Y2014 there were 20.8 million discharges from US acute-care hospitals with a diagnosis of a medical illness. Respiratory failure, heart failure, and infections were the commonest major medical diagnoses associated with risk of VTE. Overall, 7.2 million (35%) discharges met the Y2012 ACCP criteria for VTE prophylaxis for at least 6-14 days. 1.36 million patients had a diagnosis of cancer, and 2.79 million were aged ≥ 75. Based on age ≥ 75 or a history of cancer, 3.48 million discharges have an extended duration of risk of VTE and warrant thromboprophylaxis for up to 42 days according to the APEX[1] study (NCT01583218) (Table).

Conclusions: Among Y2014 US hospital discharges there are 7.2 million with acute major medical illnesses at risk of VTE as described by the ACCP. Respiratory diseases, heart failure, and infections were the commonest acute medical conditions warranting thromboprophylaxis. Almost half are at extended duration of risk of VTE based solely on age or a history of cancer.

TABLE 1 Number of Acutely Ill Medical Discharge Diagnoses from US Acute-Care Hospitals in 2014 with ACCP Guideline-Defined Risk of VTE: By Diagnosis and Age

	Overall number	Age group in years (n) 18-39	Age group in years (n) 40-59	Age group in years (n) 60-74	Age group in years (n) 75+	Cancer (n)
Number	20,815,620	5,684,465	4,867,461	4,902,047	5,361,647	
Respiratory failure / Chronic obstructive pulmonary disease	2,567,991	115,325	592,080	946,425	914,160	483,350
Heart failure	2,338,371	69,890	413,485	727,450	1,127,546	402,395
Pneumonia	1,653,541	107,505	344,915	509,035	692,085	382,025
Other infections	1,621,960	157,105	390,595	502,095	572,165	353,820
Stroke	574,850	15,675	118,960	187,240	252,975	92,790
Arthrophathy/spondylopathy	545,560	70,850	187,495	144,510	142,705	60,410
Any of the above	7,209,343	457,865	1,653,601	2,306,431	2,791,446	1,365,111
Apex 2-criteria 1	3,484,731	-	207,895	485,390	2,791,446	1,336,801

[1]Based on age ≥ 75 and/or a cancer history. VTE history (8%) and D-dimer+ (62%) in APEX were additional independent risks; these data were not available.

OC 12.2 | Venous Thromboembolism and Physical Function in the Nurses' Health Study

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Background: Physical function is integral to healthy aging and is a strong predictor of mortality in older adults. Limited research has examined the impact of venous thromboembolism (VTE) on physical function.

Aims: We prospectively examined the relationship of VTE and physical function among 82,118 women from the Nurses' Health Study, ages 46-72 at baseline in 1992; including 1,857 women with incident VTE during follow-up through 2012.

Methods: Physical function was measured by the Medical Outcomes Short Form-36 physical function scale, administered every four years starting in 1992. Participants reported physician-diagnosed VTE on biennial questionnaires. We investigated the association between an incident VTE and change in physical function score over the five 4-year periods using multivariate linear regression. We utilized linear mixed models to estimate slopes of decline over the 20-year follow-up period comparing women with vs. without VTE.

Results: We observed a substantial decrease in physical function score over 4-years comparing women with vs. without an incident VTE (multivariable adjusted mean difference=-6.89, 95% CI:-7.84,-5.93).

TABLE 1 Association between VTE, DVT, and PE and 4-year change in physical function score among women in the Nurses' Health Study.

	NHS (n=82,118)
VTE (DVT and PE)	
Age adjusted	-7.33 (-8.29, -6.38)
Multivariable adjusted	-6.89 (-7.84, -5.93)
DVT only	
Age adjusted	-6.05 (-7.41, -4.69)
Multivariable adjusted	-5.77 (-7.14, -4.42)
PE only	
Age adjusted	-8.36 (-9.69, -7.03)
Multivariable adjusted	-7.76 (-9.09, -6.43)

This difference was even greater among women reporting a pulmonary embolism (multivariable adjusted mean difference=-7.76, 95% CI:-9.09,-6.43). We also compared physical function trajectories over the 20 years of follow-up separately in participants with no VTE, and women with incident VTE during one of the 4-year periods.

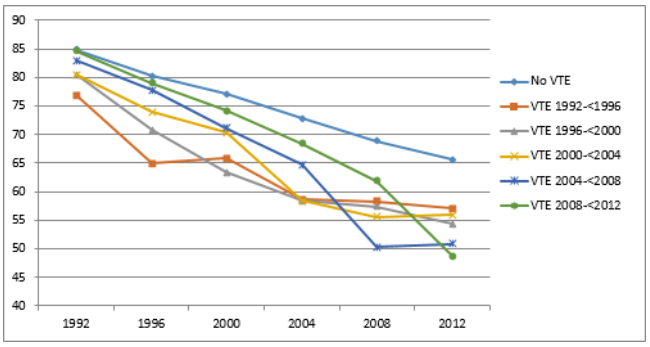

FIGURE 1 Age-adjusted physical function scores from 1992 to 2012 among women from the Nurses' Health Study

We found the sharpest decline in physical function during the time period in which the VTE occurred. Over the 20 years, those with incident VTE had worse rates of decline than those with no VTE (mean difference per 4-years=-1.71, 95% CI:-2.13,-1.28).

Conclusions: In this large cohort of women, VTE was strongly associated with physical function decline, with the greatest decline in the years immediately following the VTE. If confirmed, these results suggest it may be important to consider approaches to ameliorating functional deficits after VTE diagnosis.

OC 12.3 | Catheter-related Venous Thromboembolism in Hospitalized Pediatric Patients with Inflammatory Bowel Disease: A Retrospective Study Assessing Prevalence, Characteristics and Role of Anticoagulant Thromboprophylaxis with Enoxaparin

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Background: Hospitalized children with active inflammatory bowel disease (IBD) frequently require a central venous catheter (CVC). Since IBD confers an increased risk of venous thromboembolism (VTE), these patients have a higher risk for CVC-related VTE. Data in children are limited and likely underestimate the true incidence of these events. Furthermore, the role of anticoagulant thromboprophylaxis (AT) has not been adequately explored. In 2014, we introduced a protocol to consider initiating AT with enoxaparin after CVC placement in any patient admitted with active IBD.

Aims: To describe prevalence and characteristics of CVC-related VTE in hospitalized pediatric patients with active IBD and report efficacy and safety of AT with enoxaparin.

Methods: We conducted a retrospective study of patients who were admitted to our center in the last 2 years with active IBD and required CVC during hospitalization. Since CVCs in IBD patients are placed by interventional radiology, we used radiology data mining software to identify potential cases. The medical charts of potential cases were reviewed to identify cases that met inclusion criteria and collect rele-

TABLE Summary of characteristics and outcome measures

Characteristic	AT (N=17)[1]	No AT (N=23)	P value[2]
Female gender	11 (65%)	11 (48%)	0.3
Age (years)	14 (8-18)	14 (5-20)	0.8
IBD phenotype	Crohn's Disease [N=10 (59%)]; Ulcerative Colitis [N=7 (41%)]	Crohn's Disease [N=16 (70%)]; Ulcerative Colitis [N=7 (30%)]	0.5
Duration of hospitalization (days)	24 (10-47)	15 (3-72)	0.06
CVC-related VTE	0/17 (0%)	5/23 (22%) [3]	**0.04**
Nadir Hb during hospitalization (g/dL)	7.8 (5.8-10.8)	8.1 (5.6-13.8)	0.3
RBC transfusions during hospitalization	1 (0-7)	0 (0-7)	0.06
RBC transfusions per day of hospitalization	0.04 (0-0.19)	0 (0-0.3)	0.1

AT=Anticoagulant Thromboprophylaxis; IBD=Inflammatory Bowel Disease; CVC=Central Venous Catheter; VTE=Venous Thromboembolism; Hb=Hemoglobin; RBC=Red Blood Cell.

[1] All patients received primary AT (15 patients) or secondary AT (2 patients) with enoxaparin according to an institutional weight- and age-based dosing protocol.

[2] Continuous variables are presented as median and range and are compared using the Mann-Whitney U-test. Categorical data are presented as frequency and percentage and are compared using the Chi-square test. P value < 0.05 was considered statistically significant.

[3] All patients developed symptomatic proximal deep vein thrombosis confirmed by Doppler ultrasound.

vant data. To assess efficacy and safety outcomes, we compared the frequency of radiologically confirmed CVC-related VTE, nadir hemoglobin and red blood cell transfusion requirements in patients who received AT with enoxaparin to those who didn't. Mann-Whitney U or Chi-square tests were used to compare the two groups.

Results: A total of 40 patients met inclusion criteria. Of the 40 patients, 17 received AT with enoxaparin. Refer to summary table for results of data analysis.

Conclusions: Our study suggests a higher frequency of CVC-related VTE in hospitalized children with active IBD than previously reported. Moreover, our data suggest that AT with enoxaparin is effective and appears to be safe in this population. The results of this study support the need for a prospective study to define incidence and risk factors for VTE in children with IBD, and evaluate role of AT.

OC 12.4 | External Validation and Comparison of the Improve Risk Assessment Model with the Geneva Risk Assessment Model in the ESTIMATE Cohort

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Background: Improved thromboprophylaxis for acutely ill medical patients is needed and relies on valid predictions of thrombotic risks. The Improve risk assessment model (RAM) was only validated in North America and different classifications of low-risk patients were proposed.

Aims: To validate the Improve RAM in a Swiss cohort and to compare it to the Geneva RAM.

Methods: Medical inpatients from 3 academic and 5 non-academic hospitals were prospectively followed for 90 days in 2010-2011 (ESTIMATE study). The primary outcome was venous thromboembolism (VTE) or VTE-related death, which was adjudicated independently. We assessed and compared discriminative performance and calibration of the RAMs, using time-to-event methods taking into account the competing risk of death. Patient informed consent was obtained following the agreement of ethics committees.

Results: Of 1478 patients with a mean age of 65 years, about 60% received thromboprophylaxis during their hospitalization (median duration of 8 days). The cumulative incidence functions of VTE at 30 and 90 days were 1.1% and 1.6% (n=30), with a mortality at 90 days of 19.3% (n=292). Discriminative performances of the Improve RAM and the Geneva RAM were similar at 30 and 90 days, with 30d time-dependent AUC of 0.78 (95%CI 0.67-0.88) and 0.80 (0.72-0.88), respectively. An Improve low-risk score (< 3 points, 68% of patients) *vs*. Geneva low-risk score (< 3 points, 35% of patients) had sensitivities of 73% *vs*. 90% and 0.8% *vs*. 0.6% risk of VTE at 3 months. Among patients without thromboprophylaxis, corresponding numbers were 54% *vs*. 85% and 1.4% *vs*. 0.7%. High-risk groups had 4.7% *vs*. 2.8% risk of VTE at 3 months, respectively.

Conclusions: The discrimination of the Improve RAM is good and comparable to that of more complex scores such as the Geneva RAM. More patients are classified as low-risk in the Improve RAM, however with possibly lower sensitivity and greater VTE risks. A lower threshold to define the low-risk Improve group may be advisable.

OC 12.5 | Genetics InFormatics Trial (GIFT) of Warfarin to Prevent Deep Venous Thrombosis: Improvement in Time Spent in the Therapeutic INR Range

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Background: Whether genotype-guided dosing improves the percentage of time spent in the therapeutic INR range (PTTR) is controversial.

Aims: We conducted a multicenter, randomized controlled trial of patients ≥ 65 years of age initiating warfarin prior to elective hip or knee arthroplasty.

Methods: Participants were genotyped for 4 polymorphisms: VKORC1-1639G>A, CYP2C9*2, CYP2C9*3, and CYP4F2 V433M. For the first 11 days of therapy, warfarin dosing was guided by www.WarfarinDosing.org, which incorporated genotype in patients randomized to pharmacogenetic dosing. Recommended doses of warfarin were open label, but participants and providers were blinded to study arm. A priori, we defined a high-risk subgroup as participants for whom genetic and clinical algorithms had significantly (≥ 1 mg) different estimates of the therapeutic warfarin dose. We calculated PTTR during days 4 - 28 of therapy using a therapeutic range of 1.96 - 3.04 for patients with a target INR of 2.5, and 1.36 - 2.24 for patients with a target INR of 1.8.

Results: 1588 participants (64% female, 91% Caucasian) had at least 1 INR value after a warfarin dose and were included. The mean PTTR was 66.1% in participants randomized to genotype-guided dosing and 62.0% with clinical dosing. The mean improvement (95% CI) was 4.1%

(1.8 - 6.4); P < 0.001. Among patients with a target INR of 2.5, the mean PTTR was 57.6% with genotype-guided dosing and 53.8% with clinical dosing (mean difference 5.8% (95% CI 2.6 - 9.0); P < 0.001). Among the 654 high-risk participants, mean PTTR was 66.8% with genotype-guided and 58.9% with clinical dosing (mean difference 7.9% (95% CI 4.3 - 11.4); P < 0.001). In contrast to these benefits, there was no significant improvement in PTTR in subgroups who had a low-risk genotype or who self-identified as African-American.

Conclusions: In a cohort of mostly Caucasian patients initiating warfarin therapy, genotype-guided dosing improved INR control.

Funding: NIH (R01 HL097036) and CMS; ClinicalTrials.gov NCT01006733.

OC 25.1 | The Impact of Residual Pulmonary Obstruction on the Long-term Outcome of Patients with Pulmonary Embolism

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Background: In several patients, after a first episode of acute pulmonary embolism (PE), the risk of recurrent venous thromboembolism (VTE) is high and that of chronic thromboembolic pulmonary hypertension (CTEPH) is not negligible. Currently, there is virtually no way to identify patients with PE in whom the risk of late complications is high enough to justify indefinite anticoagulation. The impact of residual pulmonary obstruction (RPO) on the outcome of patients with pulmonary embolism (PE) is uncertain.

Aims: To determine the risk of recurrent VTE and/or CTEPH over a 3-year follow-up in a wide cohort of PE patients with and without RPO.

Methods: In a nationwide, multicentre, prospective cohort study, consecutive patients with first, objectively confirmed acute PE were prospectively followed for up to 3 years. Patients with severe cardiopulmonary diseases, low expected survival or refusal to provide informed consent were excluded. A perfusional lung scan was performed after 6 months and RPO was assessed using the Meyer score. Scintigraphic images, recurrent VTE and CTEPH were centrally adjudicated by an independent committee.

Results: 647 consecutive patients were followed for 3 years and received a lung scan at 6 months. Pulmonary perfusion defects were found in 324 patients (50.1%; 95%CI, 46.2 to 54.0). Recurrent VTE and/or CTEPH developed in 34 (10.5%) patients with RPO and in 15 (4.6%) without it leading to an adjusted hazard ratio of 2.42 (95%CI, 1.31 to 4.46).

Conclusions: Residual pulmonary obstruction, as assessed with perfusion lung scanning six months after an episode of PE, is an independent predictor of recurrent VTE and/or CTEPH.

OC 25.2 | The Impact of Increased Systolic Pulmonary Artery Pressure on the Late Outcome of Patients with Pulmonary Embolism

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Background: In several patients, after a first episode of acute pulmonary embolism (PE), the risk of recurrent venous thromboembolism (VTE) is high and that of chronic thromboembolic pulmonary hypertension (CTEPH) is not negligible. The current strategies proposed to identify high-risk patients to be treated with indefinite anticoagulation remain suboptimal and controversial. The impact of asymptomatic increased systolic pulmonary artery pressure(sPAP) on the outcome of patients with PE is uncertain.

Aims: To determine the risk of recurrent VTE and/or CTEPH over a 3-year follow-up in a wide cohort of PE patients with and without asymptomatic increased sPAP.

Methods: In a nationwide, multicentre, prospective cohort study, consecutive patients with first, objectively confirmed acute PE were prospectively followed for up to 3 years. Patients with severe cardiopulmonary diseases, low expected survival or refusal to provide informed consent were excluded. An echocardiographic examination was performed after 6 weeks and after 6 months. Increased sPAP was defined for values > 36 mmHg. Recurrent VTE and CTEPH were centrally adjudicated by an independent committee.

Results: 518 consecutive patients were followed for 3 years and received an echocardiographic examination at 6 weeks and at 6 months. An increased sPAP was found in 79 (15.3%) patients after 6 weeks and in 61 (11.8%) after six months. Recurrent VTE and/or CTEPH developed in 14 (17.7%) patients with an increased sPAP after six weeks and in 29 (6.6%) without it leading to an adjusted hazard ratio of 2.82 (95%CI, 1.46 - 5.41). Recurrent VTE and/or CTEPH developed in 12 (19.6%) patients with increased sPAP after six months and in 31 (6.8%) without it leading to an adjusted hazard ratio of 4.04 (95%CI, 2.03 - 8.04).

Conclusions: Asymptomatic increased systolic pulmonary artery pressure, as assessed with echocardiography, six weeks or six months after an episode of PE, is an independent predictor of recurrent VTE and/or CTEPH.

OC 25.3 | High Sensitivity of a Non-invasive Screening Strategy for Chronic Thromboembolic Pulmonary Hypertension after Acute Pulmonary Embolism

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Background: A non-invasive screening algorithm for chronic thromboembolic pulmonary hypertension (CTEPH) after acute pulmonary embolism (PE) consisting of a prediction score and ECG/NT-proBNP assessment (*Figure 1*) was recently constructed, aiming at faster and easier CTEPH detection than current practice.

Aims: The algorithm was applied to 54 consecutive patients with confirmed CTEPH to accurately evaluate the sensitivity of the algorithm for this rare disease. In addition, the reproducibility of the individual items of the algorithm was studied.

Methods: Two independent researchers calculated the prediction score based on clinical characteristics at PE diagnosis, and evaluated the ECG and NT-proBNP level assessed at CTEPH diagnostic work-up. Interobserver agreement for assessment of the prediction score, RV/LV ratio measurement on CTPA as well as ECG reading was evaluated by calculating kappa statistics.

Results: Median time between PE diagnosis and presentation with CTEPH was 8 months (interquartile range 5-13). 52 patients (96%, 95%CI 87-100%) had a high prediction score and/or CTEPH specific symptoms. The ECG/NT-proBNP combination was abnormal in 49 of 52 patients (94%, 95%CI 84-99%). The sensitivity of the algorithm was 91% (95%CI 79-97%), indicating that 27 of 30 cases of CTEPH would have been detected when applying the screening algorithm to 1000 random PE survivors with a 3% CTEPH incidence (projected negative predictive value 99.7%; 95%CI 99.1-99.9%). The interobserver agreement for calculating the prediction score, RV/LV ratio measurement and ECG reading was excellent with a kappa of 0.96, 0.95 and 0.89 respectively.

Conclusions: All components of the algorithm were highly reproducible. 91% of the CTEPH patients would have been identified by the algorithm, underlining its adequate sensitivity. Prospective validation of the algorithm in consecutive PE patients is required before it can be implemented in clinical practice.

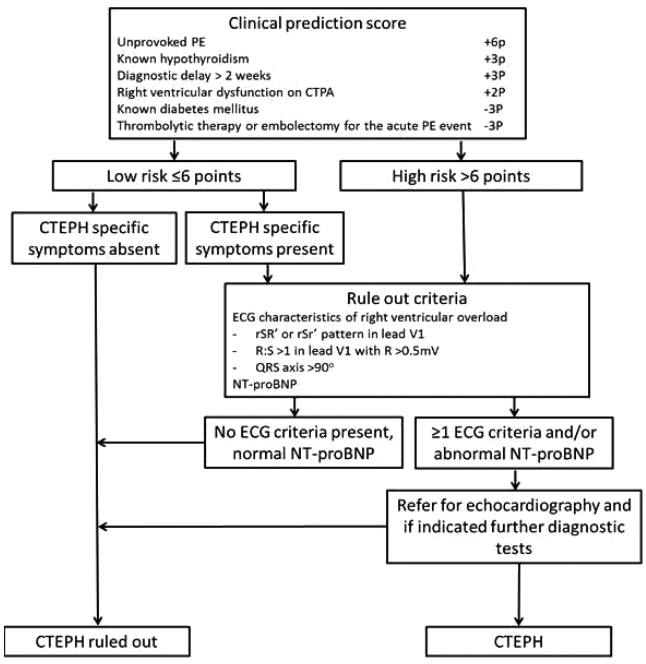

FIGURE 1 Screening algorithm for CTEPH after acute PE consisting of the CTEPH prediction score, CTEPH specific symptoms and the rule out criteria

OC 25.4 | Residual Vein Thrombosis and Serial D-Dimer for the Long-term Management of Patients with Deep Venous Thrombosis

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Background: The optimal long-term strategy for preventing recurrent venous thromboembolism (VTE) in patients with deep-vein thrombosis (DVT) is uncertain.

Aims: Assessing the value of residual vein thrombosis (RVT) and serial D-dimer determinations for identifying patients with DVT in whom anticoagulation can be safely discontinued.

Methods: In 620 consecutive outpatients with a first proximal DVT who had completed at least three momths of anticoagulation (unprovoked in 483, associated with weak risk factors in 137), the ultrasound presence of RVT was assessed and defined as an incompressibility of at least 4 mm. In 517 patients without RVT and with negative D-dimer, anticoagulation was stopped and D-dimer was repeated after one and three months. Anticoagulation was resumed in 63 of the 72 patients in whom D-dimer reverted to positivity.

Results: During a mean follow-up of three years, recurrent VTE developed in 40 (7.7%) of the 517 patients, leading to an annual rate of 3.6% (95% CI, 2.6 to 4.9): 4.1% (95% CI, 2.9 to 5.7) in individuals with unprovoked DVT, and 2.2% (95% CI, 1.1 to 4.5) in those with DVT associated with weak risk factors. Of the 233 males with unprovoked DVT, 17 (7.3%) developed events in the first year of follow-up. Major bleeding complications occurred in 8 patients while on anticoagulation, leading to an annual rate of 1.2% (95% CI, 0.6 to 2.4).

Conclusions: Discontinuing anticoagulation in patients with a first episode of proximal DVT based on the assessment of RVT and serial D-dimer leads to an overall annual rate of recurrent VTE lower than 5.0%, which is the rate reputed as acceptable by the Subcommittee on Control of Anticoagulation of the ISTH. However, in males with unprovoked DVT there is room for further improving the long-term strategy of VTE prevention.

OC 25.5 | Site and Mortality of Major Bleedings in Patients Treated with Individual Doacs

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Background: Limited direct comparisons on the prevalent site and case-fatality of major bleedings (MBs) occurring during treatment with individual direct oral anticoagulants (DOACs) are available.

Aims: To compare the prevalent bleeding site and case-fatality of MBs occurring while on treatment with different DOACs.

Methods: Patients hospitalized for DOAC-associated MB were included in a multicenter study. The primary study outcome was death at 30 days. **Results:** 302 MBs occurred while on treatment with dabigatran (72 patients), rivaroxaban (184 patients) or apixaban (46 patients). Rivaroxaban patients were younger (77 ± 11 years vs. 83 ± 7 years vs. 80 ± 9 years), had higher prevalence of previous bleeding (OR 2.33, 95% CI 1.25-4.32 and OR 3.42, 95% CI 1.51-7.74) and of venous thromboembolism as indication for treatment (23% vs. 0% vs. 6%) and a lower prevalence of previous stroke (17% vs. 29 vs. 30%) than dabigatran and apixaban patients.

Presentation as intracranial hemorrhage (ICH), gastrointestinal or genitourinary bleeding was observed in similar proportions of dabigatran, rivaroxaban and apixaban patients after adjusting for age, previous bleeding and previous stroke (Table 1). Fresh frozen plasma or prothrombin complex concentrates were used in similar proportions of dabigatran, rivaroxaban or apixaban patients.

TABLE 1 Bleeding sites of individual DOAC after adjusting for age, previous bleeding and previous stroke

	N (%)	aOR, 95% CI vs. dabigatran	aOR, 95% CI vs. rivaroxaban
Rivaroxaban intracranial hemorrhage	45 (24)	1.24 (0.55-2.83)	-
Rivaroxaban gastrointestinal bleeding	78 (42)	1.00 (0.48-2.10)	-
Rivaroxaban genitourinary bleeding	19 (10)	1.12 (0.27-4.70)	-
Apixaban intracranial hemorrhage	17 (37)	1.41 (0.51-3.88)	1.13 (0.46-2.77)
Apixaban gastrointestinal bleeding	15 (33)	0.77 (0.32-1.89)	-
Apixaban genitourinary bleeding	3 (6)	1.43 (0.23-9.03)	1.28 (0.29-5.65)
Dabigatran intracranial hemorrhage	19 (26)	-	-
Dabigatran gastrointestinal bleeding	33 (46)	0.77 (0.29-2.08)	-
Apixaban genitourinary bleeding	3 (4)	-	-

Death at 30 days occurred in 31 patients (10%). Presentation as ICH (HR 8.59; 95% CI 3.19-23.12) or shock (HR 7.37; 95% CI 2.69-20.13), INR at admission (HR 1.54; 95% CI 1.12-2.12) and previous stroke (HR 3.60; 95% CI 1.54-8.42) were independent predictors of death. No association was observed between death and treatment individual DOACs.

Conclusions: In our study on MBs while on DOACs, no difference was observed in the presentation as ICH, gastrointestinal or genitourinary bleeding across individual agents. Presentation as ICH or shock and not the individual DOAC are predictors of death at 30 days.

OC 33.1 | The Role of Time in Therapeutic Range on Bleeding Risk Prediction in Atrial Fibrillation Patients under Vitamin K Antagonist Therapy

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Background: Vitamin K antagonists (VKAs) remain widely used as oral anticoagulation (OAC) in atrial fibrillation (AF). However, bleeding risk with VKAs is closely related to the quality of anticoagulation, as reflected by time in therapeutic range (TTR).

Aims: We aim to compare the discrimination performance of four different bleeding risk scores and to investigate if adding TTR would improve their predictive value and clinical usefulness.

Methods: We included 1361 AF patients clinically stable on VKA for at least 6 months. Bleeding risk was assessed by the HAS-BLED, ATRIA, ORBIT and HEMORR$_2$HAGES scores. Major bleeding events were recorded after a median of 6.5 (IQR 4.3-7.9) years. The study protocol was approved by the Ethics Committee from our institution and performed in accordance with the Declaration of Helsinki. All patients gave informed consent.

Results: During follow-up, 250 patients suffered major bleeds and 52 patients died due to bleeding. Comparison of receiver operating characteristic (ROC) curves demonstrated that HAS-BLED had the best performance of the four tested scores (c-index: 0.62), but adding the ‚labile INR' criteria (i.e. TTR< 65%) to ATRIA, ORBIT and HEMORR$_2$HAGES increased their ability of discrimination and predictive value (c-indexes 0.75, 0.73 and 0.72, respectively), with significant improvements in reclassification and discriminatory performance (all p< 0.001). Decision curve analyses (DCA) also showed improvements of the clinical usefulness and a net benefit of the 3 modified risk scores (by addition of TTR< 65%).

TABLE 2 Comparison of the ROC curves, IDI and NRI of the modified bleeding risk scores (by addition of labile INR defined as time in therapeutic rang

	C-index	95% CI	z statistic*	p*	IDI	p	NRI	p
vs. ATRIA								
ATRIA + TTR <65%	0.751	0.727-0.774	7.514	<0.001	0.0326	<0.001	0.1527	<0.001
vs. ORBIT								
ORBIT + TTR<65%	0.733	0.709-0.757	5.087	<0.001	0.0270	<0.001	0.1097	<0.001
vs. HEMORR2HAGES								
HEMORR2HAGES + TTR <65%	0.729	0.704-0.752	4.689	<0.001	0.0159	<0.001	0.0598	0.007

CI = confidence interval; IDI = integrated discriminatory improvement; NRI = net reclassification index; TTR = time in therapeutic range. *for c-index comparison.

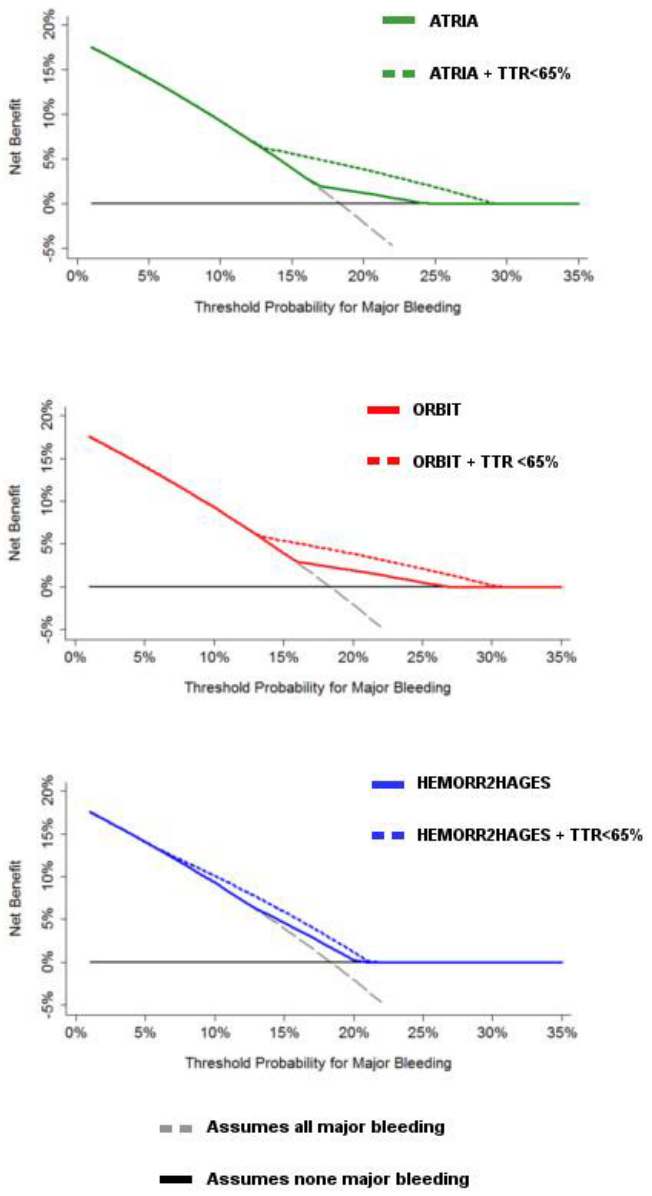

FIGURE 1 Decision curves for the original and modified bleeding risk scores (adding TTR<65%)

Conclusions: In AF patients taking VKAs, the HAS-BLED score had the best predictive ability. Adding labile INR (TTR<65%) to ATRIA, ORBIT and HEMORR$_2$HAGES scores improved their predictive value for major bleeding leading to improved clinical usefulness and higher net benefit compared to the original scores. This suggests that these 3 scores would perform suboptimally in VKA users by not considering 'labile INR' as a bleeding criterion.

OC 33.2 | Persistence of Oral Anticoagulant Treatment for Atrial Fibrillation in The Netherlands: A Surveillance Study

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Background: The efficacy of oral anticoagulant treatment is to a large degree determined by treatment adherence.

Aims: To assess the treatment persistence of direct oral anticoagulants (DOACs) and of vitamin K antagonists (VKAs) in patients with atrial fibrillation.

Methods: Observational study in two registries.

1. A database of Dutch Community Pharmacies with individual patient data on DOAC use from 1-1-2012 to 1-4-2016 (covering the whole of the Netherlands, 17.5 million inhabitants).

2. A database of the Leiden Anticoagulation Clinic with individual patient data from 1-1-2004 to 1-1-2012 (covering the greater Leiden area, n=500 000). The final study sample of 87412 patients included 13878 apixaban-, 34167 rivaroxaban-, 29288 dabigatran- and 10079 VKA users. Main outcome measures were persistence to oral anticoagulant treatment identified as time from start of DOAC treatment to withdrawal of the initial DOAC or end of study period whichever came first, or as time from start of anticoagulant treatment until completely discontinuing anticoagulant treatment.

Results: DOAC users were younger than VKA users (70 vs 73 years). In DOAC users, 11% had withdrawn from their initial DOAC treatment within 6 weeks, another 24% had withdrawn at 6 months (35% total), 43% had withdrawn at 1 year, and 71% at 4 years of follow-up. Approximately a fifth of those who had withdrawn their initial DOAC treatment switched to another anticoagulant (VKA or another DOAC). Of DOAC users, 34% had completely discontinued with their anticoagulant treatment at 1 year and 66% at 4 years. These numbers

were 22% and 36% in VKA users, respectively. Multivariable analyses showed that female sex, young age and non-adherence were predictors for discontinuation of DOAC treatment.

Conclusions: Persistence to oral anticoagulant treatment use in patients with atrial fibrillation is low and 12% (at 1 year)-35% (at 4 years) lower in DOAC users than VKA users. Further research to investigate the reasons and consequences of early stopping DOAC treatment is warranted.

OC 33.3 | Atrial Fibrillation, Stroke, and Mortality in Patients on Hemodialysis - Prospective Results of the Vienna InVestigation of AtriaL Fibrillation and Thromboembolism in HemoDIalysis Patients (VIVALDI)

O. Königsbrügge[1], E. Grilz[1], S. Schmaldienst[2], M. Auinger[3], M. Lorenz[4], R. Klauser-Braun[5], J. Kletzmayr[5], M. Antlanger[6], F. Posch[1,7], I. Pabinger[1], M. Säemann[8], C. Ay[1]

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Background: Atrial fibrillation (AF) is a major risk factor for stroke. However, it is not well investigated in patients with end-stage renal disease (ESRD) on maintenance hemodialysis (HD).

Aims: To assess the association of AF, stroke, and mortality in patients on HD.

Methods: VIVALDI is a prospective population-based study in Vienna, Austria, investigating the risk of stroke in patients on HD with approval of the ethics committee. All participating patients gave written consent. Independent experts adjudicated the composite endpoint of ischemic stroke, transient ischemic attack (TIA), and systemic embolism (SE). Presence of AF was recorded at baseline or as new AF during follow-up and modeled as a time-dependent variable for risk of the endpoint, considering death and kidney transplantation as competing risk events.

Results: The study encompassed 625 patients (median age: 66 years; 229 [36.6%] women), followed for 515 days in median. At baseline, 165 patients (26.4%) had AF and 41 patients developed AF during observation (8.9%). 59 patients (9.4%) received kidney transplants and 135 (21.6%) died. During follow-up 20 primary endpoints (3.2%) occurred (14 ischemic strokes, 2 TIAs, 4 SEs) with an incidence rate of 2.66 per 100 patient-years. In multivariable regression analysis adjusted for sex and BMI, patients with AF had a 2.5-fold increased risk of stroke (95%CI 1.0-6.0) compared to patients without AF (figure 1). Stroke was further associated with age (subdistribution hazard ratio [SHR] 1.03 per year, 95%CI 1.00-1.07), diabetes (SHR 3.1, 95%CI 1.3-7.6) and the CHA2DS2-Vasc score (SHR 1.7 per point, 95%CI 1.3-2.4). AF doubled the risk of mortality (HR 2.0, 95%CI 1.4-2.8) in multivariable regression analysis adjusted for age and sex (figure 2). The risk of death was especially high in patients with new onset AF (HR 4.7, 95%CI 2.7-8.2).

Conclusions: Patients with ESRD on HD who have AF are at increased risk of stroke and mortality. Risk evaluation using clinical characteristics can identify patients at high risk.

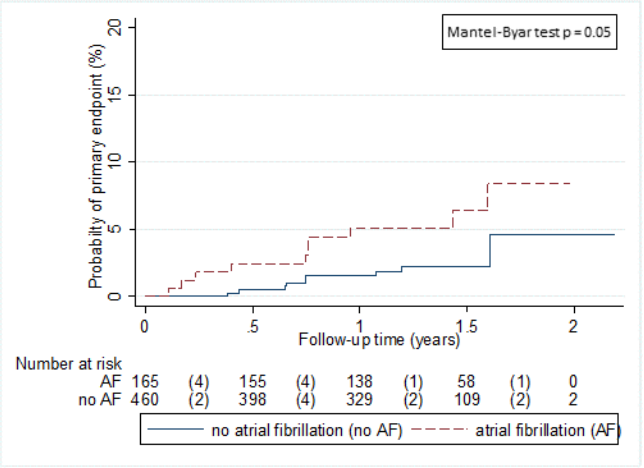

FIGURE 1 Probability of occurrence of the primary endpoint for patients with AF (red, dashed line) versus patients without AF (blue, full line)

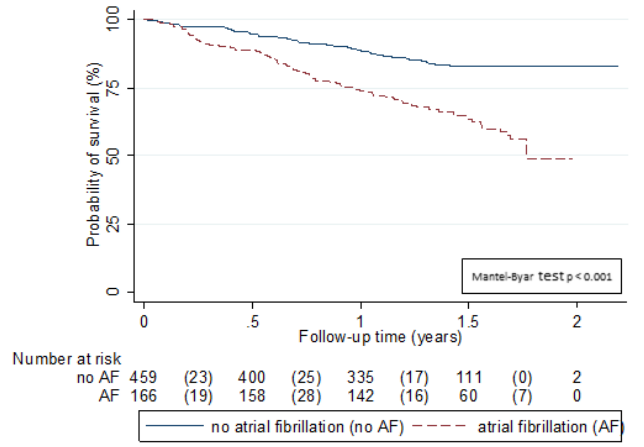

FIGURE 2 Probability of survival for patients with atrial fibrillation (red, dashed) versus patients without atrial fibrillation (blue, full line)

OC 33.4 | Direct Oral Anti-coagulants (DOACs) Are Associated with Decreased Mortality Compared to Warfarin among Hospitalized Patients with Non-valvular Atrial Fibrillation

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Sheba Medical Center, Tel Hashomer, Israel

Background: Direct Oral Anticoagulants (DOACs) are increasingly used as an alternative for traditional antithrombotic therapy for stroke prevention in patients with non-valvular atrial fibrillation (AF).

Aims: To evaluate the real world efficacy and safety of DOACs compared with Warfarin in terms of overall mortality, CVA events occurrence and gastrointestinal (GI) bleeding.

Methods: We performed a retrospective cohort study on patients admitted to medicine wards of Sheba Medical Center, a tertiary medical center, with non-valvular AF, and treated with either DOACs or Warfarin, between the years 2010 and 2016. We excluded patients with former Deep Venous Thrombosis (DVT), and solid tumors. We used propensity score matching to match, for each patient treated on DOACs, a patient of similar age, gender, Charlson comorbidity index, CHADS-VASC, and GFR, but treated with Warfarin. Mortality events were obtained from the national population registry. We used Cox proportional hazards to compare overall mortality, and the occurrence of CVA and GI bleeding leading to subsequent hospitalization.

Results: Among 7261 patients treated with oral anti-coagulants for AF or flutter, and who did not have valvular disease, DVT or solid tumors, 1813 patients were treated with DOACs, and were matched by propensity score to patients with similar baseline characteristics and treated with Warfarin. During a mean follow-up of 2.8 years, 907 mortality events, 441 CVA events, and 99 GI bleeding events were recorded. Overall mortality was significantly decreased in the DOAC group vs. Warfarin (HR=0.57; p< 0.001), as well as the number of CVA and GI bleeding events (HR=0.81; p< 0.02).

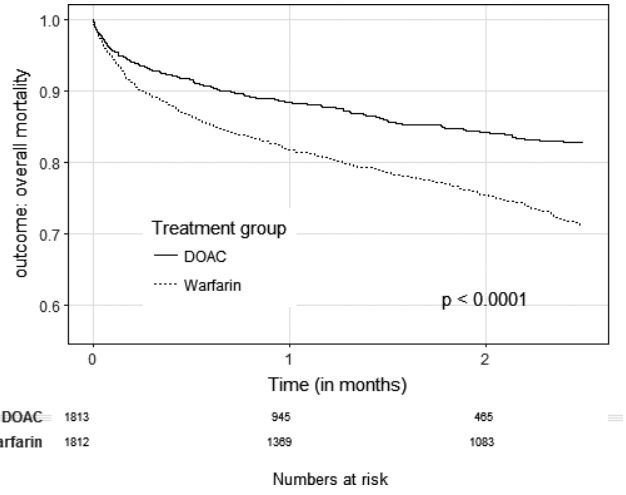

FIGURE 1 Kaplan-Meier plot: overall mortality

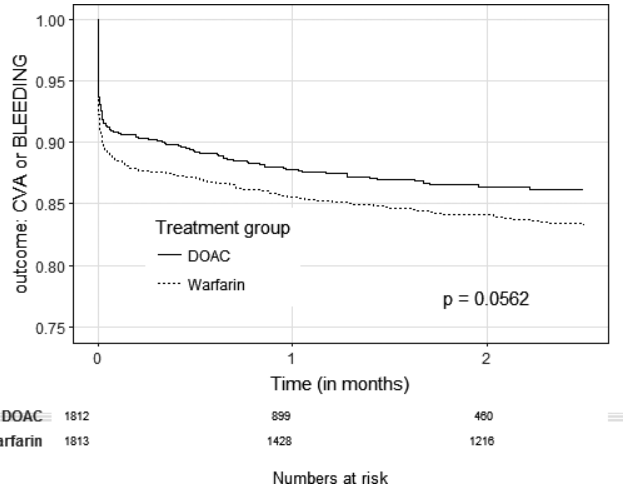

FIGURE 2 Kaplan-Meier plot: CVA and gastrointestinal bleeding events

Conclusions: In this retrospective study based on a cohort of patients hospitalized in a large tertiary medical center, and followed subsequently, decreased overall mortality was found in patients treated with DOACs, compared to patients with similar baseline characteristics and treated with Warfarin.

OC 33.5 | Perioperative Outcomes of Direct Oral Anticoagulants vs. Warfarin in Atrial Fibrillation: A Meta-analysis of Phase III Trials

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Background: Direct Oral Anticoagulants (DOACs) are surpassing Warfarin as the anticoagulant of choice for stroke prevention in Atrial Fibrillation (AF). DOACs outcomes in perioperative settings have not been well elucidated and remain a source of concern for clinicians.

Aims: To evaluate the perioperative safety and efficacy of DOACs vs. Warfarin in AF patients.

Methods: We reviewed the literature for data from Phase III randomized controlled trials comparing DOACs to Warfarin in the perioperative period among AF patients. Sub-studies from 4 trials (RE-LY, ROCKET AF, ARISTOTLE, ENGAGE-AF) were included in the meta-analysis. DOACs as a group and Warfarin were compared in terms of the 30-day pooled risk for Stroke/Systemic Embolism (SSE), Major Bleed (MB) and death, according to whether the study drug was interrupted or not perioperatively. The overall Relative Risk (RR) was estimated using a random effects model. The I^2 test was used to assess heterogeneity in RR among the studies.

Results: Under an interrupted anticoagulation strategy, there was no significant difference between DOACs vs. Warfarin for SSE [41 events/9260 procedures vs. 31/7168, RR=0.95, 95% CI= (0.59, 1.55)], MB [218/9175 vs. 136/7078, RR=1.05, 95% CI= (0.85, 1.30)], and death [69/9260 vs. 38/7168, RR=1.24; 95% CI= (0.76, 2.04)].

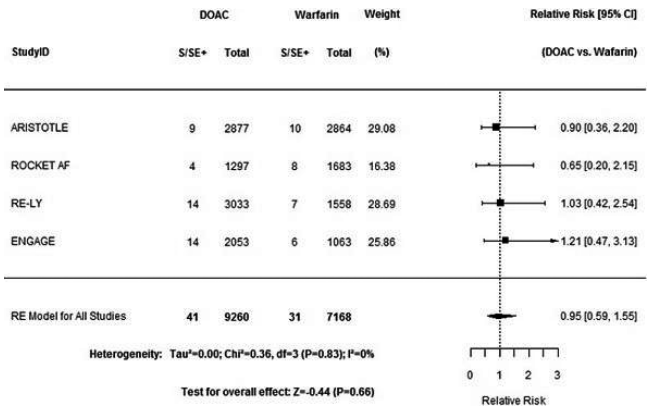

FIGURE 1 Forest Plot of Stroke/Systemic Embolism Outcomes (DOACs vs. Warfarin) under an Interrupted Perioperative Anticoagulation Strategy

In the uninterrupted strategy, DOACs had significantly fewer MB compared to Warfarin [97/4540 vs. 98/2985, RR=0.62, 95% CI= (0.47, 0.82)], and similar rates of SSE [29/4519 vs. 31/2971, RR=0.70, 95% CI= (0.41, 1.18)] and death [62/4457 vs. 54/2971, RR=0.77, 95% CI= (0.53, 1.12)].

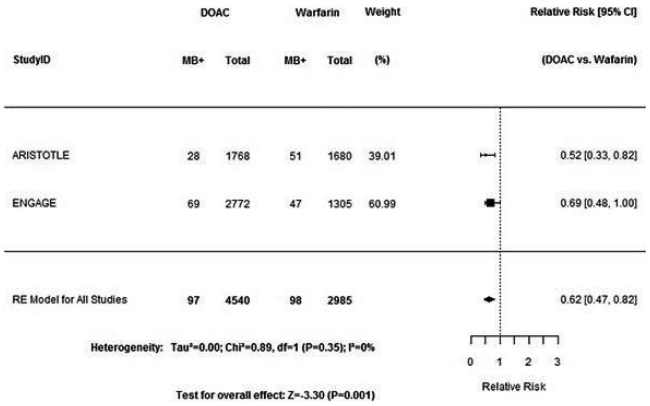

FIGURE 2 Forest Plot of Major Bleed Outcomes (DOACs vs. Warfarin) under an Uninterrupted Perioperative Anticoagulation Strategy

The studies were homogeneous (I^2= 0.0) for all calculated pooled associations except for the RR of death in the interrupted strategy (I^2= 26.3%).

Conclusions: The short-term safety and efficacy of DOACs and Warfarin are similar in AF patients perioperatively. Under an uninterrupted anticoagulation strategy, DOACs are associated with a 38% lower risk of MB compared to Warfarin.

OC 39.1 | Long-term Risk of Recurrent Venous Thromboembolism with and without Effective Antithrombotic Therapy

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Background: There is uncertainty about the appropriate duration of anticoagulation in patients with venous thromboembolism (VTE) and practice varies between countries.

Aims: To provide estimates of the risk of recurrent VTE, with and without effective antithrombotic therapy, according to the risk factor profile.

Methods: We used data from two large randomized trials (EINSTEIN-Extension and EINSTEIN-Choice) that compared rivaroxaban (20 mg or 10 mg) with placebo or aspirin (100 mg) for extended VTE treatment in patients who had already received 6 to 12 months of anticoagulation. The index VTE events were centrally classified using the following hierarchy:

1) unprovoked, or provoked with a

2) major permanent risk factor (i.e. cancer),

3) minor permanent risk factor (i.e. inflammatory bowel disease, lower extremity paralysis/paresis, congestive heart failure, BMI > 30, creatinine clearance < 50 ml/min, family history of VTE, hereditary/acquired thrombophilia),

4) minor transient risk factor (i.e. immobilization, travel > 8 h, hormonal therapy, pregnancy or puerperium, leg injury with impaired mobility, or

5) major transient risk factor (i.e. major surgery or trauma).

Results: A total of 2832 patients received rivaroxaban (10 or 20 mg) and 1721 received placebo or aspirin.

TABLE 1 Incidence of recurrent VTE according to risk factor profile of index event, n/N, %

	Rivaroxaban 10/20 mg N=2832		Placebo or aspirin N=1721	
Unprovoked VTE	19/1173	1.6%	46/711	6.5%
Provoked, major persisting risk factor	0/82	0%	3/65	4.6%
Provoked, minor persisting risk factor	18/1184	1.5%	35/714	4.9%
Provoked, minor transient risk factor	1/268	0.4%	8/177	4.5%
Provoked, major transient risk factor	0/125	0%	0/54	0%

Conclusions: Although patients with unprovoked VTE are at highest risk of recurrence, those with a minor persisting or transient risk factor also are at a high risk. In patients with these risk profiles, rivaroxaban substantially reduces the incidence of recurrence.

OC 39.2 | External Validation of the DASH Prediction Model: The TRIP (Thrombosis Research Italian Partnership) Collaboration

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[1]San Bortolo Hospital, ULSS 8 Berica, Hematology Department, Vicenza, Italy, [2]Thrombosis Centre, AOU Careggi, Florence, Italy, [3]University of Insubria, Department of Internal Medicine, Varese, Italy, [4]Thrombosis and Haemorragic Diseases Unit, IRCCS Humanitas Research Hospital, Milan, Italy, [5]Thrombosis and Hemostasis Center, Department of Immunohematology and Transfusion Medicine, Bergamo, Italy, [6]Institute of Hematology, Catholic University, Rome, Rome, Italy, [7]A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milan, Italy, [8]S.Orsola-Malpighi University Hospital, Department of Angiology and Blood Coagulation, Bologna, Italy, [9]Hemostasis and Thrombosis Center, AO Istituti Ospitalieri di Cremonas, Cremona, Italy, [10]Hemostasis and Thrombosis Center, AO Istituti Ospitalieri di Cremona, Cremona, Italy, [11]Malattie Cardiovascolari, Università di Bologna, Bologna, Italy

Background: The optimal duration of anticoagulant therapy after the first episode of unprovoked venous thromboembolism (VTE) is controversial. Several clinical prediction rules (CPR) have been developed, but few have been externally validated in an independent cohort.

Aims: We aimed at validating the DASH prediction model, in which a score ≤1 would indicate an annual recurrence risk < 5%, therefore potentially excluding the need of prolonged treatment. We were also willing to evaluate the DASH score in a predefined patient subgroup (elderly vs. younger patients).

Methods: Patients with a proximal unprovoked DVT or PE, who received a full course of VKA or DOAC (>3 months) and having D-dimer measured after treatment withdrawal were eligible. The DASH score was computed based on D-dimer after therapy withdrawal (considered positive when values exceeded a cut-off of 250 ng/ml DDU or 500 FEU), age< 50 at index event, male sex and hormone use at index event. Recurrent VTE events were symptomatic proximal or distal DVT/PE, and were analyzed with a time-dependent analysis. Observed 12 and 24-months recurrence rates were compared to recurrence rates predicted by the DASH model.

Results: We analyzed a total of 827 patients, of whom 100 (12.1 %) had an objectively documented recurrence. On average, recurrence risk factors were less represented than in the original DASH cohort, with a greater proportion of subjects having a „low-risk" (< =1) DASH score (66.3% vs. 51.6%, p< 0.001).

Figure 1, Panel A shows the observed vs. expected cumulative incidence at 2-years for all enrolled subjects, with a slope equal to 0.71 (95% CI 0.51- 1.45). Panel B shows the same data subjects >65 years (dotted line) vs. younger (continuous); c-statistic was lower for subjects >65 years (0.54) vs. younger ones (0.72).

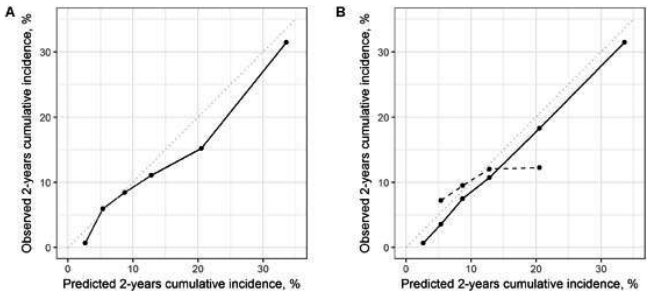

FIGURE 1 Panel A, all data; Panel B, stratified by age

Conclusions: These results confirm the validity of DASH prediction model, particularly in young subjects. The recurrence risk in elderly patients (>65 years) is >5% even with the lowest DASH scores.

OC 39.3 | Extended Anticoagulation with Two Doses of Rivaroxaban (20 mg and 10 mg) for Preventing Recurrent Venous Thromboembolism: A Benefit-risk Analysis of EINSTEIN CHOICE

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Background: Most patients with unprovoked venous thromboembolism (VTE) or with ongoing risk factors receive 6 to 12 months of anticoagulant therapy. The decision to extend therapy depends on the balance between the risk of recurrent VTE if treatment stops and the risk of bleeding if treatment continues. Information on this benefit-risk balance is limited.

Aims: The benefit-risk tradeoff of extended treatment with 20 mg or 10 mg of once daily rivaroxaban was assessed in patients with symptomatic VTE who had completed 6-12 months of anticoagulation and for whom there was equipoise regarding the need for extended anticoagulation.

Methods: One-year cumulative incidences were estimated for recurrent VTE and major bleeding with the Kaplan-Meier method. Benefits and risks were presented using the differences between treatment groups in a hypothetical population of 10,000 VTE patients followed for 1 year.

Results: A total of 1107 patients were treated with rivaroxaban 20 mg, 1127 with rivaroxaban 10 mg, and 1131 with aspirin. The cumulative incidence of recurrent VTE was 1.9% in patients in the rivaroxaban 20 mg group, 1.6% in patients in the rivaroxaban 10 mg group, and 5.0% in patients in the aspirin group. The cumulative incidences of major bleeding were 0.7%, 0.4% and 0.5% in the rivaroxaban 20 mg, rivaroxaban 10 mg, and aspirin groups, respectively. Compared with aspirin in a hypothetical population of 10,000 VTE patients followed for 1 year, treatment with 20 mg or 10 mg of rivaroxaban would result in 312 (95% confidence interval [CI], 145 to 479) and 341 (95% CI, 175 to 507) fewer recurrent VTE events (NNT=33 and 30, respectively) and in 28 (95% CI, -43 to 99) and 0 (95% CI, -60 to 59) more major bleeding events (NNH=356 and >10,000, respectively).

Conclusions: Extended anticoagulation with once daily rivaroxaban (20 mg or 10 mg) provides a clinically important benefit in terms of reduction in recurrent VTE, and both regimens have favorable benefit-risk profiles.

OC 39.4 | Prediction of Major Bleeding (MB) Risk in 2514 High Recurrence Risk Venous Thromboembolism Patients Treated Beyond 3 to 6 Months with Oral Anticoagulation Therapy (OAT)

P. Wells[1], M. Rodger[1], M. Kovacs[2], D. Anderson[3], S. Kahn[4], S. Solymoss[4], C. Kearon[5], V. Shah[6], D. Keeling[7], I. Chagnon[8], R. Zide[9], M. Carrier[1], R. Mallick[1], D. Corsi[1], G. Le Gal[1], S. Schulman[10], S. Kaatz[6]

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Background: A tool to predict the risk of MB in patients on extended OAT for venous thromboembolism (VTE), has not been developed. Identifying those with an annual MB rate over 3% is the target at which the risk of continued OAT exceeds benefit.

Aims: To develop a prediction rule for major bleeding that selects a clinically meaningful proportion of patients with a MB risk over 3%.

Methods: a multicentre, multinational prospective cohort study of extended OAT for unprovoked VTE, or provoked VTE with prior VTE. Cancer patients were excluded. Enrollment was after at least 3 months of OAT. All bleeding events were adjudicated. Univariate Cox proportional hazards analysis was used to determine the strength of association between each variable and risk of MB to permit selection of a subset of variables for the subsequent multivariate analysis. Of the 24 variables analysed 10 were found to be strongly associated with MB risk (P< 0.05) (Table 1). A multivariable Cox regression model was built using clinically important, easily measurable variables.

Results: 2514 patients enrolled with > 7100 years of observation. The mean age was 60 years, 64% were male, 92% Caucasian, mean BMI was 31, and 9% were on antiplatelet agents. Patients were followed for a mean of 2.8 years. 90% were on VKAs.121 patients (4.8%) experienced at least one MB episode. The annual rate of MB was 1.7 per 100 patient years of observation (rate constant). All variables in the derived model scored one except anemia (2) [Table 2]. 21% had a score of 2 (MB rate 3.1%, 95% CI=2.3 to 4.2%), 7% scored 3 (MB rate 4.5%, 95% CI=2.7 to 7.1%), and 1% scored ≥ 4 (MB 7.9%%, 95% CI=2.6 to 18.6%). All the fatalities from MB were in these high-risk groups. In the 71% at low risk the MB rate was 1.1%. All differences were statistically significant with p values < 0.0001. The C-index was 0.74.

Conclusions: Almost 30% of patients would score high risk (i.e. >3%) for MB and consider not continuing OAT. If validated (as planned prior to the ISTH) this could change practice.

TABLE 1 One. Significant variables in the univariate analysis

VARIABLE	NO MAJOR BLEED (N)	NO MAJOR BLEED (% OF ALL NO BLEED PATIENTS)	MAJOR BLEED (N)	MAJOR BLEED (% OF ALL MAJOR BLEED PATIENTS)	ABSOLUTE RISK (%)	HAZARD RATIO	LOWER 95% CI	UPPER 95% CI	P-VALUE
AGE > 65	893	37.3	68	57.6	7.1	2.29	1.60	3.28	<0.0001
FEMALE	849	35.4	58	49.2	6.4	1.8	1.26	2.57	0.0013
HYPERTENSION	886	37	61	51.7	6.4	1.74	1.21	2.48	0.0024
CYP2C9 *3 HETERO/ HOMO	239	10.4	21	19.3	8.1	1.93	1.20	3.10	0.0066
DVT AND PE	445	18.6	12	10.2	2.6	0.46	0.25	0.84	0.0112
ANTIPLATELET AGENT	209	8.7	23	19.5	9.9	2.32	1.47	3.65	0.0003
CREATININE CLEARANCE < 50	146	6.6	22	19.6	13.1	3.26	2.05	5.18	<0.0001
HEMOGLOBIN < 100	32	1.4	4	3.4	11.1	2.86	1.06	7.76	0.0389
STATIN USE	636	26.6	48	41	7.0	1.96	1.36	2.82	0.0003

TABLE 2 Two. Major bleeding by score in final model

POINTS	# OF PATIENTS	% OF PATIENT POPULATION	# OF MB EVENTS	MAJOR BLEED RATE PER 100 PT YEARS	LOWER 95% CI	UPPER 95% CI
0 OR 1	1638	71	51	1.07	0.8	1.4
2	485	21	40	3.1	2.2	4.2
3	158	7	19	4.5	2.7	7.1
4	24	1	5	8.0	2.6	18.6
5	5	0	0	0		

VARIABLES USED INCLUDE HEMOGLOBIN < 100, AGE > 65 YEARS, USE OF ANTIPLATELET AGENT, CREATININE CLEARANCE < 50 AND HYPERTENSION

OC 39.5 | External Validation of the VTE-BLEED Score for Predicting Major Bleeding in Stable Anticoagulated Patients with Venous Thromboembolism

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Background: One of the main determinants of establishing the optimal treatment duration of patients with venous thromboembolism (VTE) is the risk of major bleeding during long-term anticoagulant therapy. The 6-variable VTE-BLEED score was recently developed to enable estimation of this bleeding risk.

Aims: This study aimed at externally validating VTE-BLEED.

Methods: This was a post-hoc study of the randomized, double-blind, double-dummy, HOKUSAI-VTE study that compared edoxaban versus warfarin. VTE-BLEED was calculated in all 8,240 study patients. The number of adjudicated major bleeding events during ‚stable anticoagulation', i.e. occurring after day 30, in patients with low (total score < 2 points) and high risk of bleeding (total score ≥2 points) were compared for the overall study population, patients randomized to edoxaban or warfarin, and for important patient subcategories.

Results: During ‚stable' anticoagulation, major bleeding occurred in 1.02% (40/3,903) and 0.82% (32/3,899) of patients treated with warfarin and edoxaban respectively. For the overall study population, the risk of bleeding in the low and high risk groups were 0.51% and 2.03% respectively, for an Odds Ratio of 4.04 (95%CI: 2.51-6.48). Odds Ratios were 2.53 (95%CI: 2.15-2.99) and 1.35 (95%CI: 1.16-1.58) for warfarin and edoxaban respectively. VTE-BLEED was consistently able to identify patients at a 2.5 to 11-fold higher bleeding risk across all the predefined subcategories as well as for the treatment period between day 30 from enrollment to day 180, and beyond day 180.

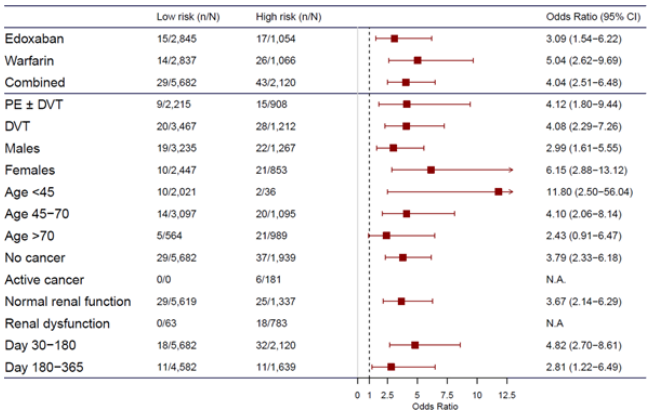

FIGURE 1 Performance of VTE-BLEED in the predefined cohorts and subcategories during ´stable´ anticoagulation

Conclusions: Patients identified as high risk by VTE-BLEED had a 4-fold increased risk of bleeding during the chronic phase of treatment.

OC 52.1 | Evaluation of Interventions for Implementation of Thromboprophylaxis in Hospitalized Patients at Risk for Venous Thromboembolism (VTE): An Updated Cochrane Systematic Review and Meta-analysis of Randomized Controlled Trials (RCT)

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Background: While numerous RCTs have shown that the use of thromboprophylaxis (TP) in hospitalized patients at risk for VTE is safe, effective, and cost-effective, TP remains underutilized. System-wide interventions may be more effective to improve the use of TP than relying on individual providers' prescribing behaviors.

Aims: In this update to our 2013 Cochrane review, we aimed to determine the effectiveness of various system-wide interventions designed to increase the use of TP in hospitalized medical and surgical patients at risk for VTE, focusing on RCTs only.

Methods: We conducted a systematic literature search to identify RCTs assessing interventions designed to increase use of TP and/or decrease incidence of VTE in hospitalized patients. We searched Medline, Embase, BIOSIS, CINAHL, Cochrane, Web of Science, LILACS, clinicaltrials.gov, CENTRAL and DARE from inception to July 2015. Data were extracted on study design, setting, intervention, and outcomes, including proportions receiving prophylaxis (RP). We categorized the interventions into 4 groups: education, any alerts, computer alerts, and multifaceted interventions. We performed a random effects meta-analysis if 3 or more studies were available for an intervention group and an outcome. Heterogeneity was assessed using I^2 statistic.

Results: Among 12,920 records identified by our search, 16 RCTs were assessed for eligibility and 11 RCTs (N= 55,197 patients) were included in our review. Among the RCTs, there were sufficient data to pool one outcome (RP) for 3 intervention types (any alerts, computer alerts, and multifaceted (Table).

Conclusions: We found statistically significant improvements in prescription of prophylaxis associated with any alerts and computer alerts. The results of our review will help physicians, hospital administrators and policy makers make practical decisions about adoption of specific system-wide measures to improve prevention of VTE.

Funded by: Canadian Institutes of Health Research.

TABLE Meta-Analysis Results

Intervention	Outcome	Number of Studies	Number of Patients	Risk Difference (RD)(95% CI)	I^2 Statistic for RD	Relative risk (RR) (95% CI)
Any Alerts*	Received Prophylaxis	5	6,523	0.20 [0.15, 0.24]	65%	1.80 [1.37, 2.36]
Computer Alerts*	Received Prophylaxis	3	3,890	0.18 [0.16, 0.21]	0%	1.96 [1.46, 2.64]
Multifaceted **	Received Prophylaxis	5	25,742	0.02 [-0.00, 0.04]	0%	1.04 [0.97, 1.10]

Interventions were categorized into 4 groups: education (e.g., grand rounds, self-administered course), any alerts (e.g., electronic, human), computer alerts, (i.e., electronic alert only), and multifaceted interventions (e.g., combination of education, audit and feedback, and alert). *Clustered trials did not provide sufficient data (intra-cluster correlation (ICC) or adjusted confidence intervals) for us to pool cluster adjusted estimates. **ICCs were available for 4/5 trials included in this meta-analysis. An ICC of 0.00 was used for the 5th trial. All trials in the meta-analysis were clustered designs.

OC 52.2 | Extended Venous Thromboembolism Prophylaxis Comparing Rivaroxaban to Aspirin Following Total Hip or Knee Arthroplasty (EPCAT II)

D. Anderson[1], M. Dunbar[1], S. Kahn[2], S. Pleasance[3], P.-A. Vendittoli[4], J. Murnaghan[5], D. Zukor[6], P. Gross[7], M. Forsythe[8], S. Pelet[9], W. Fisher[2], E. Belzile[10], S. Dolan[11], M. Crowther[12], E. Bohm[13], S. MacDonald[14], W. Gofton[15], P. Kim[15], P. Andreou[1], S. Doucette[1], C. Theriault[1], A. Abianui[3], M. Carrier[15], D. Coyle[15], M. Kovacs[14], M. Rodger[15], P. Wells[15]

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Background: Deep vein thrombosis (DVT) and pulmonary embolism (PE) are common complications post total hip (THA) and total knee arthroplasty (TKA). Prophylaxis with anticoagulants such as rivaroxaban extended beyond hospital discharged is commonly prescribed. We reasoned aspirin (ASA) would be an attractive alternative for extended prophylaxis because of its potential efficacy, low cost, convenience and few side effects.

Aims: To determine if ASA was as effective and safe as rivaroxaban for extended prophylaxis in patients already having received five days rivaroxaban post THA or TKA.

Methods: This was a 15 centre, double-blind, randomized controlled trial following THR or TKR. All patients received rivaroxaban 10mg orally once daily until post-operative day 5 and then were randomized to continue rivaroxaban 10 mg daily or aspirin 81 mg daily. Prophylaxis was continued for an additional 9 days following TKA or 30 days following THA. Patients were followed for 90 days for the development of symptomatic proximal DVT and PE or bleeding complications. No screening tests were performed.

Results: 3424 patients were included in the intention to treat analysis. This included 1804 patients undergoing THA and 1620 patients receiving TKA. Mean age was 62.8 and 47.8% of patients were male. 12 (0.70%) patients randomized to rivaroxaban experienced DVT or PE compared with 11 (0.64%) of patients randomized to ASA (-0.06%, 95% CI -0.66% to 0.55%; P < 0.0001 for non-inferiority and 0.84 for superiority). 17 (0.99%) of patients receiving rivaroxaban and 22 (1.3%) of patients receiving ASA experienced clinically important bleeding (0.30%, 95% CI 1.07% to -0.47%; P = 0.43). 5 (0.29%) patients receiving rivaroxaban and 8 (0.47%) of patients receiving aspirin experienced major bleeding complications (0.18%, 95% CI 0.65% to -0.29%; P = 0.42).

Conclusions: For patients receiving five days of rivaroxaban following THR or TKR, extended prophylaxis with ASA was at least as effective and safe as rivaroxaban.

OC 52.3 | A landmark analysis of the APEX study comparing extended duration betrixaban with standard duration enoxaparin in hospitalized medically ill patients

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Background: Medically ill patients are at elevated risk for venous thromboembolism (VTE), with the majority of VTE events occurring after hospital discharge. While standard duration in-hospital thromboprophylaxis decreases the risk of in-hospital VTE, subsequent out of hospital thrombotic events occur when thromboprophylaxis is discontinued after discharge.

Aims: The aim of this APEX sub-analysis is to evaluate the efficacy and safety of extended duration betrixaban vs standard duration enoxaparin in the occurrence of symptomatic VTE (symptomatic DVT, non-fatal PE, and VTE related death) and major bleeding during three separate time periods throughout the study: the standard prophylaxis period, the extended betrixaban period, and the post-betrixaban follow-up period.

Methods: The survival analysis was performed according to the treatment periods and events in previous periods were censored from subsequent ones.

Results: During the standard prophylaxis period, VTE event rates were similar in the betrixaban and enoxaparin arms (HR=0.67, 95% CI=0.24-1.89, p=0.45). Administration of extended duration betrixaban was associated with a decrease in symptomatic events compared to enoxaparin following the standard prophylaxis period through day 35 (betrixaban = 25, enoxaparin = 42, HR = 0.61 (0.38-0.98), p = 0.041) and through day 42 (betrixaban = 27, enoxaparin = 44, HR = 0.61, 95% CI = 0.38-0.99, p = 0.041), as well as the post-betrixaban follow-up period (betrixaban = 2, enoxaparin = 12, HR = 0.17, 95% CI = 0.04-0.74, p = 0.007). No increase in major bleeding was seen at any time point, Table.

Conclusions: Compared to standard duration enoxaparin, betrixaban had similar VTE and major bleeding rates in the standard prophylaxis period. Extended duration thromboprophylaxis with betrixaban significantly reduced symptomatic VTE following hospital discharge in the extended prophylaxis period as well as following discontinuation of study drugs, consistent with a legacy effect, and was not associated with increased major bleeding.

TABLE Symptomatic VTE and Major Bleeding (Kaplan Meier Analysis Hazard Ratios) for the three time periods

	Betrixaban	Enoxaparin	Hazard Ratio (95% CI)	p-value
Standard Prophylaxis Period (Day 1-14)				
Symptomatic VTE	6/3714	9 /3716	0.67 (0.24-1.89)	0.45
Major bleeding	9/3714	9/3716	1.07 (0.43-2.71)	0.88
Extended Duration Betrixaban Period (Day 6-35)				
Symptomatic VTE	25/3661	42/3658	0.61 (0.38-0.98)	0.041
Major bleeding	16/3645	12/3632	1.33 (0.63-2.81)	0.45
Follow-up period, no prophylaxis (Day 35 to 77)				
Symptomatic VTE	2 /3495	12/3493	0.17 (0.04-0.74)	0.007
Major bleeding	0/3488	0/3510	-	-

OC 52.4 | Rivaroxaban Use in Extensive Superficial Thrombophlebitis

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Background: Superficial thrombophlebitis (STP) of the legs is now recognised as venous thromboembolic disease. Anticoagulation is required to prevent thromboembolic complications. Data regarding the use of the direct oral anticoagulant drugs in extensive STP is currently unavailable.

Aims: The primary aim was to validate the efficacy of treatment with rivaroxaban in patients with extensive STP. The secondary aim was to assess bleeding complications.

Methods: Over a 24 month period (November 2014 - November 2016) patients with extensive STP were identified using our DAWN VTE database. Extensive STP was defined as being greater than 5cm in length and more than 3cm from the deep venous system. These patients were treated with either low molecular weight heparin (LMWH) or rivaroxaban 15mg once daily for 6 weeks (Rx). Treatment failure was defined as progression to deep vein thrombosis (DVT), non-resolution requiring further treatment or STP recurrence within 100 days. Bleeding complications were defined as a bleeding episode leading to hospital admission or drop in mean haemoglobin level.

Results: 281 patients had extensive STP. 184 (65.5%) were treated with rivaroxaban, 91 (32.4%) were treated with LMWH. 6 patients receiving warfarin are excluded. 16 patients (8.6%) in the rivaroxaban group met the criteria for treatment failure compared to 19 patients (20.9%) in the LMWH group: progression to DVT (n= 8, 1 Rx vs 7 LMWH), non-resolution with need for further treatment of STP (n= 11, 7 Rx vs 4 LMWH), recurrence within 100 days (n=16, 8 Rx vs 8 LMWH). There is a statistically significant difference between the groups for treatment failure (p=0.004). There were no reported bleeding complications in either group.

Conclusions: The use of rivaroxaban appears to be as safe and effective in the treatment of extensive STP as low molecular weight heparin from our small study. It simplifies treatment and represents a saving in drug costs and medical resource.

OC 52.5 | Relationship between DOAC Levels and Subsequent Thromboembolic Events

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Background: Apixaban, dabigatran and rivaroxaban (DOAC) are administered at fixed dose in relation to clinical indications, individual characteristics and renal function without need of laboratory monitoring. Nevertheless, DOAC plasma concentrations show a high inter-individual variability both at trough and peak level. A post-hoc analyses of phase III trials indicates a relationship between DOAC plasma levels, as measured at trough, and thrombotic and bleeding complications in the follow up period. Thus, it might be informative to check for DOAC levels at steady state in real world patient population to explain bleeding/thrombotic events.

TABLE 1 Clinical characteristics and Ctrough drug levels in the study population

	Dabigatran	Rivaroxaban	Apixaban
Pts (n°)	163	153	194
Age (yr)mean ±SD	75.6±8.7	74.5±6.6	76.3±6.7
Gender (M/F)	91/72	95/58	103/91
CHADS2 Score mean±SD	2.3±1.0	2.4±1.3	2.2±1.1
Events n° (%)	5 (3) - 4 stroke - 1 IMA	3 (1.9) - 2 IMA - 1 TIA	2 (1.0) - 1 DVT - 1 Systemic embolism
Biological variability (all patients) Ctrough (ng/ml) mean (min-max)	90.4 (0.05-324)	48.9 (0.00-337)	122.5 (7.9-342)
Drug levels in patients with thrombosis Ctrough (ng/ml) mean (min-max)	55.2 (13.9-91.0)	25.9 (23.0-28.0)	78 (45.0-112)

TABLE 2 Thromb\oembolic complications and Ctrough drug levels

	Drug	Thromboembolic Complication	DOAC level Ctrough (ng/ml) mean (min-max)
Pt 1	dabigatran	Stroke	13
Pt 2	dabigatran	Stroke	40
Pt 3	dabigatran	Stroke	53
Pt 4	dabigatran	Stroke	78
Pt 5	rivaroxaban	TIA	26
Pt 6	dabigatran	IMA	91
Pt 7	rivaroxaban	IMA	23
Pt 8	rivaroxaban	IMA	28
Pt 9/Pt 10	apixaban	Systemic embolism/DVT	112/44

Aims: To assess the relationship between DOAC trough anticoagulant level, measured within the first month of treatment, and thromboembolic events observed during 1 year follow up.

Methods: Consecutive naïve patients with Non Valvular Atrial Fibrillation referred to 4 Italian Anticoagulation Clinics, were enrolled in the study for the START-Laboratory Register. Age, $CHADS_2$ Score, HAS-BLED, kidney and liver function, concomitant medications were recorded. DOAC measurement at trough after 2 weeks from starting the drug and within the first month of treatment and clinical follow up were entered into a structured data base.

Diluted thrombin time calibrated for dabigatran, anti-FXa calibrated for rivaroxaban or apixaban were performed to determine plasma drug concentration.

Results: Between September 2015 and September 2016, 510 consecutive patients (163 dabigatran, 153 rivaroxaban, 194 apixaban) were enrolled. Main clinical characteristics, DOAC levels and thrombotic complications are in tables 1-2. We observed 10 thrombotic events (2%), all occurred after the first 6 months of treatment. All events were recorded in patients with drug trough levels below the mean value of biological variability, calculated for each drug.

Conclusions: Our data show a relationship between low DOAC levels and thrombotic events. Further prospective analysis will be needed to confirm this preliminary observation.

OC 60.1 | Short and Long Term Incidence of and Risk Factors for Post Thrombotic Syndrome after a First Deep Vein Thrombosis

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Background: The reported short-term incidences of post-thrombotic syndrome (PTS) after deep vein thrombosis (DVT) vary and the long-term incidence is unknown. Furthermore, studies reporting on risk factors for PTS are contradictory.

Aims: To assess the 0 to 1- and 1 to 7-year PTS cumulative incidence (CInc) in patients with a first DVT, and to evaluate the effect of several risk- and treatment factors.

Methods: Patients with a first DVT of the lower limb included in the MEGA study completed a questionnaire 1 and 7 years after the event. PTS was assessed by a clinical classification score based on the Villalta score. The CInc of PTS between 0 to 1- and 1 to 7-year was determined. The effect of possible risk factors was assessed by determining risk ratios (RR), adjusted for confounders if appropriate.

Results: Questions regarding PTS were completed by 1657 out of 3306 patients in the first questionnaire; 361 patients were diagnosed with PTS, for a 1-year CInc of 21.8% (95%CI 19.9-23.8). Of the 1244 patients without PTS at 1 year who completed the second questionnaire, 117 additional patients developed PTS, for a 9.4% 1-7 year CInc (95%CI 7.9-11.2). At 1 year, the RR for women vs. men was 1.5 (95%CI 1.2-1.9); for height < 165cm vs. 165-180 cm 1.5 (95%CI 1.2-1.9) and for 180-195cm vs. 165-180cm 1.3 (95%CI 1.0-1.7); for weight 85-100kg vs. 60-85kg 1.4 (95%CI 1.1-1.7) and for >100kg vs. 60-85kg 1.5 (95%CI 1.2-2.0). At 7 years, the same association was found for female sex and weight but not for height (*table 1).* Patients with PTS had more often been treated with oral anticoagulation for a period of >180 days and wore elastic compression stockings more frequently than patients without PTS (*table 2).* Provoked/unprovoked DVT, thrombus location, pregnancy, hormone use, factor V Leiden, prothrombin 20210A and FXIII mutation did not affect risk of PTS, either at 1 or 7 years.

Conclusions: The risk of PTS remains substantial up to seven years after a first venous thrombosis, and is highest in women and overweight individuals.

TABLE 1 Risk factors associated with PTS 1 and 7 years after DVT diagnosis. *Adjusted for age and sex. # Adjusted for sex, age and height

	1 year, n	PTS, n	RR (95%CI)	RR adjusted (95%CI)*	7 years, n	PTS, n	RR (95%CI)	RR adjusted (95%CI)*
Woman	872	234	1.7 (1.4-2.0)	1.5 (1.2-1.9)	644	72	1.5 (1.0-2.1)	1.4 (0.9-2.0)
Height (cm) <165	203	68	1.6 (1.3-2.1)	1.5 (1.2-1.9)	111	11	1.0 (0.6-1.9)	0.9 (0.5-1.8)
165-180	892	183	1 (ref)	1 (ref)	711	69	1 (ref)	1 (ref)
180-195	480	90	0.9 (0.7-1.1)	1.3 (1.0-1.7)	369	29	0.8 (0.5-1.2)	1.0 (0.6-1.7)
>195	34	7	1.0 (0.5-2.0)	1.4 (0.7-2.8)	13	1	0.8 (0.1-5.3)	1.0 (0.1-7.0)
Weight (kg) <60	79	19	1.2 (0.8-1.2)	1.0 (0.7-1.6)#	53	1	0.3 (0.0-1.7)	0.2 (0.0-1.4)#
60-85	847	169	1 (ref)	1 (ref)	640	50	1 (ref)	1 (ref)
85-100	461	101	1.1 (0.9-1.4)	1.4 (1.1-1.7)#	357	41	1.5 (1.0-2.2)	1.7 (1.2-2.6)#
>100	224	63	1.3 (1.0-1.7)	1.5 (1.2-2.0)#	154	17	1.4 (0.8-2.4)	1.7 (1.0-2.8)#

TABLE 2 Treatment factors associated with PTS. *Adjusted for age and sex

	1 year, n	PTS, n	RR (95%CI)	RR adjusted (95%CI)*	7 years, n	PTS, n	RR (95%CI)	RR adjusted (95%CI)*
Duration of oral anticoagulation ≤90 days	60	8	0.7 (0.4-1.3)	0.7 (0.4-1.3)	39	4	1.3 (0.5-3.4)	1.4 (0.5-3.6)
90-180 days	820	157	1 (ref)	1 (ref)	652	52	1 (ref)	1(ref)
180-365 days	595	149	1.3 (1.1-1.6)	1.4 (1.1-1.6)	440	47	1.3 (0.9-1.9)	1.4 (0.9-2.0)
>365 days	181	47	1.3 (1.0-1.8)	1.4 (1.1-1.9)	113	14	1.6 (0.9-2.7)	1.6 (0.9-2.8)
Frequency of elastic compression stockings use always	1079	243	1 (ref)	1 (ref)	802	90	1 (ref)	1 (ref)
most of the time	188	46	1.1 (0.8-1.4)	1.0 (0.8-1.35)	65	11	1.5 (0.9-2.7)	1.5 (0.9-2.7)
sometimes	100	31	1.4 (1.0-1.9)	1.3 (1.0-1.8)	41	4	0.9 (0.3-2.3)	0.9 (0.3-2.2)
never	280	39	0.6 (0.5-0.8)	0.6 (0.5-0.9)	292	10	0.3 (0.2-0.6)	0.3 (0.2-0.6)

OC 60.2 | Rivaroxaban Reduces the Rate of Post-Thrombotic Syndrome After DVT - a Cross-Sectional Study Comparing Rivaroxaban to Warfarin

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Background: Post thrombotic syndrome (PTS) is a common sequel after DVT. Poor quality treatment with Vitamin K antagonists (warfarin) is a risk factor for PTS.

Aims: To assess whether treatment with a direct oral factor Xa inhibitor, rivaroxaban, may lower the rate of PTS as compared to warfarin treatment.

Methods: In a cross sectional study we compared the 2-year rate of PTS in patients treated with rivaroxaban versus conventional anticoagulation with enoxaparin and warfarin in 309 patient with first time DVT. PTS was assessed using Patient Reported Villalta Scale (Utne et al Thromb Haemost, 115 (2), 361-7) 24 (+/- 6) months after DVT. PTS was defined as a score >4 points. Health-related quality of life (HRQoL) was assessed by EQ-5D and VEINES-QOL/Sym. Chi square test was performed to compare the rate of PTS between the two groups. Multiple logistic regression was performed to adjust for possible confounders. Regional etics committee approved the study.

Results: Mean age of 309 patients was 61 years (SD 14). Median observation time from diagnosis of DVT (index event) to study inclusion was 25 (interquartile range (IQR) 23-29) months. One hundred and sixty-one (52%) patients were treated with rivaroxaban and 148 (48%) with enoxaparin/warfarin. The prevalence of PTS was 45% versus 59% in patients treated with rivaroxaban versus enoxaparin/warfarin, respectively (p = 0.01). The difference in range of PTS between the two treatment groups remained significant (OR 0.5, 95% CI: 0.3-0.9; p=0.02) in the multivariate analysis after adjustment for possible confounders. Patients treated with rivaroxaban had better HRQoL as assessed by both EQ-5D index value (p=0.05), EQ-VAS (p=0.003), and by VEINES QOL/Sym (p=0.002/0.004).

TABLE Rate and severity of post-thrombotic syndrome according to the Patient Reported Villalta scale

PTS (n%)	Warfarin (n%)	Rivaroxaban (n%)
No	61 (41)	89 (55)
Mild	46 (31)	35 (21)
Moderate	26 (18)	28 (17)
Severe	15 (10)	9 (6)

Conclusions: Treatment of acute DVT with rivaroxaban resulted in statistically significant lower risk of developing PTS and better HRQoL as compared to treatment with enoxaparin/vitamin K antagonist.

OC 60.3 | Development and Validation of a Practical Two-step Prediction Model for Post-thrombotic Syndrome

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Background: Post-thrombotic syndrome (PTS) occurs in 20-50% of patients following deep vein thrombosis (DVT). Optimizing preventive therapy requires identification of patients at risk for PTS.

Aims: To develop a prediction model for PTS for use in the acute phase of DVT, and secondary use 6 months after the DVT.

Methods: Data from a cohort of 479 DVT patients from the Maastricht University Medical Center+ were used in multivariate logistic regression analyses. The obtained models were internally validated using standard bootstrapping techniques. Indices of the overall performance, discriminative ability, calibrations and characteristics of the models were determined. All analyses were done according to current ISTH definition, as well as the Prandoni definition of PTS.

Results: Depending on PTS definition (ISTH followed by Prandoni), PTS occurence at 6 months was 33.3% and 13.0%, and at 24 months 49.9% and 24.6%. Variables in the baseline model were: age, body mass index, gender, varicosities, DVT recurrence, smoking status and thrombus location. In the secondary model additional variables were residual thrombosis and spontaneous DVT. After internal validation the AUCs were 0.67 and 0.72 for baseline models, and 0.62 and 0.72 for secondary models, all indicating good discriminative ability. Calibration plots showed well calibrated predictions. Efficiency was greatest for the models using the Prandoni definition. At a threshold of 15%, 313/451(69,4%) patients were allocated to a low risk for PTS at the cost of 20/313 (6,4%) missed cases. For the ISTH definition this was 41/451(9.1%) at the cost of 2/41 (4,9%) missed cases.

Conclusions: A model based on readily accessible baseline clinical characteristics can accurately predict the baseline risk for developing PTS at 6 months after DVT. Additional clinical characteristics accurately predict the PTS risk in sub-acute DVT. Risk prediction is more accurate and efficient when the Prandoni definition is used.

OC 60.4 | Development of a Clinical Prediction Model for the Post-Thrombotic Syndrome in a Prospective Cohort of Patients with Proximal DVT: The SOX-PTS Index

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Background: Post-thrombotic syndrome (PTS) is a chronic complication that develops in 20-50% of patients after deep vein thrombosis (DVT). While individual PTS risk factors have been identified, the ability to predict which DVT patients are likely to develop PTS remains limited.

Aims: To develop a clinical prediction index for PTS in patients with proximal DVT.

Methods: The derivation cohort consisted of participants in the SOX trial, a randomized placebo controlled trial of elastic compression stockings vs. placebo stockings to prevent PTS after a first proximal DVT. Outcome was the occurrence of PTS using Ginsberg's criteria. Baseline characteristics evaluated as potential predictors of PTS included sex, age, BMI, ethnicity, anatomic extent of DVT, type of DVT (unprovoked vs. provoked), baseline Villalta score severity category, smoking, right vs. left leg DVT and family history of VTE. Potential predictors were evaluated in a multivariable logistic regression analysis with backward selection. Multiple imputation was done for missing outcome data. Final model performance was assessed for discrimination using the c-index and for calibration using calibration plots. Internal validation was done via nonparametric bootstrapping.

Results: The final model includes 3 independent predictors and has a range of possible scores from 0-4 (Table1; Figure1]). High-risk predictors were:

1) index DVT in iliac vein;

2) BMI>35 kg/m^2; and

3) moderate-severe Villalta severity category at DVT diagnosis.

Compared to patients with a score of 0, those with a score of 4 had an odds ratio of 5.9 (95% CI 2.1-16.6) for developing PTS.

TABLE 1 Clinical prediction index for PTS development in patients with first proximal DVT

Predictor Variables	OR for PTS	95% CI	Points
Iliac DVT	1.96	1.08-3.57	1
Non iliac DVT	1.00		0
BMI≥35	2.17	1.27-3.72	2
BMI<35	1.00		0
Baseline Villalta score category >14 (severe)	2.64	1.41-4.96	2
Baseline Villalta score category 10-14 (moderate)	2.00	1.21-3.29	1
Baseline Villalta score category 0-9 (none or mild)	1.00		0

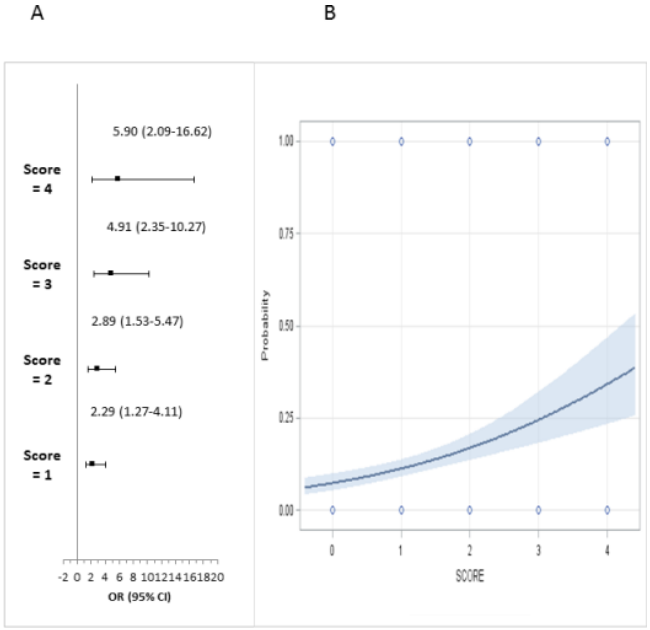

FIGURE 1 A. Odds ratios for PTS according to final score. B. Predicted probabilities with 95% CI according to maximum likelihood estimates

Conclusions: To our knowledge, this is the first clinical prediction index for PTS. We identified three independent predictors that, when combined, predicted risk for PTS after a first proximal DVT. The SOX-PTS index requires external validation before it can be considered for clinical use to inform prognosis or help select DVT patients for measures to prevent PTS.

OC 60.5 | No Compression, Multilayer Compression Bandaging or Compression Hosiery in the Acute Phase of Deep Vein Thrombosis in Relation to the Villalta Score, Health-related Quality of Life, and Costs at 3 Months after the Diagnosis

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Background: Deep vein thrombosis (DVT) is a serious condition that affects quality of life and is associated with substantial costs ,especially if its complication post-thrombotic syndrome (PTS) occurs. The effectiveness of compression therapy in the acute phase of DVT is undetermined.
Aims: To assess the impact of compression therapy in the acute phase of DVT on Villalta score, quality of life, and costs.
Methods: A cohort of 864 patients with proximal DVT (nested within the IDEAL DVT study), received multilayer compression bandaging (MCB), compression hosiery (CH) or no compression (NC) in the acute phase of DVT. Signs and symptoms were assessed by the subjective, objective and total Villalta score three months after DVT, a lower score indicating less signs and symptoms. Six weeks and three months after DVT diagnosis, quality of life was measured using the EQ-5D, SF6D and VEINES-QoL. Utility and summary scores were calculated as well as costs.
Results: MCB and CH had lower objective Villalta scores than the NC group (1.47+1.57 and 1.60+1.67 vs 2.21+2.14) p0.000. The subjective and the total Villalta scores were similar across the groups. Six weeks after diagnosis, HRQOL was better in the CH group (EQ-5D 0.86±0.18, SF6D 0.77±0.13, VEINES-QoL 51.47±9.45) compared to MCB (EQ-5D 0.81±0.31, SF6D 0.74±0.13, VEINES-QoL 49.50±10.2),p0.027, p0.008, p0.022 and NC (EQ-5D 0.81±0.24, SF6D 0.73±0.12(p0.002), VEINES-QoL 49.06±9.45(p0.016)). At three months, the CH group performed better on SF6D (0.80±0.12) compared to NC (0.78±0.12) p0.049. Mean therapy costs per patient were highest in the MCB group, followed by HC, and NC.
Conclusions: Compression significantly reduced the objective Villalta by 15%. CH was associated with better quality of life than NC and MCB. Therapy costs are substantially lower with CH than MCB; therefore compression with CH seems to be the preferred option. Implications of lower objective Villalta score at three months as a result of MCB and CH for the development of PTS at a later stage have to be awaited.

OC 73.1 | VTE Risk Factors in an Irish Urban Obstetric Population

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Background: Risk factors for pregnancy-associated venous thromboembolism (VTE) are well reported. However, the prevalence of VTE risk factors in our population is less well described. A deep understanding of the burden of VTE risk within a population is essential when developing and implementing strategies to prevent pregnancy-associated VTE.
Aims: To estimate the prevalence of risk factors for postpartum VTE in an Irish urban population.
Methods: Data were extracted from electronic VTE risk assessments which were routinely completed after delivery in the Rotunda Hospital, Dublin, between Sept 2014 and Dec 2016. Data were imported into SPSS Statistical Software for analysis. Descriptive statistics were used to explore the prevalence of VTE risk factors among the population.
Results: We studied VTE risk factors in 16,218 women, representing 83% of women who attended our institution in the study period. The majority of women (n=13,239, 82%) had at least one risk factor for VTE (Range=0-8, median=2). Half of the women assessed had 2 or more VTE risk factors and therefore would warrant prophylactic anticoagulant therapy according to recent guidelines from the Royal College of Obstetricians and Gynaecologists.

TABLE 1 Number of VTE risk factors recorded in postpartum VTE risk assessments in the Rotunda Hospital (Sept 2014-Dec 2016)

Number of risk factors	Number of women (%) (n=16,218)
0	2979 (18.4%)
1	5081 (31.3%)
2	4594 (28.3%)
3	2474 (15.3%)
4	774 (4.8%)
5	241 (1.5%)
6	54 (0.33%)
7	16 (0.1%)
8	5 (0.03%)

BMI >25kg/m^2, age over 35 years, parity greater than 3, caesarean delivery and instrumental delivery were the most common VTE risk factors recorded (Table 2). In women deemed to be at highest risk of VTE, additional risk factors included post-partum haemorrhage, delivery less than 37 weeks gestation, prior history of VTE and stillbirth. In 16% of women (n=2,663), VTE risk only arose in the intra-partum or post-partum period.

Conclusions: Risk factors for VTE are common and VTE risk is dynamic. Repeated VTE risk assessment is essential throughout pregnancy and into the postpartum period to ensure risk is accurately assessed. Thresholds for initiating prophylactic anticoagulation in the postpartum period must take into account the burden of VTE risk within the population.

TABLE 2 Frequency of VTE risk factors recorded in postpartum VTE risk assessments in the Rotunda Hospital (Sept 2014-Dec 2016)

Patient Related VTE Risk Factors	Number of women (n=16,218)	% (95% CI)	Pregnancy Related VTE Risk FactorS	Number of women (n=16,218)	% (95% CI)	Delivery Related VTE Risk Factors	Number of women (n=16,218)	% (95% CI)
BMI 25kg/m2 or above	5593	34.5% (34.1-34.8)	Parity 3 or above	2989	18.4% (18.2-18.7)	Caesarean Section	5025	31.0% (30.7-31.3)
Age 35 years or above	5380	33.2% (32.8-33.5)	IUGR	256	1.6% (-)	Instrumental Delivery	2486	15.3% (15.1-15.5)
Smoker	1048	6.5% (6.4-6.6)	Multiple Pregnancies	244	1.5% (-)	Delivery at less than 37 weeks´ gestation	806	5.0% (4.9-5.0)
Varicose Veins	298	1.8% (1.8-1.9)	Pre-eclampsia	212	1.3% (-)	PPH 1000ml or transfusion	513	3.2% (3.1-3.2)
High Risk Family History	28	1.8% (1.7-1.8)	Antenatal LMWH indicated	68	0.4% (-)	Prolonged labour	292	1.8% (-)
Significant Medical Co-morbidities	247	1.5% (-)				Manual removal of placenta	204	1.3% (1.2-1.3)
Immobility	214	1.3% (-)				Stillbirth	81	0.5% (-)
Prior VTE	84	0.5% (-)				Systemic Infection	76	0.5% (-)
Thrombophilia	67	0.4% (-)	VTE= Venous Thromboembolism, BMI = Body Mass Index, IUGR=Intrauterine Growth Restriction, PPH=Postpartum Haemorrhage, LMWH = Low Molecular Weight Heparin					

OC 73.2 | Low Persistence to Direct Oral Anticoagulants at Routine Treatment for Acute Venous Thromboembolism

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Background: Recently, direct oral anticoagulants (DOACs) became the treatment of choice for venous thromboembolism (VTE) in the Netherlands. The main advantages over vitamin K antagonists (VKAs) are that DOACs give possibly less major bleeding and that monitoring nor titration are needed. A drawback of no monitoring is the risk of decrease in drug adherence and persistence.

Aims: To audit treatment persistence of DOAC use in patients identified with acute VTE.

Methods: All patients from the administrative database of Dutch Community Pharmacies from 1 January 2012 - 1 April 2016 who initiated DOAC treatment for VTE in 1538 pharmacies (79% of total) in the Netherlands were included. Persistence to DOACs was identified as time from start of DOAC treatment to discontinuation (stopping DOAC or switch to another DOAC or VKA). Since the minimal

treatment duration with DOAC for acute VTE is 3 months, a cut-off point of 2 months treatment duration was chosen as the period in which discontinuation would be surely premature.

Results: Of 2048 patients, 115 patients used apixaban, 1593 rivaroxaban and 340 dabigatran. The 2-month cumulative incidence of discontinuing DOAC was 27%(95%CI 25-29%) (figure 1). 23% (122 of 531) of these patients switched to an alternative treatment, of whom 118 to VKA and 4 to another DOAC, while the others stopped permanently. In univariate logistic regression analysis, predictors for stopping DOAC or switching to alternative treatment were previous use of VKAs (odds ratio [OR] 1.8; 95%CI 1.2-2.8), type of DOAC (OR for rivaroxaban 2.3 (95%CI 1.4-3.8), OR for dabigatran 3.1 (95%CI 1.8-5.4), both compared to apixaban) and female sex (OR 1.2; 95%CI 0.9-1.4).

Conclusions: The cumulative incidence of stopping DOAC treatment for acute VTE or switching to another anticoagulant within 2 months was 27%. Further research to investigate the reasons and consequences of early stopping is urgently needed.

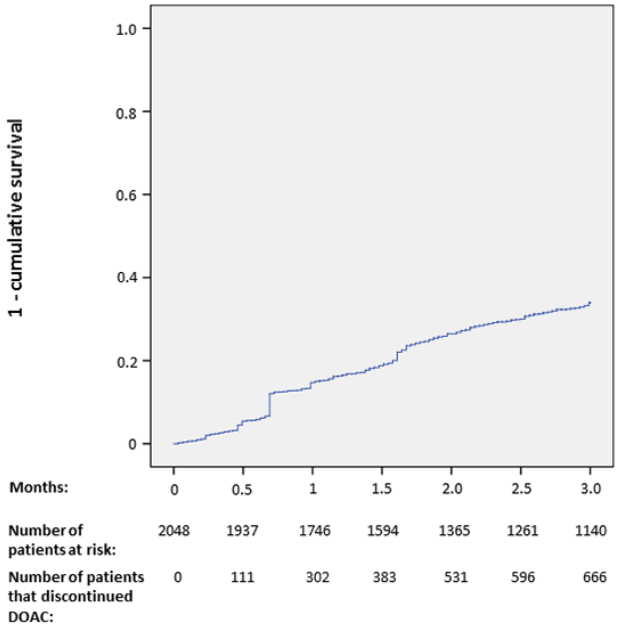

FIGURE 1 Cumulative incidence of discontinuing DOAC within the first 3 months after diagnosis of venous thrombosis

OC 73.3 | Management Strategies and Long-term Outcomes in Patients with Isolated Distal Deep Vein Thrombosis: Findings from the XALIA Study

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Background: Isolated distal deep vein thrombosis (IDDVT) accounts for 30-50% of all DVT diagnosed in the lower limbs. Recent studies report conflicting results on the long-term risk of recurrent venous thromboembolism (VTE) and bleeding; thus, the optimal treatment of IDDVT remains controversial.

Aims: To report the results of a subgroup analysis of patients with IDDVT from XALIA.

Methods: XALIA was a prospective, non-interventional study of rivaroxaban in treatment of acute VTE. Patients aged ≥18 years scheduled to receive ≥3 months of anticoagulation with rivaroxaban or standard of care (SOC) were eligible. Patients were followed for ≥12 months. We describe baseline characteristics, management strategies and unadjusted incidence rates of recurrence, major bleeding and all-cause mortality in patients with IDDVT compared with patients with proximal DVT with or without distal vein involvement (PDVT). Only events on treatment were considered.

Results: Overall, 1004 patients with IDDVT and 3098 with PDVT were enrolled; 641 (63.8%) and 1683 (54.3%) received rivaroxaban, respectively. Table 1 shows selected baseline characteristics. Annualized symptomatic recurrent VTE rates with IDDVT were 1.85% (95% CI 0.60-4.31) for rivaroxaban and 3.1% (1.00-7.20) for SOC. For PDVT, recurrence rates were 2.7% (1.79-3.84) and 4.5% (3.23-5.97), respectively. Major bleeding rates with IDDVT were 1.5% (0.40-3.76) for rivaroxaban and 3.1% (0.99-7.13) for SOC, and 1.1% (0.57-1.92) and 3.1% (2.10-4.38), respectively, for PDVT. All-cause mortality was 0.4% (0.01-2.05) and 4.3% (1.71-8.76), respectively, with IDDVT, and 0.9% (0.44-1.69) and 5.6% (4.27-7.30) with PDVT.

Conclusions: Patients with IDDVT were younger, less fragile and less frequently had cancer than patients with PDVT. Patients with IDDVT more often received rivaroxaban and had lower recurrence rates than patients with PDVT. Bleeding rates were similar between the two groups. Rivaroxaban was safe and effective in patients with IDDVT.

TABLE 1 Baseline demographics and clinical characteristics.

Characteristic N (%) unless stated	IDDVT (N=1004)	PDVT (N=3098)
Age (years), mean (SD)	56.5 (17.1)	61.0 (16.7)
Female	551 (54.9)	1394 (45.0)
Creatinine Clearance <50 ml/min	27 (2.7)	173 (5.6)
Cancer	78 (7.8)	352 (11.4)
Known thrombophilia	54 (5.4)	177 (5.7)
Previous VTE	241 (24.0)	749 (24.2)
Previous major bleeding	17 (1.7)	70 (2.3)
Previous hospitalization	159 (15.8)	441 (14.2)
Provoked DVT	416 (41.4)	1053 (34.0)

OC 73.4 | Impact of BMI on Clinical Outcomes in Daily Care DOAC Recipients - Results of the Prospective Dresden NOAC Registry (NCT01588119)

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Background: Dose adjustment of direct oral anticoagulants (DOAC) for overweight patients is not recommended despite the potential for relative underdosing. At present, the impact of body mass index (BMI) on the effectiveness and safety of DOAC is unclear.

Aims: To evaluate the impact of BMI on clinical outcomes in daily care DOAC recipients.

Methods: Patients from a prospective, non-interventional registry were stratified according to BMI (table 1) and cardiovascular (CV), major bleeding events (MB) and all-cause mortality were evaluated. All outcome events were centrally adjudicated using standard scientific definitions.

Results: Between November 1st 2011 and June 30th 2016, 3273 patients were enrolled into the registry (64.0% received rivaroxaban; 18.9% apixaban; 10.6% dabigatran, 6.4% edoxaban). The mean duration of follow-up was 932±485 days (median 1047 days). With increasing BMI (range 13.7-57.2 kg/m^2), the proportion of patients receiving standard (vs. reduced) DOAC dose increased from 64.7% (underweight) to 78.3% (obesity class II/III; table 1).

Only one patient (BMI 42.2 kg/m^2) received higher dose because of high BMI (rivaroxaban 2x20mg from diagnosis until day 60, followed by 1 x 20 mg).

Although cardiovascular risk profile increased with rising BMI (table 1), rates of all clinical outcomes (CV, MB, all-cause-mortality) were lowest in overweight and obese patients, followed by normal-weight patients (figure 1 a-d).

Underweight patients had considerably higher rates of CV and MB and demonstrated an excess mortality, probably due to a higher prevalence of previous stroke, renal impairment and malignant disease, or DOAC dose reduction, although these observations were based on small numbers only.

Conclusions: In a large set of real-life DOAC recipients we found no indication that elevated BMI is associated with a lack of DOAC effectiveness or safety. Consistent with epidemiological data, increased BMI seems to be associated with a better survival also in DOAC recipients, if compared to underweight patients.

TABLE 1 Patient characteristics of all patients and BMI subgroups

	All patients n=3273	Underweight (<18.5) n=17	Normal (18.5-24.9) n=838	Overweight (25-29.9) n=1384	Obese class I (30-34.9) n=698	Obese class II/III (≥35) n=336
Age, years (mean±SD)	70.6±13.6	66.5±25.1	70.6±16.0	71.4±12.6	70.3±12.0	67.9±12.8
Heart failure, n (%)	824/3273 (25.2)	2/17 (11.8)	190/838 (22.7)	331/1384 (23.9)	188/698 (26.9)	113/336 (33.6)
Arterial hypertension, n (%)	2452/3273 (74.9)	6/17 (35.3)	536/838 (64)	1041/1384 (75.2)	573/698 (82.1)	296/336 (88.1)
Diabetes, n (%)	986/3273 (30.1)	2/17 (11.8)	171/838 (20.4)	375/1384 (27.1)	268/698 (38.4)	170/336 (50.6)
Peripheral arterial occlusive disease/coronary artery disease, n (%)	547/3273 (16.7)	1/17 (5.9)	143/838 (17.1)	221/1384 (16)	118/698 (16.9)	64/336 (19)
Prior TIA, stroke, or systemic embolism, n (%)	394/3273 (12)	3/17 (17.6)	110/838 (13.1)	163/1384 (11.8)	79/698 (11.3)	39/336 (11.6)
Impaired renal function, n (%)	283/3273 (8.6)	3/17 (17.6)	71/838 (8.5)	112/1384 (8.1)	64/698 (9.2)	33/336 (9.8)
active or history of cancer, n (%)	447/3273 (13.7)	4/17 (23.5)	132/838 (15.8)	193/1384 (13.9)	76/698 (10.9)	42/336 (12.5)
Reduced / standard dose, n (%)	891/2381 (27.2/72.7)	6/11 (35.3/64.7)	255/583 (30.4/69.6)	376/1008 (27.2/72.8)	182/516 (26.1/73.9)	72/263 (21.4/78.3)

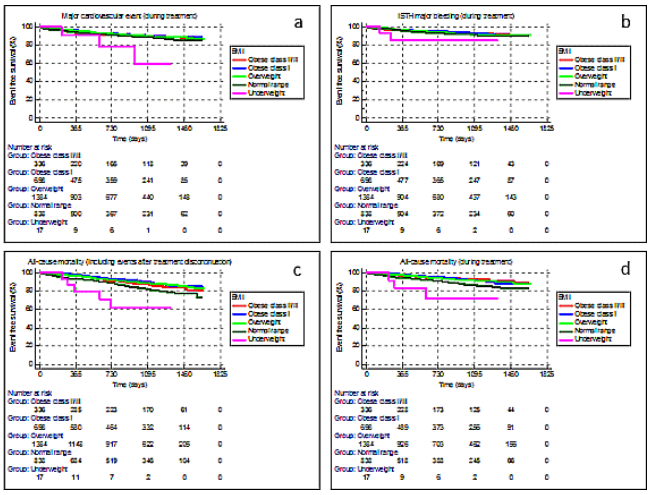

FIGURE 1 Major cardiovascular (a), major bleeding (b) events and all-cause mortality (intention to treat; c and during treatment; d) according to BMI classes

OC 73.5 | Risk Factors for Death Following Major Bleeding Whilst on Warfarin or Direct Oral Anticoagulants: Results from a Prospective UK Study (ORANGE)

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Background: Major bleeding (MB) is an important complication of oral anticoagulant (OAC) therapy with potentially fatal outcomes. ORANGE was a UK multicentre prospective study that catalogued MB events in patients on warfarin or direct OACs (DOACs).

Aims: To identify the risk factors for death within 30 days of MB episode on OACs.

Methods: MB was defined as bleeding leading to hospitalisation and: death; transfusion of ≥2 units of red cells; ≥20 g/L drop in haemoglobin; bleeding into a critical organ; administration of haemostatic agents. Patients' age, sex, OAC regimen, co-morbidities linked to elevated thrombotic or bleeding risk, bleed sites and provocation were potential risk factors.

Results: We analysed the first MB episode of 2192 patients (53% male; median [IQR] age 80 [72-86] years) from 32 hospitals between Oct 2013 and Sep 2016. Patients (%) were on: warfarin (81); dabigatran (2); rivaroxaban (13); apixaban (4). OAC indications (%) were one or more (so total >100%) of: atrial fibrillation (72), venous thromboembolism (21), metal heart valve (10), others (8). Intracranial, gastrointestinal and other bleeds made up 44%, 32% and 24% respectively of cases. Outcomes at 30 days: 65% discharged; 20% died (median [IQR] days alive: 3 [1-8]); 12% inpatient; 3% lost to follow-up. Logistic regression (n=2132) showed no evidence that OAC type (p=0.99) or indication (p>0.34) were independently associated with mortality. Intracranial haemorrhage (ICH) was associated with 4.3-fold [95% CI: 3.0-6.0] higher odds of death compared with other bleeds. Unprovoked bleeding was associated with over twice the odds of death compared with bleeding from trauma (p< 0.001), surgery (p=0.09) or falls (p< 0.001). Age ≥75 years was also associated with death (p=0.003).

Conclusions: Hospital mortality following MB on OACs is 20%; the adjusted odds of death were similar for warfarin and DOACs. ICH, unprovoked bleeding and age ≥75 years were the strongest predictors of fatality.

OC 76.1 | High Affinity FXII Inhibitor Macrocycle Peptide for Safe Anticoagulation Therapy

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Background: Coagulation factor XII (FXII), a serine protease that initiates the contact system, has a *highly conserved* amino acid sequence, suggesting a physiologic function. Mechanistic *in vivo* research indicates that FXII contributes to thrombosis by triggering excessive coagulation. Inhibiting FXII reduces thrombosis without increasing the bleeding risk, a major side-effect of currently used anticoagulants.

Aims: To characterize the first high affinity small molecule FXII inhibitor *in vitro* and *in vivo* in mice.

Methods: Bicyclic peptide synthesis, protease inhibition assays for the following serine proteases: tPA, uPA, factor XIa, plasma kallikrein, thrombin, plasmin, trypsin, factor VIIa, factor Xa, trypsin and factor XIIa, structural model and structure analysis, plasma stability assays, coagulation testing, pharmacokinetics, $FeCl_3$-induced thrombosis mouse model on mesenteric arterioles.

Results: We have generated a highly selective FXII inhibitor based on a macrocyclic peptide format (MW< 2000 kDa). Its potency and stability were improved using various approaches based on unnatural amino acid incorporation. It showed high inhibitory affinity and selectivity with a high stability in plasma (K_i=380±80pM, >200,000-fold selectivity, $t_{1/2}$ plasma >96h) and prolonged intrinsic coagulation in human, mouse and rabbit plasma (EC_{2X} human=1μM). Pharmacokinetic studies in mouse and rabbit showed activity *in vivo* with no signs of toxicity or abnormal bleeding. We then recorded thrombus formation in mouse mesenteric arterioles by intravital

microscopy, a thrombosis model sensitive to defects in the intrinsic pathway of coagulation. The peptide substantially reduces thrombus formation (peptide:3/9(33%), control: 7/8(87%) P< 0.05), full occlusion (peptide:0/9(0%), control:5/8(63%), P< 0.05), time to thrombus formation (peptide:20±3.6min, control:9.6±5.7min).

Conclusions: Our data suggest that FXII inhibition by a peptide macrocycle can potentially offer a safe anticoagulation therapy.

OC 76.2 | Improving the Therapeutic Index with JNJ-375: A Novel Long Acting Exosite-1 Thrombin Inhibitor In Animal Models

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Background: An antibody (Ab) against thrombin exosite-1 was identified from a 54 year-old female who presented with a traumatic subacute subdural hematoma and markedly prolonged coagulation tests at a hospital in Cambridge, UK. The patient made a complete recovery without intervention and had no abnormal bleeding during 8-years of follow-up, despite continuing to present with grossly prolonged coagulation tests. The Ab was characterized by scientists at Cambridge University and the resulting IP was licensed to XO1 Limited, a small biotech company that was later acquired by Janssen Pharmaceuticals, Inc. JNJ-375 is a human IgG4 antibody designed to mimic the patient's IgA antibody, with the same binding properties. JNJ-375 specifically binds to the exosite-1 region on thrombin and does not inhibit the active site.

Aims: Here we compare the antithrombotic efficacy and bleeding risk (therapeutic index, TI) of JNJ-375 with apixaban, a marketed factor Xa inhibitor.

Methods: In rat and monkey models.

Results: In the rat AV-shunt model thrombosis, JNJ-375 inhibited thrombosis (50%) at 0.3 mg/kg and the positive control apixaban (58%) at 1 mg/kg. In the rat tail transection model, JNJ-375 and apixaban significantly increase bleeding at 10 (TI=33) and 3 mg/kg (TI=3), respectively. In the FeCl3 model of venous thrombosis in cynomolgus monkeys, JNJ-375 and apixaban inhibit thrombosis significantly (> 80%) at 1 and 0.1 mg/kg respectively. In the cynomolgus monkey liver laceration bleeding model, JNJ-375 and apixaban significantly increase bleeding time at 40 (TI=40) and 1 mg/kg (TI=10), respectively.

Conclusions: The animal studies described here, demonstrate an improved TI of JNJ-375 over apixaban. In addition to the potential of a lower bleeding risk, JNJ-375 with a projected once monthly dosing regimen (Vs BID for apixaban), likely leading to steady anticoagulation for the duration of the dosing interval may provide better efficacy compared to existing agents. JNJ-375 is currently being investigated in early clinical studies.

OC 76.3 | Different Effects of Heparin and Bilvalirudin on Thrombin-induced Platelet Activation via PAR1 and PAR4

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Background: Heparin and bivalirudin are extensively used as antithrombotic agents during percutaneous coronary interventions (PCI). Although several large clinical studies have been performed, there is currently no consensus regarding which one of the two is associated with a superior clinical outcome. Heparin and bivalirudin inhibit thrombin by different mechanisms. Heparin bind strongly to exosite II, wheres bivalirudin inhibit thrombin via dual interaction with exosite I and the active site.

Aims: To characterize the inhibitory effects of heparin and bivalirudin on thrombin-induced platelet activation via PAR1 and PAR4.

Methods: Intracellular calcium measurements and flow cytometry was used to monitor platelet response to thrombin stimulation. Experiments were conducted in the presence of different inhibitors to distinguish between the contribution of PAR1 and PAR4 to platelet activation.

Results: At physiological levels of antithrombin, thrombin inhibition with heparin resulted in a transient calcium spike with a modest inhibition of peak height but no prolonged calcium mobilization, consistent with selective activation of PAR1. This finding was corroborated by experiments conducted in the presence of specific inhibitors of PAR1 and PAR4.

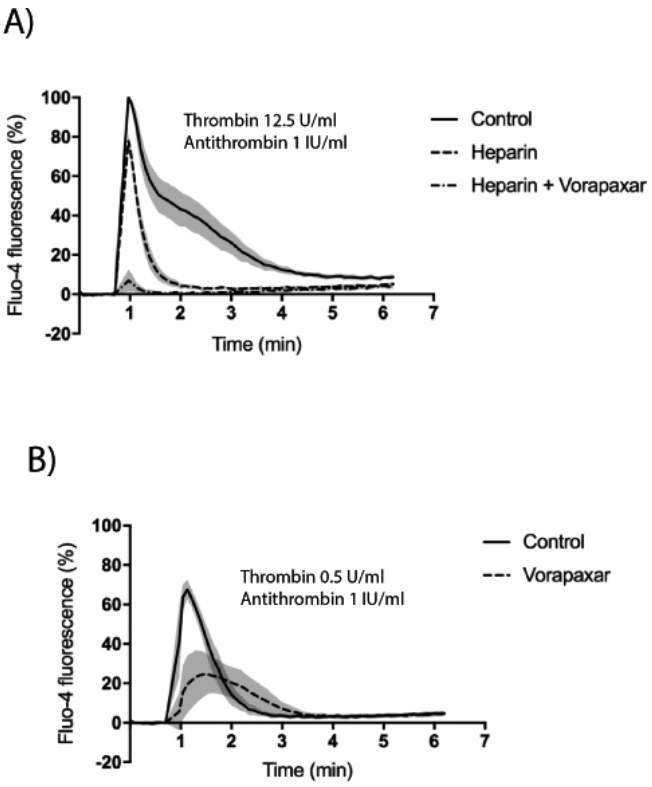

FIGURE 1 Thrombin-triggered calcium transients were measured on washed platelets alone or together with different antagonists as indicated. n ≥ 3

In contrast, thrombin inhibition with bivalirudin resulted in biphasic calcium mobilization, with an initial peak resulting from residual PAR1 activation, and a second delayed peak observed after 10-30 min which was found to be caused primarily by PAR4 activation, supposedly due recovery of thrombin activity as the bivalirudin-Arg3-Pro4 bond is cleaved.

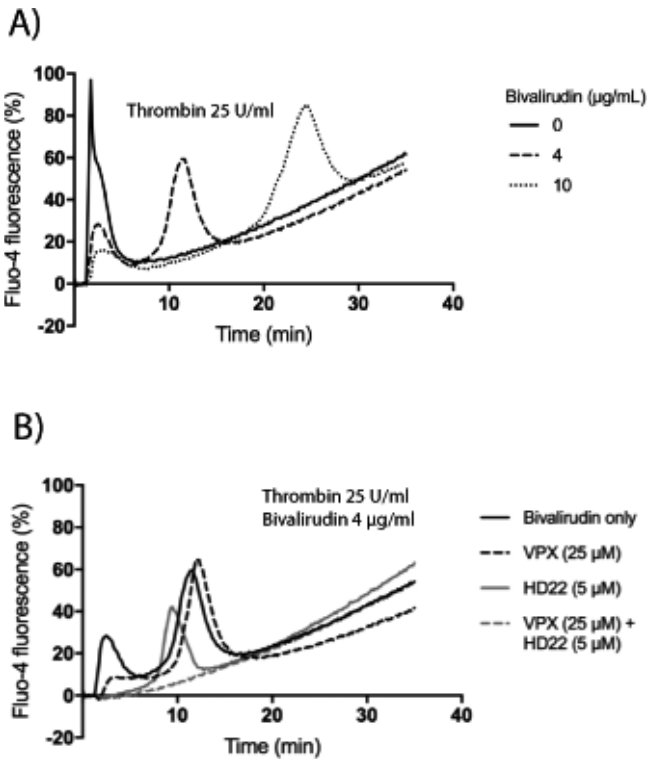

FIGURE 2 Thrombin-triggered calcium transients were measured on washed platelets alone or together with different antagonists as indicated. n ≥ 3

Conclusions: Our study shows that heparin and bivalirudin have different inhibitory effects on thrombin-induced platelet activation via PAR1 and PAR4. These findings could be of clinical relevance in the light of recent findings suggesting a higher rate of stent thrombosis after PCI with bivalirudin, and varying contributions of PAR receptors to thrombin-induced platelet activation among patients from different ethnic groups.

OC 76.4 | Pharmacokinetic and Pharmacodynamic Modeling of Andexanet Alfa Dose to Reverse the Anticoagulant Activity of Factor Xa Inhibitors in Patients with Acute Major Bleeding

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Background: Andexanet alfa is being investigated for reversal of anticoagulation by factor Xa (FXa) inhibitors. A PK/PD model, developed in healthy subjects, predicted the andexanet regimen required to reverse anticoagulation by FXa inhibitors.

Aims: To validate the PK/PD model using interim data from the ANNEXA-4 study in patients with acute major bleeding.

Methods: In ANNEXA-4, an ongoing prospective, open-label study, bleeding anticoagulated patients received andexanet IV bolus (400 or 800 mg) followed by 120-min infusion (4 or 8 mg/min). Anti-FXa activity was measured before andexanet administration (baseline), at end of bolus (EOB), end of infusion, and 4, 8, and 12 h after infusion. The relationship between baseline anti-FXa activity and reversal in healthy subjects was derived from the PK/PD model and used to provide a predicted percent reversal for patients with acute major bleeding.

Results: From the first interim analysis of ANNEXA-4, 73 patients (apixaban, 39; rivaroxaban, 34) had plasma levels available for model qualification, although ~7 did not meet criteria for inclusion into safety and ~27 did not meet criteria for efficacy analysis. The mean observed percent reversal of anti-FXa activity for rivaroxaban and apixaban was well predicted by the healthy subject PK/PD model; the point estimates fell within the 90% confidence intervals of predicted values. The percent reversal at EOB for rivaroxaban and apixaban were 74.4 [58.3-90.7] and 83.9 [75.3-92.5], compared to 76.3 and 84.1 predicted by the model (Tables 1 and 2). The predicted reversal fit within the observed confidence intervals through the first 4 h for rivaroxaban and apixaban, and extended through all evaluated time points for rivaroxaban and slightly outside of post 4-h time points for apixaban, possibly due to higher baseline anti-FXa activity levels for apixaban.

Conclusions: The PK/PD model in healthy subjects closely predicted the extent of reversal of anti-FXa activity for apixaban and rivaroxaban in patients with major bleeding.

TABLE 1 Comparison of mean predicted percentage of anti-FXa reversal vs. observed reversal for rivaroxaban at different time points

Time	N	Predicted percent reversal	Observed percent reversal [95% CI]
End of bolus (EOB)	32	76.4	74.4 [58.3 to 90.4]
End of infusion	33	78.3	77.4 [67.6 to 87.2]
4 hrs	32	31.8	32.0 [18.0 to 45.9]
8 hrs	33	40.7	43.6 [35.0 to 52.2]
12 hrs	32	51.0	56.9 [50.2 to 63.7]

TABLE 2 Comparison of mean predicted percentage of anti-FXa reversal vs. observed reversal for apixaban at different time points

Time	N	Predicted percent reversal	Observed percent reversal [95% CI]
End of bolus (EOB)	34	84.1	83.9 [75.3 to 92.5]
End of infusion	36	81.4	84.2 [77.3 to 91.1]
4 hrs	33	34.4	27.6 [10.2 to 45.0]
8 hrs	32	40.2	30.6 [25.1 to 36.1]
12 hrs	36	48.6	32.8 [26.1 to 39.5]

OC 76.5 | Plasma Level of Apixaban is Inversely Correlated with Hemoglobin Level due to Apixaban Binding to Deoxy-hemoglobin

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Background: Apixaban (APX) was associated with a significantly lower risk of major bleeding than warfarin. However, in the ARISTOTLE trial, a hematoctit < 45% was found as the most relevant risk factor for bleeding (HR=1.38). According with this finding, In a pilot study we found and inverse relationship between plasma level of APX and Hb concentration in patients treated with this drug. Moreover, APX molecule shows structural analogies with bezafibrate, a lipid-lowering agent to treat hyperlipidaemia, which is known to bind to deoxy-hemoglobin.

Aims: This study is aimed at investigating in vitro the possible direct interaction of apixaban with human hemoglobin.

Methods: The oxygen saturation curve of Hb was studied at 577 nm with a tonometer. The P_{50} of oxygen binding was measured as a function of APX concentration. The right docking pose of APX into the binding site of Hb was accomplished using Docking Server, which integrates AUTODOCK 4.0 program for docking calculation. Semiempirical, quantum mechanical methods were used for ligand set up, utilizing MOPAC software.

Results: APX concentrations causes progressive right-shifts of the oxygen dissociation curve of dilute Hb solution. This implies that APX binds to the deoxy form of Hb with a calculated Kd in the micromolar range at 25 °C. In silico analysis showed that APX binds to the deoxy Hb central water cavity, where also bezafibrate binds. In particular, the piperidyl- moiety of APX fits into a cavity surrounded by residues α1Thr134, α 2Pro95, α 2Thr137, α 2Tyr140, α 2Arg141, and β1Trp37 of Hb. The phenoxy ring of APX engages in hydrophobic contacts with Hb α1Lys99, α2Arg141, β1Tyr35 and β1Trp37.

Conclusions: APX interacts with deoxy-Hb and reduces its affinity for oxygen. Thus, in venous circulation, where about 30% of Hb is in its deoxy-form, the hemoprotein can buffer the amount of APX that is able to pass through the red cell membrane due to its high lipophilicity. This phenomenon could significantly change the level of the available drug in the circulation.

PATHOGENESIS OF THROMBOEMBOLISM

OC 21.1 | Development and External Validation of a Risk Assessment Model for Cancer-associated Venous Thromboembolism

I. Pabinger[1], N. van Es[2], G. Heinze[3], F. Posch[1], N. Kraaijpoel[2], H.R. Büller[2], C. Ay[1], on behalf of the contributors to the Vienna Cancer and Thrombosis Study (CATS) and the Multinational Cohort Study to Identify Cancer Patients at High Risk of Venous Thromboembolism (MICA)

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Background: Venous thromboembolism (VTE) is a frequent complication in cancer patients.

Aims: To develop and externally validate a risk assessment model for cancer-associated VTE.

Methods: Two prospective cohorts of patients with solid cancer, the Vienna Cancer and Thrombosis Study (CATS, n=1,423), and the Multinational cohort study to Identify CAncer patients at high risk of VTE (MICA, n=832), were used for development and external validation of the risk assessment model (**Table 1**). The primary outcome was symptomatic or unsuspected, objectively confirmed VTE at 6 months of follow-up. The cumulative 6-month incidence was 5.7% in CATS (95%CI: 4.5-6.9), and 6.3% (4.7-8.2) in MICA. Tumor sites were categorized into low/moderate, high, and very high VTE risk sites according to Khorana et al. (Blood. 2008).

TABLE 1 Baseline characteristics of the two study populations (total n=2,255)

Cohort	CATS (n=1,423)	MICA (n=832)
Variable	Median [25th-75th percentile], or Absolute count (percent)	Median [25th-75th percentile], or Absolute count (percent)
Age at entry (years)	61.7 [51.6 - 68.5]	63.7 [55.9-70.3]
Female Gender	651 (45.8%)	354 (42.5%)
Body mass index (kg/m²)	25.0 [22.1-28.3]	24.7 [22.5-27.4]
Newly-diagnosed malignancy	1,008 (70.8%)	454 (54.6%)
---Low/Moderate VTE risk tumor site (Breast, Prostate)	379 (26.6%)	271 (32.6%)
---High VTE risk tumor site (Lung, Esophagus, Colorectal, Kidney, Lymphoma, Gynaecologic, Bladder, Other sites)	863 (60.7%)	408 (49.0%)
---Very high VTE risk tumor site (Stomach, Pancreas)	181 (12.7%)	153 (18.4%)
D-Dimer (µg/mL)	0.7 [0.4-1.5]	0.9 [0.5-2.0]

Results: In the CATS cohort, a penalized likelihood regression (LASSO) was used to select predictive variables from a broad set of clinical and laboratory factors, which led to a final model including two variables, the tumor site category (hazard ratio (HR) per 1 category increase=1.96, 95%CI: 1.41-2.72, p=0.0001), and D-Dimer (HR per doubling=1.32, 1.12-1.56, p=0.001). The model discriminated well between cancer patients with and without VTE (C-Index=0.67, 95%CI 0.64-0.68) and was appropriately calibrated. As compared to the Khorana score, the new model correctly re-classified a significant proportion of patients according to their outcome (net reclassification improvement=0.44). In the external validation cohort (MICA study), the resulting nomogram (**Figure 1**) demonstrated good discrimination (C-Index=0.66, 95% CI: 0.58-0.72) and good calibration. Tumor site specific D-Dimer cut-offs over a range of VTE risk thresholds will be presented at the meeting.

Conclusions: An externally validated risk assessment model of only one clinical factor (tumor site) and one biomarker (D-Dimer) predicts

the risk of VTE in cancer patients. This simple model can aid physicians in selecting patients for thromboprophylaxis.

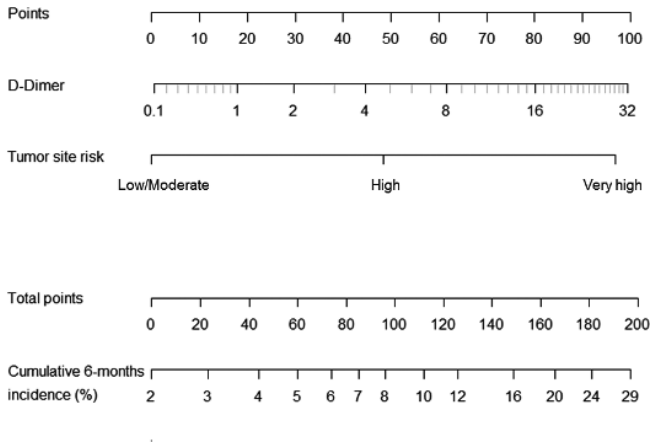

FIGURE 1 Externally-validated nomogram for predicting the risk of VTE in patients with cancer.

OC 21.2 | Comparison of Risk Prediction Scores for Cancer-Associated Venous Thromboembolism: A Prospective Cohort Study

N. van Es[1], M. Di Nisio[2], G. Cesarman[3], A. Kleinjan[1], H.-M. Otten[4], I. Mahé[5], I.T. Wilts[6], D.C. Twint[7], E. Porreca[8], O. Arrieta[3], A. Stépanian[9], K. Smit[7], M. De Tursi[8], S.M. Bleker[10], R. Nieuwland[1], P.W. Kamphuisen[6,11], P.M. Bossuyt[1], H.R. Büller[1]

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Background: In ambulatory patients with solid cancer, thromboprophylaxis to prevent venous thromboembolism (VTE) is not recommended. Several risk prediction scores have been developed to identify cancer patients at high risk of VTE, but their clinical usefulness remains a matter of debate.

Aims: To evaluate and directly compare the performance of the Khorana, Vienna, PROTECHT, and CONKO scores (Table) in predicting VTE in ambulatory cancer patients.

Methods: In this multinational, prospective cohort study, patients with advanced cancer were enrolled when they had recently started or were scheduled for chemotherapy. Patients receiving anticoagulants or adjuvant chemotherapy were excluded. The primary outcome was objectively confirmed, symptomatic or incidental deep vein thrombosis or pulmonary embolism over a 6-month follow-up period. The discriminatory performance (c-statistic), the positive predictive value, and the difference between low and high risk patients at the positivity threshold of 3 points were evaluated in a competing risks analysis.

Results: A total of 876 patients with nine different stage III or IV tumor types were enrolled, of whom 260 (30%) had not yet received chemotherapy. Fifty-three patients (6.1%) developed VTE. C-statistics of the scores ranged from 0.50 to 0.57. At the positivity threshold of 3 points, the scores classified 13-34% of patients as high risk; the 6-month VTE incidence in these patients ranged from 6.5% (95% CI, 2.8-12%) for the Khorana score to 9.6% (95% CI, 6.6-13%) for the PROTECHT score. High risk patients had a significantly higher risk of VTE if defined on the Vienna CATS (subhazard ratio 1.7; 95% CI 1.0-3.1) or PROTECHT scores (subhazard ratio 2.1; 95% CI 1.2-3.6; Figure).

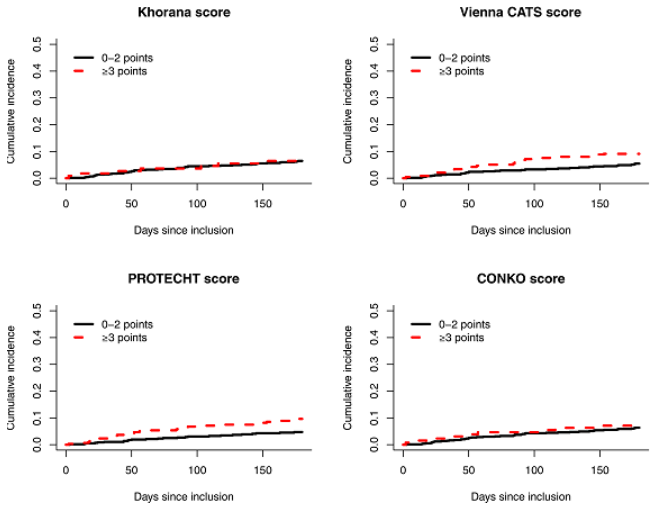

FIGURE Cumulative VTE incidence in low and high risk patients according to the four scores

Conclusions: The prediction scores performed relatively poorly in predicting VTE in cancer patients. The Vienna CATS and PROTECHT scores appear to discriminate better between low and high risk patients, but research is needed to further improve their performance prior to introduction in clinical practice.

TABLE Risk prediction scores

Item	Khorana score (points)	Vienna CATS score (points)	PROTECHT score (points)	CONKO score (points)
Very high risk tumor / high risk tumor	+2 / +1	+2 / +1	+2 / +1	+2 / +1
Hemoglobin <10 g/dL or ESA use	+1	+1	+1	+1
White blood cell count >11 x 10^9/L	+1	+1	+1	+1
Platelet count ≥350 x 10^9/L	+1	+1	+1	+1
Body mass index >35 kg/m2	+1	+1	+1	+1
D-dimer >1.44 g/L	-	+1	-	-
Soluble P-selectin >53.1 g/L	-	+1	-	-
Gemcitabine / platinum-based chemotherapy	-	-	+1 / +1	-
WHO performance status ≥2	-	-	-	+1

OC 21.3 | External Validation of a Risk Score for Occult Cancer in Patients with Unprovoked Venous Thromboembolism: Results from an Individual Patient Data Meta-Analysis

N. van Es[1], L. Bertoletti[2], L. Jara-Palomares[3], G. Le Gal[4,5], H.-M. Otten[6], A. Piccioli[7], P. Religa[8,9], R. Lecumberri[10], V. Rieu[11], M. Rondina[12], M. Beckers[13], P. Robin[14], P. Prandoni[15], M. Di Nisio[16], P.M. Bossuyt[17], H.R. Büller[1], M. Carrier[4], P.-Y. Salaun[18]

[1]Academic Medical Center, Department of Vascular Medicine, Amsterdam, The Netherlands, [2]Centre Hospitalo-Universitaire de Saint-Etienne, Service de Médecine Vasculaire et Thérapeutique, Saint-Etienne, France, [3]Instituto de Biomedicina de Sevilla (IBiS), Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Virgen del Rocio Hospital, Medical Surgical Unit of Respiratory Diseases, Sevilla, Spain, [4]Ottawa Hospital Research Institute, Department of Medicine, Ottawa, Canada, [5]Centre Hospitalo-Universitaire de Brest, Département de Médecine Interne et Pneumologie, Brest, France, [6]Slotervaartziekenhuis, Department of Internal Medicine, Amsterdam, The Netherlands, [7]University Hospital Padua, Departments of Cardiovascular Sciences and Medicine, Padua, Italy, [8]Karolinska Institutet, Department of Medicine, Stockholm, Sweden, [9]Warsaw Medical University, Department of Medicine, Warsaw, Poland, [10]Clinica Universidad de Navarra, Hematology Service, Pamplona, Spain, [11]Centre Hospitalo-Universitaire Estaing, Department of Internal Medicine, Clermont-Ferrand, France, [12]University of Utah, Division of General Internal Medicine, Salt Lake City, United States, [13]University Hospital Leuven, Department of Hematology, Leuven, Belgium, [14]Centre Hospitalo-Universitaire de Brest, Brest, France, [15]University Hospital Padua, Padua, Italy, [16]G. D'Annunzio University, Dipartimento di Medicina e Scienze dell'Invecchiamento, Chieti, Italy, [17]Academic Medical Center, Department of Clinical Epidemiology, Biostatistics, and Bioinformatics, Amsterdam, The Netherlands, [18]Centre Hospitalo-Universitaire de Brest, Service de Médecine Nucléaire, Brest, France

Background: Unprovoked venous thromboembolism (VTE) may be the first sign of occult cancer. A prediction score to identify VTE patients at increased risk of an occult cancer diagnosis was recently proposed (Table).

Aims: To evaluate the performance of the risk prediction score for occult cancer detection in patients with unprovoked VTE.

Methods: Combined data from 10 prospective studies that evaluated cancer screening in patients with unprovoked VTE were used. As in the derivation study, cases were defined as patients in whom cancer was diagnosed between 30 days and 2 years of follow-up. No information was available on chronic lung disease within our database. Therefore, 1 point was assigned to all patients who were former or current smokers as a proxy. Using random effect meta-analyses, the score's discriminatory performance (c-statistic), positive predictive value, and association with occult cancer detection were evaluated. A multivariable analysis was performed to explore the predictive performance of the separate items.

Results: The risk score for occult cancer diagnosis could be calculated in 1,869 of 2,371 patients (79%), who were enrolled in five studies. Twenty-nine patients were excluded because they were diagnosed with cancer within 30 days. Of the remaining 1,840 patients, 65 (3.5%) were diagnosed with cancer over a median follow-up of 402 days. The pooled c-statistic of the continuous score was 0.59 (95% CI 0.52-0.67). Of the 446 patients (24%) classified as ‚high risk' (≥3 points), 26 (5.8%) were diagnosed with cancer compared to 39 of 1,394 (2.8%) low-risk patients (pooled hazard ratio 2.1; 95% CI 1.3-3.4; P=0.004; Figure). In the multivariable analysis, age >70 years was the strongest predictor of occult cancer detection (P=0.0024).

Conclusions: The risk score had a poor overall discriminatory performance. However, when used dichotomously, it was able to discriminate between low and high risk patients. The performance of the score was largely driven by the item age >70 years.

TABLE Risk prediction score of occult cancer detection in patients with venous thromboembolism (Jara-Palomares et al., Chest, 2016)

Item	Points
Male sex	+1
Age >70 years	+2
Chronic lung disease	+1
Anemia (<13 g/dL in males or <12 g/dL in females)	+2
Platelet count ≥350 x 10^9/L	+1
Previous venous thromboembolism / recent surgery	-1 / -2
Classification	
Low risk	≤2
High risk	≥3

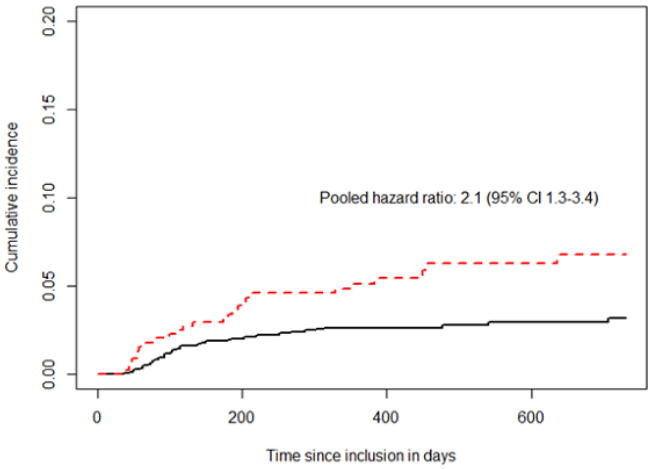

FIGURE Risk of cancer diagnosis stratified by dichotomous risk score

OC 21.4 | External Validation of a Prognostic Score for Occult Cancer in Patients with Venous Thromboembolism

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Background: The benefits of a diagnostic workup for occult cancer in patients with venous thromboembolism (VTE) are controversial. In a recent study, a prognostic model to identify VTE patients at increased risk for occult cancer was proposed with an internal validity.

Aims: We used the RIETE database to validate the score in a subsequent cohort of patients.

Methods: The following variables scored 1 point: male sex, chronic lung disease and platelet count ≥350 x 10^9/L each; 2 points: age >70 years and anaemia; -1 point: previous VTE; -2 points: recent surgery. We dichotomized patients as having low (≤2 points) or high risk for cancer (≥3 points). We calculated the area under the receiver-operating characteristic curve, and hazard ratio of occult cancer in high-risk score patients.

Results: From 2014 to 2016, 11,695 VTE patients were recruited. At 12 months, there were 2,580 eligible patients. Of these, 93 (3.6%; 95%CI: 2.9 to 4.4%) were diagnosed with occult cancer. Mean age was 64.7 ± 17.6 years, and 51% were female. The most frequent sites were: colorectal (16.5%), lung (14.6%) and hematologic (14.6%). Among 1,849 patients scoring ≤2 points, 46 (2.5%) had occult cancer. Among 731 patients scoring ≥3 points, 47 (6.4%) had cancer (hazard ratio 2.7; 95% CI 1.8-4.1). C-statistic was 0.65 (95% CI 0.59-0.71). At 24 months, 103 of 628 eligible patients (16.4%; 95%CI: 13.6 to 19.5%) were diagnosed with occult cancer. Among 429 patients scoring ≤2 points, 53 (12.4%) had occult cancer. Among 429 scoring ≥3 points, 53 (25.1%) had cancer (hazard ratio 2.3; 95% CI 1.5-3.7; p< 0.01). C-statistic was 0.63 (95% CI 0.57-0.69).

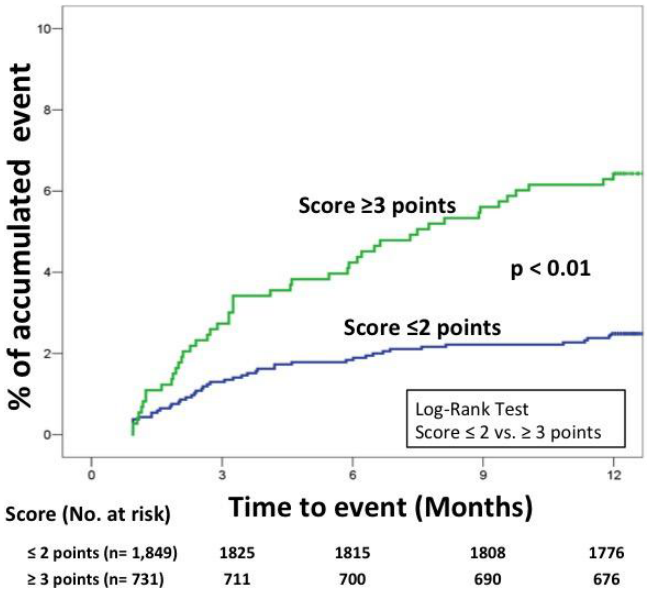

FIGURE 1 Cumulative incidence of occult cancer over 2 years attending score (≤2 vs. ≥3 points). Time-to-event data

Conclusions: We externally validated our score at 12 and 24 months.

OC 21.5 | Thrombin Generation Predicts for Early Cancer Recurrence in Breast Cancer Patients Undergoing Post-surgical Adjuvant Therapy: A Prospective Study

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Background: Breast cancer screening has greatly increased early diagnoses, so that cancer treatments by surgery and systemic adjuvant chemotherapy (SAC) can be anticipated and reduce the risk of disease recurrence (DR). However, local relapses and distant metastasis may occur in 2-7% and 20% of resected patients, respectively, in the 10 years following surgery. In this setting, the identification of prognostic biomarkers for DR can help to identify high-risk patients who might most benefit from more aggressive therapy.

Aims: This study wants to evaluate whether hemostatic biomarkers may be prognostic of early DR in a group of breast cancer patients undergoing post-surgical SAC.

Methods: Plasma samples from 588 limited-resected breast cancer patients (8M/580F), enrolled in the Italian, prospective, multicenter HYPERCAN study (AIRC 5x1000 grant #12237), were obtained at enrollment before starting SAC and after 1, and 2 years follow-up. Samples were tested for fibrinogen, D-dimer, and thrombin generation (TG). Clinical data and information regarding surgery, cancer subtype, and treatment were recorded. DR was routinely monitored by imaging during post-treatment surveillance.

Results: At enrollment, fibrinogen levels were in the normal range, while D-dimer and TG were increased. During follow-up, D-dimer significantly diminished over time, while no modifications occurred in the other biomarkers. After 2 years follow-up, 5.7% of patients presented with DR. A TG peak value ≥394 nM at enrollment was an independent risk factor for early DR, also after correcting for gender, age and triple-negative subtype (HR=2.28; 95% CI 1.25-4.16; p< 0.01). Differently, DR was not associated with fibrinogen and D-dimer levels.

Conclusions: Our data show, for the first time, the prognostic significance of TG measurement on the risk of early DR in resected breast cancer patients. With a proper validation, this biomarker can be a candidate to tailor a risk-adapted adjuvant treatment.

OC 32.1 | Genetic Associations of a Clinical Pulmonary Embolism among Those with a Venous Thromboembolism: The INVENT Consortium

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Background: Pulmonary emboli (PE) can be life-threatening sequelae of deep vein thrombosis (DVT). Little is known about the genetic risk factors for clot embolization in those with DVT.

Aims: We used genome-wide markers from 12 studies of incident venous thromboembolism (VTE) to investigate genetic risks of developing a PE among those with DVT.

Methods: Participants were 8,905 adults (63% women, average age 56 years) who experienced a first VTE in each study. For these analyses, the outcome was a PE diagnosis (n = 3662, 41% of all VTE), with or without a concomitant DVT diagnosis, among those with an incident VTE. The comparison group was those whose incident VTE was a DVT only. In each study, the association between 1000-genomes imputed genetic variation and PE was assessed with an additive genetic model using logistic regression that adjusted for age, sex, and study-specific design variables. Data were meta-analyzed across studies using fixed-effects models with inverse-variance weighting. The threshold of genome-wide statistical significance was set at 5.0E-8.

Results: After applying quality control procedures, 1 locus exceeded the threshold of genome-wide significance: rs6025 (factor V Leiden variant; minor allele frequency [MAF] 0.074): odds ratio (OR) 0.72; p-value 8.2E-11. A high-signal locus (rs9485137; MAF 0.15; OR 1.20; p-value 3.9E-7) was located in *STXBP5-AS1*, which is antisense RNA to *STXBP5*. Several studies have previously reported that the factor V Leiden variant is more strongly associated with DVT alone than with PE. Our results add further evidence and provide an estimate of the difference in relative odds. The *STXBP5-AS1* association is novel. This locus is anti-sense RNA to *STXBP5*, a gene previously implicated in the risk of incident VT. The rs9485137 variant was not in linkage disequilibrium with the *STXBP5* variants.

Conclusions: We identified genetic variation that may be associated with the embolization of clots among those who experience a VTE; replication of the *STXBP5-AS1* locus is needed.

OC 32.2 | Genetic Footprint of Endothelial Cells in Chronic Thromboembolic Pulmonary Hypertension

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Background: Studies in mice have shown that thrombus neovascularization plays a role during venous thrombus resolution. In specimens from patients with Chronic Thromboembolic Pulmonary Hypertension (CTEPH), characterized by thrombus non-resolution and increased fibrosis, endothelial cell-poor thrombi were observed.

Aims: To determine the genetic footprint of CTEPH endothelial cells and their role in the accumulation of thrombofibrotic material.

Methods: Visually identified endothelial cells outgrown from CTEPH specimens were processed for whole gene microarray analysis. Human Umbilical Vein Endothelial cells (HUVECs) and Human Pulmonary Arterial Endothelial cells (HPAECs) were used as control.

Results: Microarray analysis revealed that out of 26.808 genes examined, the expression of 105 genes was misregulated in CTEPH vs. HUVECs and vs. HPAECs (False Discovery Rate= 0.05). Endothelial (VWF, CDH5, ICAM2) and apoptotic (CASP2, CCND1) markers were expressed on similar level. However, certain proliferation markers (CCNA1, CCND1) were upregulated in CTEPH cells. Pathway analyses using two databases (Gene Onthology, Reactome) revealed misregulation of diverse genes in 14 pathways of biological processes. In brief, CTEPH endothelial cell profiling exhibited a ‚pro-fibrotic', ‚pro-coagulable' and ‚pro-inflammatory' phenotype, as suggested by the upregulation of genes involved in extracellular matrix production (COL1A1, COL1A2, COL3A1, ACTA), degradation (TIMP1,

MMP1, FBN2, CD44, CTSS, CTSK) and cell-matrix adhesion (VCAM1, POSTN, APOD), collagen catabolism (CTSS, CTSK, MRC2, ADAMTS2) or fibril organization (COL1A1, COL1A2, COL3A, ADAMTS2) or procoagulable factors (F3, THBS2) and genes involved in platelet activation (ADAMTS18, MYL9). Results were confirmed via qPCR on the cellular and by immunohistochemistry on the tissue level in CTEPH specimens.

Conclusions: We expect that the microarray data will provide a useful basis for further analyses of selected molecules to better understand the pathophysiology of this disease.

OC 32.3 | Genetic Associations with Recurrent Venous Thromboembolism: A Meta-analysis of Candidate SNPs in 4 Studies

M. Blondon[1], M. Germain[2], S. Armasu[3], A. van Hylckama Vlieg[4], K. Wiggins[5], P.-E. Morange[6], F. Rosendaal[7], D.-A. Tregouët[8], M. de Andrade[3], N. Smith[5]

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Background: Predicting the risk of recurrence in patients with venous thromboembolism (VTE) is critical when assessing the duration of anticoagulation. Genetic polymorphisms are hypothesized to contribute to the individual susceptibility to recurrent VTE.

Aims: To assess, using a meta-analysis of 4 large studies of recurrent VTE, the impact on recurrence of polymorphisms known to associate with incident VTE.

Methods: We identified 25 SNPs in 19 genes (*ABO*, *F2*, *F5*, *F11*, *F13*, *VWF*, *SERPINE1* (PAI-1), *PROC*, *PROCR*, *FGG*, *SERPINC1 (AT)*, *GP6*, *HIVEP1*, *STXBP5*, *KNG1*, *KLKB1*, *TC2N*, *TSPAN15*, and SCL44A2), all of which have been established as causes of incident VTE. Using an additive genetic model that adjusted for sex and age, we tested for association with recurrent VT in 4 studies of VTE in European-ancestry participants: the HVH study (a US HMO-based cohort study), the MARTHA study (a French tertiary center-based case-control study), the MEGA study (a Dutch regional cohort study), and the Mayo study (a US hospital-based cohort study). Relative risk (RR) estimates were meta-analyzed using fixed-effect models. Informed consent was obtained from all participants and protocol approval by medical ethics committee.

Results: Overall, the 4 studies included 8079 participants (57% women) of whom 1674 (20.7%) had a recurrent VTE. Among the 25 candidate SNPs, 7 were nominally associated with recurrent VTE at an alpha-level of 0.05 (Table), in *ABO*, *F5*, *F2*, *F13*, *FGG*, *VWF*, and *STXBP5*. A conservative Bonferroni correction for multiple tests (p-value threshold 0.05/25 = 0.002) identified 3 variants reaching significance: *FV* rs6025 (RR 1.23), *ABO* rs8176719 (RR 1.15) and *STXBP5* rs1039084 (RR 1.16).

TABLE 1 Nominally-significant meta-analytic associations of candidate SNPs with risks of recurrent VTE

GENE	SNP	RISK ALLELE FREQUENCY	META-ANALYTIC HR/OR (95%CI)	P VALUE
ABO	rs8176719 (O blood group)	0.42-0.50	1.15 (1.07-1.24)	<0.001
F5	rs6025 (Leiden)	0.07-0.10	1.23 (1.09-1.37)	0.001
F2	rs1799963 (G20210A)	0.02-0.03	1.36 (1.08-1.71)	0.01
F13	rs5985	0.74-0.77	1.10 (1.00-1.22)	0.047
FGG	rs2066865	0.28-0.33	1.08 (1.0-1.17)	0.04
STXBP5	rs1039084	0.55-0.58	1.16 (1.08-1.24)	<0.001
VWF	rs1063856	0.37-0.40	1.09 (1.01-1.17)	0.03

Conclusions: Several candidate SNPs are associated with the risk of recurrent VTE, including a novel finding in STXBP5. Given the modest individual effect size, determining these SNPs individually is unlikely to confer clinical utility. Future research should focus on the refinement and validation of genetic scores for recurrence.

OC 32.4 | A New Genetic Risk Score for Predicting Venous Thromboembolism Events in Cancer Patients Receiving Chemotherapy

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Background: Risk scores for prediction of VTE associated with chemotherapy (Chemo) show a low/moderate discrimination capacity. Thrombo inCode (TiC) is a tool for predicting the risk of VTE that combines a Genetic Risk Score (GRS) with clinical parameters of the patient.

Aims: To assess whether an adapted version of TiC (TiC-Onco) shows a better predictive capacity than the Khorana score (KS) to identify patients at high risk of VTE associated with Chemo.

Methods: Prospective, observational study including 406 patients with colon, stomach, pancreas and lung cancer receiving Chemo. Informed consent was obtained from all of the participants and the study was approved by a recognized medical ethics committee. The

analysis was performed at 6 months of followed-up. Three predictive models were compared: KS; KS+tumor stage and TiC. Prediction capacity was assessed in terms of AUC, LH+ and LH-.

Results: VTE incidence at 6 months was 18.8%. KS AUC was 0.569 (95% CI, 0502-0637, p = 0.065), sensitivity 21.33, specificity 81.54, LH+ 1.16, LH- 0.96. Discrimination increased significantly with TiC-Onco model AUC 0.751 (95% CI 0.687-0.816, p < 0.0001), sensitivity 73.0, specificity 66.26, LH+ 2.16, 0.41 LH-. TiC-Onco discrimination was significantly higher than KS (p < 0.001). 59 patients who were classified as low and intermediate risk by KS developed VTE. TiC would have identified 41 of them as high-risk for developing VTE.

Conclusions: TiC-Onco has a better predictive capacity to identify patients with a high risk of thrombosis associated with Chemo compared to KS.

OC 32.5 | Prothrombotic Genotypes and the risk of Major Bleeding Events in the First Year after Incident Venous Thromboembolism

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Background: Prediction of bleeding risk in patients with acute venous thromboembolism (VTE) is pivotal in decisions on duration of anticoagulant treatment (ACT). Prothrombotic genotypes are strongly predictive of venous thrombosis when combined in the de Haan-score, but it remains unsettled whether they protect against major bleeding (MB) during anticoagulation.

Aims: To investigate the impact of the pro-thrombotic genotypes (FVL [rs6025], F2 [rs1799963], ABO [rs8176719], FGG [rs2066865] and F11 [rs2036914]) individually and combined on bleeding risk.

Methods: Patients with a first VTE (n=674) aged 28 to 102 years, were sampled from the general population (The Tromsø study). Prothrombotic genotypes were determined in DNA isolated from whole blood. Patients with major bleeding according to ISTH criteria within one year after the VTE-diagnosis were identified through systematic review of medical records. Cox proportional hazards regression models were used to calculate hazard ratios (HRs) by individual and combined (de Haan 5-SNP score) risk alleles modeled continuously and in ordinal categories.

Results: Within one year of diagnosis, 60 (8.9%) patients had a bleeding event. No risk allele was individually associated with bleeding risk. In combined analyses (de Haan score) adjusted for age, sex and duration of anticoagulation, the hazard ratio per risk-allele increase was 0.98 (95% CI 0.78-1.25) and there was no trend for decreased risk of MB across increasing categories of risk alleles (0-1, 2, 3-4, and ≥ 5 risk alleles). There appeared some difference in the one-year cumulative incidence of MB between patients carrying 0-2 (7.4% (95% CI 5.1-10.6) and ≥ 3 (4.6% (95% CI 2.5-8.1) risk alleles according to de Haan score.

Conclusions: We found no clear association between individual and combined prothrombotic risk alleles and risk of major bleeding within the first year after a venous thrombotic event. These findings suggest that prothrombotic genotypes do not protect against iatrogenic bleeding.

OC 46.1 | Combined Effects of GP6 rs1613662 and Pre-cancer Platelet Count on the Risk of Cancer-related Venous Thromboembolism

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Background: Cancer is a strong risk factor for venous thromboembolism (VTE). GP6 encodes a glycoprotein collagen receptor that plays a key role in platelet function, and a missense mutation (rs1613662) in this gene is associated with VTE risk. Platelet count is associated with an increased risk of VTE in cancer patients but not in cancer-free subjects.

Aims: To study the joint effects of GP6 rs1613662 and cancer on risk of VTE stratified by pre-cancer platelet count.

Methods: Cases with a first VTE (n=629) and an age-weighted sub-cohort (n=1862) were recruited from three surveys of the Tromsø study (inclusions in 1994-95, 2001-02 and 2007-08, and follow-up through 2012). Rs1613622 was genotyped in DNA isolated from whole blood. VTEs were considered cancer-related if they occurred up to 6 months before or within 2 years after a cancer diagnosis. Pre-cancer platelet count was dichotomized using the median value ($246x10^9$/L). Cox regression was used to determine age- and sex adjusted hazard ratios (HR) for VTE by cancer status, risk alleles and platelet count.

Results: There were 167 cancer-related VTEs and 354 sub-cohort members were diagnosed with cancer during the study period. The GP6 G allele frequency was 0.15. Cancer patients with 1 or 2 risk alleles had an 11.9-fold (95% CI 8.6-16.9) and 14.2-fold (95% CI 6.3-32.0) increased risk of VTE, respectively, when compared to cancer-free subjects with no risk alleles. In cancer patients with platelet counts >$246x10^9$/L, the risk was 14.1-fold (95% CI 9.1-22.0) increased in those with 1 risk allele and 24-fold (95% CI 8.8-65.7) in those with 2, when compared to cancer-free subjects with no risk alleles. There was

no association between GP6 and VTE in cancer patients with platelet counts ≤246x10^9/L.

Conclusions: Our findings show an allele-dependent increased risk of VTE by GP6 rs1613662 in cancer patients with high platelet count. This suggests that both platelet count and platelet activity play a key role in the pathogenesis of VTE in cancer patients.

OC 46.2 | Incidence and Risk Factors for Central Venous Catheter-related Venous Thromboembolism in Breast Cancer Patients under (Neo)adjuvant Chemotherapy: The CAVECCAS Study

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Background: In Breast Cancer (BC) patients under (neo)adjuvant chemotherapy, higher risk of venous thromboembolism may be partly related to central venous catheters (CVC).

Aims: To analyse the incidence and risk factors for catheter related thrombosis (CRT).

Methods: The CAVECCAS study is a prospective, multicenter cohort of non-metastatic invasive BC patients undergoing insertion of a single lumen CVC. All patients underwent Doppler US before and 7, 30 and 90 days (D) after CVC insertion. Symptomatic CRT were objectively confirmed. A 6-month clinical follow-up was performed. D-Dimers, thrombin generation, platelet-derived microparticles (Pd-MPs) and Pd-MPs expressing phosphatidyl serin (Pd-MP/PS+) were measured before and at D2 after CVC insertion. Individual thrombophilia risk factors were analysed by a nested case-control study. All subjects provide informed consent to participate in the study which was approved by a recognized medical ethics committee.

Results: Among 524 patients, the overall CRT (14 symptomatic, 46 asymptomatic) incidence rate was 2.18 cases/100 patient-months. The CRT cumulative probability was 9.6% at 3 11.5% at 6 months. After CVC insertion, D-Dimers increased (p< 0.0001) with no significant increase in thrombin generation parameters, while Pd-MPs (p< 0.0001) and Pd-MP/PS+ decreased (p=0.021). Age >50 years (OR, 1.80; 95% CI, 1.01-3.22), BMI> 30 kg/m² (OR, 2.64; 95% CI, 1.46-4.76) and comorbidities (OR, 2.05; 95% CI, 1.18-3.56) were strongly associated with CRT. Using multivariate analysis, BMI>30 kg/m² (OR, 2.66; 95%CI, 1.46-4.84) and lobular carcinoma histology (OR, 2.56; 95%CI, 1.32-4.96) remained the only significant CRT risk factors. Thrombophilia did not account for CRT.

Conclusions: In this large prospective study of BC patients receiving chemotherapy, only clinical parameters identified high risk CRT patients who may be considered for CRT thromboprophylaxis.

OC 46.3 | Statin Use and Venous Thromboembolism in Cancer: A Large, Propensity Score Matched Cohort Study in the United States

J. Brown[1], S. El-Refai[2], V. Adams[3], P. Black[4], J. Talbert[2]

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Background: Statins have been shown to have a protective effect for venous thromboembolism in the general population suggesting a potential role for statins in VTE prevention.

Aims: To assess the association between statins and the risk for cancer-associated deep vein thrombosis (DVT) and pulmonary embolism (PE).

Methods: Patients with newly diagnosed cancer were identified and followed for up to 1 year in a national healthcare claims database. Three treatment groups were identified based on medication use in the 6-months prior to cancer diagnosis: statin users (treatment group), non-statin cholesterol lowering medication users (active control), and an untreated group. Pairwise propensity score matched groups were compared using competing risks survival models for DVT and PE outcomes reporting the hazard ratios (HR) between the treatment groups. Sensitivity analyses assessed the influence of dose intensity, age, and individual medications on outcomes.

Results: The total cohort included 287,107 patients, which, after matching, were similar on baseline characteristics within each treatment group comparison. The overall model showed a protective effect for statins compared to no treatment, which was attributed to leukemia (DVT, HR=0.77) and colorectal cancers (DVT HR=0.87, PE HR=0.83) when stratified.

There were no protective effects for PE.

With only moderate-to-high statin doses, renal cancers showed an additional protective benefit from DVT with statin use (HR=0.84). There were no differences in outcomes between statins and non-statins and no individual statins produced results different from the class effect.

Conclusions: In this large propensity score matched sample of patients with cancer, statins were shown to have a small protective effect in renal cancer, colorectal cancers, and leukemias for the risk of DVT but not for PE. The lack of effect was consistent across statin dose intensity and was also not found for any of the sensitivity analyses included.

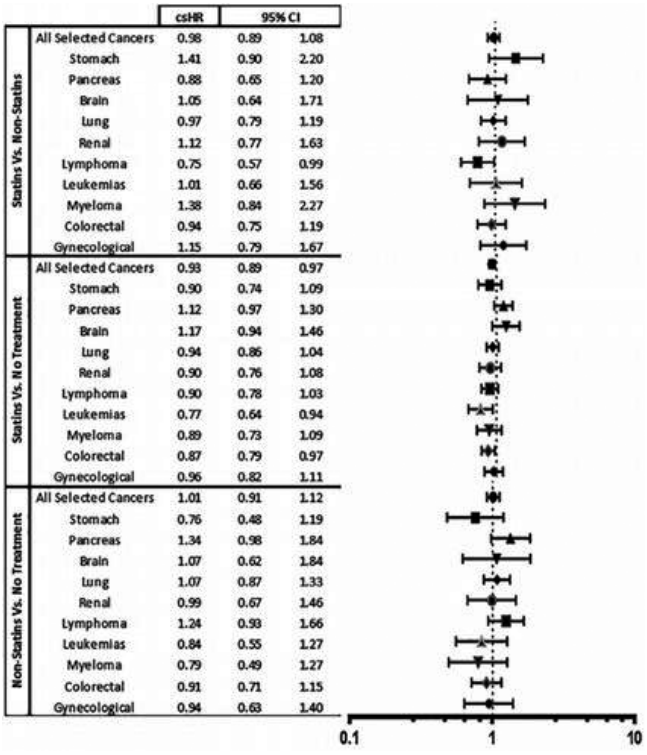

FIGURE 1 Statin treated, non-statin treated, and no treatment hazard ratios of the rate of deep vein thrombosis

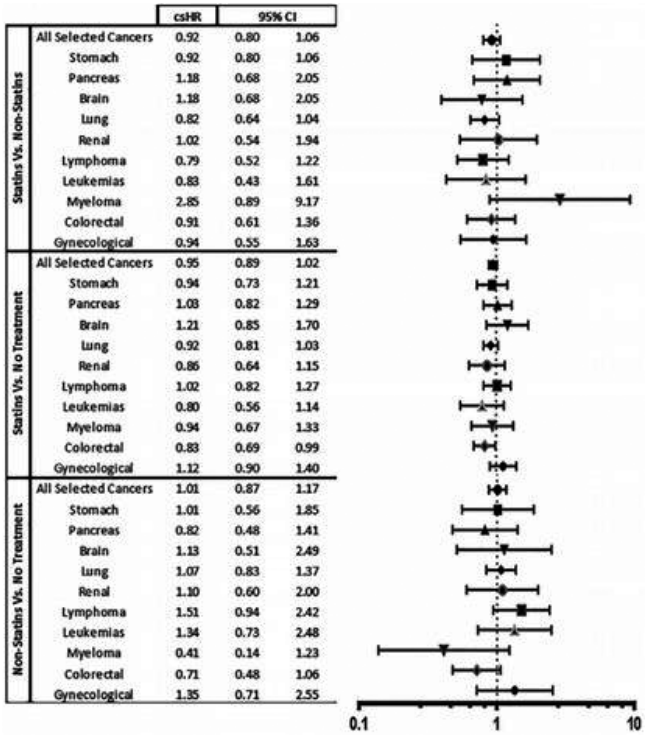

FIGURE 2 Statin treated, non-statin treated, and no treatment hazard ratios of the rate of pulmonary embolism

OC 46.4 | Arterial Thromboembolism in Patients with Cancer: Frequency, Risk Factors and Mortality

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Background: While the relevance of venous thromboembolism (VTE) in patients with cancer is well-recognized, much less is known about arterial thromboembolism (ATE) in these patients.

Aims: To describe the rates of ATE in patients with cancer, explore clinical risk factors and investigate all-cause mortality.

Methods: In this observational cohort study, we included patients with newly diagnosed malignant disease or progressive disease after remission. All patients were prospectively followed for a maximum of 2 years. Study endpoint was symptomatic ATE, which was adjudicated by a committee.

Results: 1387 patients (52.3% male; mean age: 59 years), recruited between Oct. 2003 and Sept. 2013, were included in this analysis. The median observation time was 1.8 years, 125 (9.0%) developed VTE, and 585 (42.2%) patients died during this time. 34 (2.5%, table 1) patients developed ATE within 2 years (13 [38.2%] had myocardial infarctions, 13 [38.2%] strokes and 8 [23.6%] peripheral arterial events). The cumulative 3-month, 6-months, 12 months and 24 months risk of ATE was 0.9% (95%CI: 0.5-1.5), 0.9% (0.5-1.6), 1.6% (1.0-2.4), and 2.6% (1.8-3.5), respectively. In univariable competing risk regression analysis, male sex (subdistribution hazard ratio [SHR]=2.2, 1.1-4.6, p=0.04), higher age (SHR per 10 year increase=1.8, 1.4-2.3, p< 0.001), lung cancer (SHR=2.8, 1.4-5.6, p=0.005), and renal cell carcinoma (SHR=3.5, 1.1-11.5, p=0.04) were associated with a higher ATE risk. In multivariable analysis, lung cancer (SHR=2.9, 1.4-6.2, p=0.005) and higher age (SHR per 10 years increase=1.8, 1.4-2.4, p< 0.001) were independently associated with ATE. In multistate modeling, the occurrence of ATE was associated with a 2.6-fold increased risk of mortality from any cause (hazard ratio [HR]=2.6, 95%CI: 1.6-4.3, p< 0.001).

Conclusions: In contrast to VTE, ATE is a less frequent complication in patients with cancer. Patients with cancer who develop ATE are at an increased risk of mortality.

TABLE 1 ATE rate in different tumor types

Primary Organ	No. of Patients (%)	No. of Patients with ATE (%)
Lung	233 (16.8)	12 (5.2)
Breast	228 (16.4)	0 (0.0)
Brain	193 (13.9)	4 (2.1)
Haematologic Malignancies	175 (12.6)	2 (1.1)
Gastric & Pancreatic	155 (11.2)	3 (1.9)
Colorectal	153 (11.0)	2 (1.1)
Kidney	37 (2.7)	3 (8.1)
Others	213 (15.4)	8 (3.8)

OC 46.5 | Effect of Low Molecular Weight Heparin on Survival of Patients with Resected Non-small Cell Lung Cancer: The Tinzaparin in Lung Tumors (TILT) Randomized Phase III Trial

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Background: The antitumoral effects of low-molecular weight heparins remain controversial in the clinical setting. Most previous studies included various cancer types and/or advanced cancers.

Aims: To assess the effect of tinzaparin on overall survival (OS) of patients with non-metastatic resected non-small cell lung cancer (NSCLC).

Methods: Patients with completely resected stage I, II or IIIA NSCLC were randomized within 8 weeks of surgery in a multicenter open trial with blinded adjudication of outcomes (NCT00475098). Randomization was stratified on pathological stage (I vs II-III). Patients in both groups received usual care, patients in the experimental group received subcutaneous tinzaparin 100 IU/kg once a day for 12 weeks. The primary endpoint was OS. Follow-up was planned until 3 years after the inclusion of the last patient.

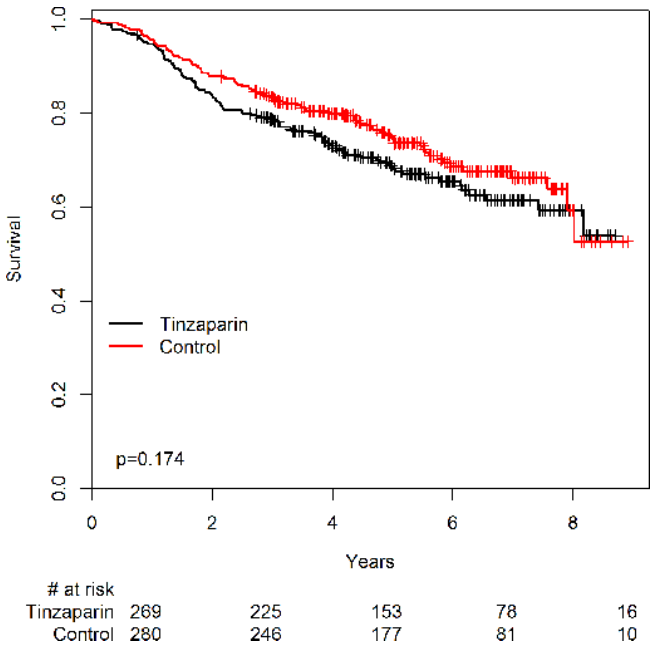

FIGURE Overall survival after randomisation

Results: A total of 553 patients were included, 4 withdrew consent, the remaining 549 patients were randomized to tinzaparin (n= 269) or control (n= 280). Mean age ±SD was 61.6±8.9 years, 356 patients (64.8%) were men, 358 patients (65.2%) had adenocarcinoma, 359 (65.4%) and 190 patients (34.6%) had pathological stages I and II-III, respectively. Median follow-up was 5.7 years. OS was not significantly different between groups (Hazard ratio [HR]=1.24; 95% confidence Interval: 0.92-1.68, p=0.17, figure). In a preplanned subgroup analysis, there was no significant difference in OS between groups in patients with stage I disease (HR=0.97; 0.64-1.47, p=0.90); in patients with stage II-III disease, OS was significantly lower in the tinzaparin group (HR=1.61; 1.03-2.54, p=0.04) but disease-free survival was not significantly different (HR=1.01; 0.64-1.59, p=0.97), and cancer-specific mortality was similar (28/95 *vs* 24/95 patients in the tinzaparin and control groups, respectively).

Conclusions: After complete resection of stage I-IIIA NSCLC, tinzaparin on top of usual care had no detectable impact on overall survival and disease-free survival.

OC 48.1 | Venous Thromboembolism Risk Assessment in 2214 Relatives from 651 Families with Known Thrombophilia Defects

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Background: Clinical assessment of venous thromboembolism (VTE) relies on several biomarkers including thrombophilia screening based on 5 defects (antithrombin (AT), protein C (PC), protein S (PS) deficiencies, factor V Leiden (FVL) and prothombin mutation (PTM)). However the clinical VTE pattern often shows wide heterogeneity within relatives of a VTE affected family despite they carry the same thrombophilia defect.

Aims: To assess whether common risk factors for VTE explain the clinical VTE heterogeneity observed in families with inherited thrombophilia.

Methods: Individuals were recruited at the Marseille Hospital Center between 1990 and 2013. A thrombophilia screening was systematically performed together with ABO blood group determination and genotyping of 11 polymorphisms selected because they were reported to associate with VTE in the general population. Individuals were split into 3 groups according to the thrombophilia screening: no defect (group 1), mild thrombophilia (FVL heterozygous or PTM heterozygous = group 2) and severe thrombophilia (AT, PC, PS deficiencies, FVL homozygous, PTM homozygous, combined defects = group 3). A multivariate survival analysis using Cox's regression was performed from which a score was derived.

TABLE 1 Survival multivariate analysis. 1Body mass index. Reference groups used for estimating hazard ratio = No thrombophilia (group 1), F11 rs20369

	N (%)	Hazard Ratio (95% CI)	p-value		N (%)	HR (95% CI)	p-value
Mild thrombophilia (group 2)	793 (47.3)	2.29 (1.45-3.62)	0.0004	BMI1 30-35 kg.m-2	103 (6.1)	1.79 (1.16-2.77)	0.008
Severe thrombophilia (group 3)	184 (11.0)	5.45 (3.32-8.93)	<10-4	BMI ≥ 35 kg.m-2	34 (2.0)	2.69 (1.24-5.82)	0.01
F11 rs2036914 - CT/CC	1266 (75.5)	1.63 (1.06-2.51)	0.03	Current smoking	403 (24.0)	1.83 (1.25-2.67)	0.002
FGG rs2066865 - CT	616 (36.8)	1.67 (1.18-2.35)	0.004				
FGG rs2066865 - TT	133 (7.9)	2.04 (1.20-3.45)	0.008				
Blood group A or B	1026 (61.2)	1.37 (0.93-2.02)	0.11				
Blood group AB	94 (5.6)	2.68 (1.52-4.74)	0.0007				

A trend towards an increased incidence of VTE was observed according to the score: from 0 to 10.7 per 1000 PY ($p=2.3.10^{-8}$).

TABLE 2 VTE incidence according to the score derived from multivariate analysis

Score	Number of events	Follow-up (years)	Incidence (per 1000 person-years)	p-value
0	0	2046	0	
1	13	10769	1.21	
2	44	18365	2.40	
3	55	17068	3.22	
4	26	5879	4.42	
5	9	1406	6.40	
6-7	4	373	10.72	2.3.10-8

Of note this increase held in the different groups of thrombophilia severity. The incidence raised 31.2 per 1000 PY in group 3 relatives with a score of 6-7.

Results: 2214 relatives from 651 families were included among whom 246 (11%) had a personal VTE history. VTE incidence was 1.2, 2.9 and 5.4 per 1000 person.years (PY) in groups 1, 2 and 3 respectively ($p=9.7.10^{-12}$). Obesity, smoking, ABO blood group, F11_rs2036914 and FGG_rs2066865 were statistically associated with VTE and included in a score.

Conclusions: Taking into account common environmental and genetic risk factors appeared to improve VTE risk assessment in relatives from families with thrombophilia.

OC 48.2 | Coagulation Factor 12 and the Risk of Recurrent Venous Thromboembolism: A Prospective Cohort Study

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Background: Factor 12 (F12) plays a major role in the activation of the contact system. Low F12 does not cause bleeding. High F12 confers an increased risk of ischemic stroke in young women, is protective against myocardial infarction in men, and is not related to first venous thromboembolism (VTE). F12 is considered a promising target for further anticoagulant strategies.

Aims: To assess the relationship between F12 and the risk of VTE in high risk patients, i.e. in patients with first, unprovoked VTE.

Methods: We followed patients with a first, unprovoked VTE after anticoagulation withdrawal for an average of 6 years. We excluded patients with major thrombophilia, cancer or requirement for indefinite anticoagulation for other reasons. Study endpoint was symptomatic recurrent VTE. We used cumulative incidence methods to estimate the VTE recurrence risk and uni- and multivariate Cox proportional-hazards models to analyze the association between F12 and recurrence risk.

TABLE 1 Relativerisk of recurrent VTE according to quartiles of F12

F XII (%)	Pt (n)	Rec (n)	HR (95% CI)	HR (95% CI) adjusted for age, sex, VTE site, F5 Leiden, F 2 G20210A
< 91	199	56	1.0 (ref)	1.0 (ref)
> 91-111	199	73	1.2 (0.8-1.2)	1.1 (0.7-1.5)
> 111-132	212	64	1.0 (0.7-1.1)	1.0 (0.7-1.4)
> 132	208	72	1.1 (0.8-1.2)	1.1 (0.7-1.5)

Results: We included 818 patients (66% men, mean age 53 years) with F12 between 24% and 212%. 265 patients (32%) had recurrence. The hazard ratio (HR) of recurrence was 1.0 (95% CI 0.99-1.01) for each 1% increase of F12 and was unchanged after adjustment for age, sex, VTE site, F5 Leiden and F2 G20210A. The cumulative incidence of recurrence was similar when patients were compared according to F12 quartiles (Figure 1). Compared to patients with lowest F12 (< 91%), those with higher levels did not have an increased recurrence risk (Table 1). The recurrence risk among patients with F12 >153% (90^{th} percentile) or < 73% (10^{th} percentile) was comparable to those with lower/higher F12 [HRs 0.9 (0.6-1.3) and 1.1 (0.7-1.7)].

Conclusions: F12 levels do not affect the recurrence risk in patients with a first, unprovoked VTE.

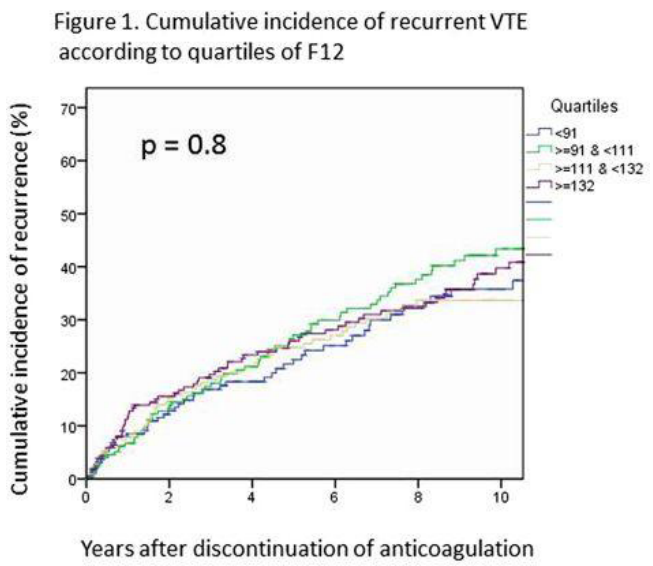

FIGURE 1 Cumulative incidence of recurrent VTE according to quartiles of F12

OC 48.3 | Indications and Outcomes after IVC Filter Insertion for Primary Prophylaxis of Pulmonary Embolism: A Population-based Cohort Study

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Background: Inferior vena cava filter insertion (**filter**) for primary prevention of pulmonary embolism (**PE**) in patients without venous thromboembolism (**VTE**) is of unproven efficacy or safety.

Aims: To determine indications and estimate outcomes after a filter placement for primary VTE prophylaxis among Olmsted County (OC), MN residents, 1996-2015.

Methods: Using Rochester Epidemiology Project resources, we identified all OC residents with a filter, reviewed their complete medical records in the community and recorded demographic and clinical characteristics, type of filter placed and outcomes, including filter retrieval and complications, survival and cumulative VTE incidence. Age-, sex-specific VTE incident rates of the general OC population were used to calculate the expected number of VTE events and the standardized morbidity (i.e., VTE) ratio (95% CI).

Results: Over the 20-year period, 134 residents received a filter (85% retrievable) for primary VTE prophylaxis (**Figure**). Trauma (92%) was the most common indication (**Table**). Twenty-nine residents developed VTE (27 DVT; 2 PE). One-year survival and 3- and 12-month VTE cumulative incidence were 89%, 16%, and 21%, respectively. The standardized morbidity ratio for VTE was 47.1 (95% CI: 31.5, 67.6). Of 115 filters eligible for retrieval, 78 were removed, removal failed in 3, 9 transitioned to a permanent filter, 15 lacked documentation of removal, and 10 patients died or had persisting contraindications to anticoagulant use. Filter complications (n=2; 1% incidence at 3 months) included strut embolization with cardiac tamponade and duodenal perforation.

Conclusions: IVC filter insertion for primary prophylaxis was performed mainly post-trauma. Subsequent incident VTE was 47-fold higher than the general OC population of similar age and sex. Filter complications were rare but life-threatening. Conditional on surviving 14 days, 34% of potentially retrievable filters had not been removed at one year.

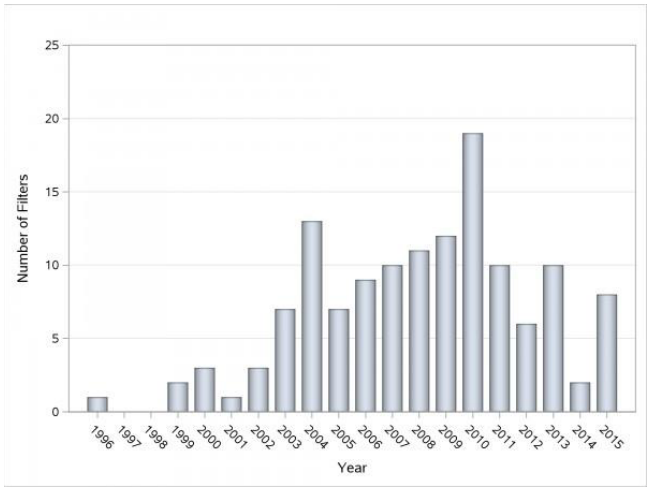

FIGURE Annual Number with IVC Filter Insertion for Primary VTE Prophylaxis Among Olmsted County, MN Residents, 1996-2015

TABLE Demographic and Baseline Clinical Characteristics of Olmsted County, MN Residents with IVC filter insertion for Primary Prophylaxis (n=134), 1996-2015

Patient age, mean ± SD (range)	45.1 ± 21.0 (16 - 89)
Male gender, n (%)	85 (63%)
BMI, kg/m2; median (range)	27.4 (17.8 - 52.3)
Trauma†	123 (92%)
Chronic cardiac disease, n (%)	22 (16%)
Chronic pulmonary disease, n (%)	16 (12%)
Active cancer, n (%)	5 (4%)
Therapeutic anticoagulation, n (%)	2 (2%)
Aspirin use, n (%)	21 (16%)

OC 48.4 | Derivation and Validation of a Prognostic Model for VTE Development in Outpatients Setting: A Nested Case-control Study

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Background: Consistent data have suggested that most episodes of venous thromboembolism (VTE) occurred in outpatients setting. Unfortunately, only a few studies have evaluated factors potentially associated with an increased risk of VTE in this setting and, to date, no score potentially able to identify the group of patients who may deserve an antithrombotic prophylaxis has been developed.

Aims: To assess potential risk factors for VTE in the outpatients setting.

Methods: Using the Health Search - IMS Health Longitudinal Patients Database, we followed a cohort of consecutive adult patients until the occurrence of one of these events, whichever came first: VTE (DVT and PE; event date), death from any cause, end of registration with GP, or end of the study period. The cohort was randomly divided into two cohorts containing approximately two-third (development) and one-third (validation) of patients, respectively; the presence of potential risk factor for VTE in cases and in controls was compared and a clinical score was developed and validated. Several sensitivity analysis were performed

Results: A total of 1,359,880 patients (53.4% women) met the study inclusion criteria. During follow-up, we identified 16317 cases of VET, yielding an overall incidence rate of 1.38 (95% CI: 5.0-6.4) per 1000 person-years. When the score was categorized in deciles and applied to the validation cohort, it was able to explain 27.9% of the variation for VTE occurrence. In terms of discrimination, AUC was 0.82 (95% CI: 0.82-0.83). The calibration measure revealed a margin of error lower than 10% in the 70% of the population. Results of sensitivity analyses substantially confirmed the finding of principal analysis.

Conclusions: Our clinical score, developed and validated, on a large population of outpatients demonstrated a good accuracy in predicting the risk of developing a VTE in this setting.

OC 48.5 | Increased Incidence of Venous Thromboembolism in Californians with Severe Asthma

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Background: Chronic inflammatory diseases are associated with activation of coagulation and increased risk of venous thromboembolism (VTE). Asthma is a chronic inflammatory disease of the airways, marked by acute exacerbations. Severe asthma patients have a prothrombotic state as defined by higher biomarker levels of hemostasis and inflammation. There are limited population-based studies determining the association of asthma and VTE.

Aims: Determine the incidence of VTE in patients with asthma exacerbation requiring emergency department (ED) or in-patient care.

Methods: Using ICD-9 codes, we identified a cohort of adult patients (> 18 years) with a diagnosis of asthma during 2005-2013 in the California Patient Discharge Dataset and Emergency Department Utilization Dataset. We calculated incidence rates of VTE and used Cox proportional hazards regression to analyze factors associated with VTE and the association of VTE with mortality. Results are presented as adjusted hazard ratios (HR) and 95% confidence intervals (CIs).

Results: There were 387,860 asthma patients with 4,433 VTE events. The incidence rate of VTE was 226 per 100,000 person-years. Of the VTE events, 57.6% (n=2,553) were pulmonary emboli, 19.1% were proximal deep vein thromboses (DVT), 12.8% were distal DVT, and 10.5% were DVTs of the lower extremity not otherwise specified. Almost all (94.4%) of the VTE events were unprovoked. Compared to Non-Hispanic Whites, African Americans had a higher risk (HR=1.22, CI: 1.11-1.34) and Hispanics (HR=0.71, CI: 0.64-0.78) and Asians (HR=0.51, CI: 0.43-0.61) had a lower risk of VTE. VTE was associated with an increased risk of death (HR=2.26, CI 2.00-2.55).

Conclusions: In this large cohort of asthma patients, the incidence rate of VTE was twice as high as previously described in the general population. These data suggest adult asthma patients with acute exacerbations requiring ED or inpatient care are at a higher risk of VTE, which may increase their risk of mortality.

PEDIATRICS

OC 11.1 | Development of a Predictive Risk Model for Deep Vein Thrombosis in Pediatric Oncology Patients

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Background: Pediatric oncology patients are at increased risk for deep vein thrombosis (DVT). Determining a predictive model to identify DVT risk is critical in order to target prophylactic interventions to children at risk.

Aims: To develop a predictive model for DVT in pediatric oncology patients using single nucleotide polymorphisms (SNPs) and clinical variables.

Methods: A population based nested case control study in 7 Canadian centers was conducted. SNPs were selected from candidate SNPs in 33 hemostasis-related genes and a concurrent genome wide association study (GWAS). To build a logistic risk model, the following clinical

variables were included: age, blood group, cancer diagnosis and type of chemotherapy received. In a first step, relevant predictors were determined by backward logistic regression in the initial sample set based on the minimized AIC (Akaike information criterion). Additionally, 10 SNPs were selected from the GWAS and candidate gene association analysis of DVT in pediatric oncology patients. Then, coded genotypes were added as predictors to the dataset, resulting in a combined dataset of 312 subjects (105 cases, 207 controls). Cross validated logistic regression was used for model training and backward feature selection in a training set, which was 70% of the subjects, as implemented in the ´caret´ R-package.

Results: The best model was selected with respect to AIC and contains the SNP genotypes of rs1800378, rs2028002, rs9332653, rs34631763, rs7837156 and rs10764406 as well as clinical variables. When applying the model to the remaining 30% of subjects in the test sample set, we determined an area under the ROC-curve (AUC) of 0.80 (0.74-0.86) and an accuracy of 0.7204 (for predictive risk threshold of 0.5). The model has a positive predictive value of 0.60 and negative predictive value of 0.75.

Conclusions: Using SNPs found to be associated with DVT in pediatric oncology patients, we propose a model for prediction of DVT risk in children undergoing chemotherapy.

OC 11.2 | External Validation of a Venous Thromboembolism Risk Prediction Tool for Critically Ill Children - The Cleveland Score

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Background: Pediatric venous thromboembolism (VTE) in critically ill children is associated with increased mortality, prolonged length of stay, and decreased ventilator-free days. We developed the "Cleveland Score" model to predict the risk of developing VTE in critically ill children with central venous catheters (CVCs). A validated bedside prediction tool may help improve outcomes through identification of patients benefiting from screening and prophylaxis.

Aims: To perform an external validation of the Cleveland Score using a cohort of critically ill children with central venous catheters.

Methods: With IRB approval, the Virtual Pediatric Systems, LLC database was interrogated for children < 18 years old admitted to pediatric intensive care units (PICU). All children had a CVC. VTE that were "active" but not "present on admission" were included. The "Cleveland Score" model was derived by logistic regression using data from 01/2009-09/2014. The major categories include age, sex, race, primary diagnosis, type of surgery, past medical history and CVC type. The model was validated using data from 10/2014-9/2016. Statistical measures included calculating the area under ROC curve, the concordance index and Brier score.

Results: The derivation cohort included 158,299 children of which 1623(1.0%) developed VTE. The validation cohort contained 57,976 patients of which 834(1.4%) patients developed VTE. Utilizing the validation cohort, this model had a concordance index of 0.782 (95% CI: 0.767, 0.798)(Fig. 1)

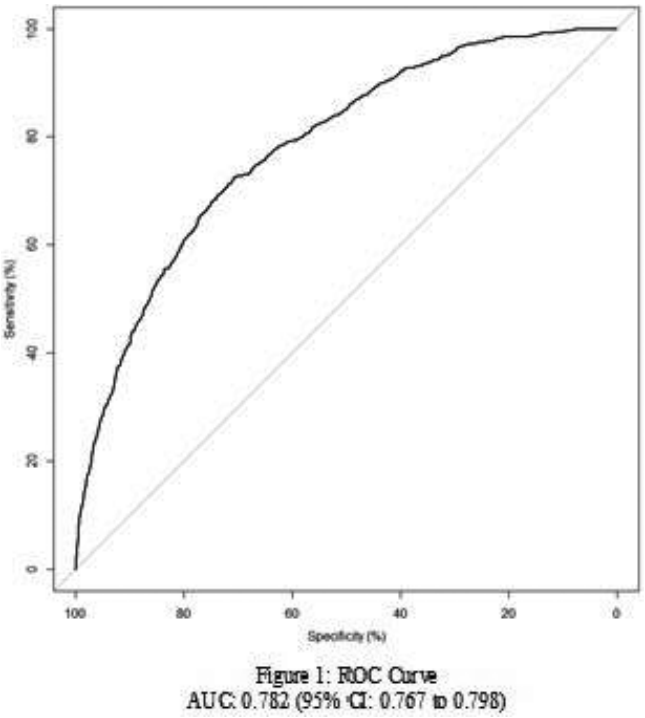

FIGURE 1 ROC Curve

and a Brier score of 0.0140. A score of 0 indicates a perfect association between the predicted and actual event. Predicted and actual VTE rates in the derivation and validation cohort are shown in Table 1.

TABLE 1

Risk Category	Predicted VTE risk	Derivation Cohort (n=158,299)		Validation Cohort(n=57,976)	
		n	Actual VTE(%)	n	Actual VTE (%)
Normal Risk	<0.3%	52973	73 (0.14%)	35411	179 (0.51%)
Moderate Risk	0.3%-0.7%	51788	204 (0.39%)	12886	200 (1.55%)
High Risk	>0.7%	53538	1346 (2.51%)	9679	455 (4.70%)
C-Statistic(95% CI)		0.826 (0.807-0.844)		0.782 (0.767-0.798)	

Analyzing specific groups within the dataset, the AUC of post-operative patients, cardiovascular diagnosis, and PICC lines, were 0.829, 0.865 and 0.745 respectively.

Conclusions: The Cleveland Score is externally validated and performs well to predict VTE in critically ill hospitalized children with CVCs.

OC 11.3 | External Validation of Risk Prediction Models for Catheter-associated Thrombosis in Critically Ill Children

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Background: Risk prediction models for central venous catheter (CVC)-associated thrombosis in critically ill children were recently developed and internally validated using recursive partitioning (Model A) and logistic regression (Model B) methods with data from 3 centers in the United States (derivation cohort). The external validity of these models is unclear.

Aims: To externally validate the risk prediction models using data from critically ill children admitted to an Australian center.

Methods: Secondary analysis was performed in a prospective cohort study of critically ill children requiring a CVC (validation cohort). Predictive variables in the models (i.e., recent surgery, predicted risk of mortality, age, blood transfusion within 24H of CVC insertion and CVC location) were extracted from the study database. Discrimination and calibration of the models were expressed as areas under the receiver operating characteristic curves (AUROC) and Hosmer-Lemeshow chi-square test statistic, respectively.

Results: Data from 146 children (median age, 8 mo.) were used. A total of 32 children developed CVC-associated thrombosis. Both models had poor discrimination (AUROC-Model A: 0.44 [95% CI: 0.24-0.54]; Model B: 0.49 [95% CI: 0.37-0.61]). Model A (chi-square: 22.54, p< 0.001), but not Model B (chi-square: 14.01, p=0.12), had poor calibration. Stratification based on heart disease, suggested that Model B had better discrimination in those without heart disease (AUROC: 0.59 [95% CI: 0.40-0.79] vs. 0.40 [95% CI: 0.26-0.54]).

Conclusions: External validation should be performed to confirm the external validity of risk prediction models. The models for CVC-associated thrombosis performed poorly when validated in this Australian cohort, likely reflecting demographic variance between cohorts. Refining the risk prediction model using a larger cohort of children with a breadth of demographic variables is the next step in developing of a robust risk prediction model.

OC 11.4 | Thrombophilia in Children to Predict Recurrent Line-related Thrombosis: Time to Choose Wisely

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Background: The role of thrombophilia to predict recurrent line-related deep vein thrombosis (LR-DVT) with subsequent line placements after a first LR event in children remains unclear.

Aims: To investigate the association between thrombophilia and recurrent LR-DVT.

Methods: Children with a first episode of objectively confirmed LR-DVT from 1998 to 2014 and complete thrombophilia testing were included in the study. Recurrent (local or distant) LR-DVT with subsequent line placements was the main outcome. Thrombophilia was classified as minor (heterozygous FVL/PTG, high Lp(a), high FVIII levels), major (AT, PS, PC deficiency, ACLA/LAC+, homozygous FVL or PTG), or none. Analysis was conducted using logistic regression with a generalized estimating equation approach for correlated data. Ethics approval was obtained.

Results: 201 patients had 858 central venous lines (CVL, median 3 CVL/patient, range 1-23 lines) placed for a median of 4 days (range 1-1794 days); 59% were males. The most common underlying conditions were cardiac disease (35%), cancer (16%), and complex defects (14%). In 32% of the CVLs, patients did not receive anticoagulation (AC); in the remaining cases, patients received AC (either prophylaxis or treatment). 66% of the lines were temporary CVLs and 26% were PICCs. Whereas 78% of children had negative thrombophilia tests, 16% had minor and 6% had major thrombophilia.

24% of patients (49/201) had at least one recurrent LR-event (5% local, 17% distant, 2% both). Only time of line permanence was associated with recurrent LR-events (OR 1.01 for every 10 days of line permanence; 95% CI 1.00-1.02, p=0.04). Conversely, thrombophilia, age at the time of line insertion, sex, underlying condition, year of line insertion, type of line (PICC vs CVL) and AC (prophylaxis or treatment) were not.

Conclusions: Thrombophilia was not predictive of recurrent DVT during subsequent line insertions in this cohort of children with previous LR-DVT, suggesting that thrombophilia testing in these patients is not warranted.

OC 11.5 | Predictive Factors for Central Venous Catheter-related Thrombosis and Long Term Sequelae

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Background: Elevated D-dimer and Factor VIII levels are reported to predict poor outcomes, such as post thrombotic syndrome (PTS), in children with known thrombosis. Recently, an American cohort showed Factor VIII activity was predictive of asymptomatic central venous catheter (CVC)-related thrombosis in critically ill children.

Aims: To determine if D-dimer, Factor VIII levels and the probability of death score (pim2) are predictive of asymptomatic CVC-related thrombosis and PTS in critically ill children.

Methods: A prospective cohort study recruited children admitted to a paediatric intensive care unit (PICU) requiring a CVC. The study was approved by the hospital ethics committee and informed consent was obtained. Participants had a (blinded) ultrasound of the blood vessel in which the CVC was placed, plasma collection and clinical data was collected (phase I). PTS assessment and an ultrasound were performed approximately 24 months after CVC insertion (phase II).

Results: Neither Factor VIII, D-dimer or the pim2 score were predictive of CVC-related thrombosis in our cohort (Table 1). There was a statistically significant difference in Factor VIII and D-dimer levels between children with CVCs placed in their femoral veins compared to those with jugular CVCs (Table 2). No increased risk of clinically significant PTS was found related to D-dimer levels (p=0.2, OR 1.029 (0.66 - 1.57)) and pim2 scores (p=0.1).

TABLE 1 ^Mann Whitney U test; >Paediatric Index of Mortality 2

Factors	Normal	Thrombus	P value (2 tailed)	Odds ratio (95% CI)	P value for OR
Phase I					
Factor VIII	36	9	0.5^	0.99 (0.98 - 1.00)	0.2
D-dimer	111	32	0.2^	1.06 (0.9-1.26)	0.5
PIM2 score>	109	31	0.1^	1.02 (0.99-1.04)	0.09
Phase II					
Factor VIII	28	4	0.8^	1.0 (0.9-1.01)	0.9
D-dimer	101	15	0.1^	0.9 (0.58-1.4)	0.6
PIM2 score>	98	16	0.1^	1.02 (0.98-1.05)	0.4

TABLE 2 SD: standard deviation; *Mann Whitney U, >Paediatric Index of Mortality 2

	Jugular CVC		Femoral CVC		P value
	n	Median(range)/ Mean(SD)	n	Median(range)/ Mean(SD)	
D-dimer	150	0.59 (0.27-10.83)	35	2.35 (0.27-20.0)	<0.001*
Factor VIII	48	160.5 (59-516)	8	259 (116-399)	0.007*
PIM2 score> (%)	146	1.92 (0.14-100)	34	3.2 (0.30-90.6)	0.035*

Conclusions: This study shows D-dimer, Factor VIII and pim2 scores not predictive of acute CVC-related thrombosis or PTS in critically ill children. Children with femoral CVCs represented a subgroup with overall higher acuity shown by a significantly higher pim2 score, elevated D-dimers and factor VIII levels. These characteristics among children with femoral CVCs in PICU indicate a higher risk of mortality and could be useful in future studies identifying populations at risk of thrombosis.

OC 64.1 | Extracorporeal Membrane Oxigenation (ECMO) in Neonates and Children: Prospective Single Center Study of Argentina

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Background: ECMO is used in patients(pts) with severe respiratory(r) or cardiac(c) failure. Thrombotic and hemorrhagic complications are major causes of morbidity and mortality. The hemostasis disturbance assessment and antithrombotic therapy (ATT) are topics of debate.

Aims: To describe clinical characteristics, ATT and outcomes of a cohort of pts supported with ECMO at a tertiary care center.

Methods: From Jan 2010 to Dec 2016, consecutive pts < 18y old on rECMO or cECMO were studied. ATT was based on ELSO guidelines. Demographic, clinical and laboratory (lab) data, therapy and outcomes were prospectively registered. Data are expressed in median(range).

Results: 119pts with venoarterial ECMO were recruited. 4pts treated for < 12hours were excluded. rECMO:54pts (47%), 30males (56%), age:2d (7h-146d), 33pts (61%) had preoperative diaphragmatic hernia, days on ECMO :5 (1-26). Antithrombin replacement (ATR):41pts (76%). Unfractioned heparin (UFH)doses and lab data are shown in Table 1. aXa correlated with UFH doses (r=0.27, p=0.0008) whereas ACT did not (r=0.06, p=0.5052). Within therapeutic ranges, ACT/aXa were concordant 21.6% of times and ACT/APTT 2.8% of times. Intracranial hemorrhage (ICH) 16pts(30%), thrombosis18pts (33%). Survival 31pts (57%). cECMO: 61pts (53%),34males (56%),age7,6m (1d-174m),45pts (74%) had postoperative congenital heart disease, days on ECMO:4(1-12), ATR 33pts(53%), UFH dose and lab data are shown in Table 2. aXa correlated with UFH dose (r=0.42,p=< 0.001) whereas ACT did not (r=0.15, p=0.1398). Within therapeutic ranges, ACT/aXa were concordant 8.5% of times and ACT/APTT 11.2% of times. Major bleeding 29pts (48%),27/29 from surgical site; thrombosis 13pts (21%).Survival 33pts (54%).

TABLE 1 Respiratory ECMO: UFH doses and laboratory results in median (range)

	UFH doses (U/Kg/h)	Activated clotting time (ACT) (sec)	anti-Factor Xa activity level (aXa) (IU/mL)	Activated partial thromboplastin time (APTT) (sec)	Platelet count ($x10^9L^{-1}$)	Prothrombin time (%)	Fibrinogen (mg/dL)	Factor V (%)	Antithrombin (%)
Day 1 n=54	48 (5-90)	189 (165-575)	0,7 (<0,1-2,8)	240 (50->300)	110 (93-309)	40 (10-55)	175 (100-345)	36 (17-126)	34 (10-110)
Day 2 n=53	60 (20-87)	184 (165-240)	0,8 (0,2-2,0)	233 (84->300)	110 (50-183)	53 (10-84)	210 (139-608)	61 (19-136)	46 (23-101)
Day 3 n=48	55 (20-97)	182 (152-202)	0,7 (<0,1-1,9)	112 (57->300)	113 (35-211)	63 (24-92)	224 (157-409)	74 (24-217)	59 (33-100)
Day 4 n=37	53 (22-83)	185 (175-196)	0,8 (0,1-1,9)	199 (74->300)	94 (49-153)	61 (41-99)	248 (83-510)	80 (43-180)	56 (14-96)
Day 5 n=27	45 (20-90)	186 (161-200)	0,7 (0,2-1,4)	180 (37->300)	97 (69-177)	65 (49-98)	252 (146-419)	80 (47-122)	60 (31-111)

TABLE 2 Cardiac ECMO: UFH doses and laboratory results in median (range)

	UFH doses (U/Kg/h)	Activated clotting time (ACT) (sec)	anti-Factor Xa activity level (aXa) (IU/mL)	Activated partial thromboplastin time (APTT) (sec)	Platelet count ($x10^9L^{-1}$)	Prothrombin time (%)	Fibrinogen (mg/dL)	Factor V (%)	Antithrombin (%)
Day 1 n=61	15 (5-72)	177 (135-229)	0,2 (<0,1-2,2)	102 (42->300)	105 (18-174)	39 (16-76)	201 (60-464)	35 (13-94)	39 (10-70)
Day 2 n=53	20 (5-51)	177 (101-250)	0,3 (<0,1-1,2)	100 (37->300)	97 (18-301)	50 (21-96)	240 (110-464)	50 (14-118)	41 (10-83)
Day 3 n=44	23 (8-72)	174 (125-250)	0,3 (<0,1-2,0)	109 (41->300)	87 (31-144)	58 (18-86)	264 (76-536)	67 (17-145)	48 (11-78)
Day 4 n=34	27 (5-45)	171 (149-200)	0,4 (<0,1-1,3)	122 (42->300)	77 (18-143)	62 (35-89)	275 (76-554)	76 (29-123)	54 (23-100)

Conclusions: In this cohort, most of the pts on ECMO survived. However, high rates of bleeding and thrombosis were observed. ICH and surgical site bleeding were the most frequent hemorrhagic complications in neonates on rECMO and in children on cECMO respectively. aXa assay was a superior correlate of heparin doses than APTT or ACT.

OC 64.2 | Can Coagulation Parameters Guide the Way in Neonatal and Pediatric Extracorporeal Membrane Oxygenation?

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Background: Coagulation problems complicate extracorporeal membrane oxygenation (ECMO), a lifesaving procedure for severely respiratory or circulatory compromised children. There is no international evidence-based guideline for the management of coagulation during ECMO.

Aims: To outline the coagulation complications that arise during neonatal and pediatric ECMO in a high-volume ECMO center. Secondly, to analyze coagulation parameters with respect to complications and survival.

Methods: Analysis of prospectively collected data in 79 children treated with ECMO between September 2011-August 2015. In our protocol, anticoagulation with unfractionated heparin was monitored with APTT. Values of ACT, APTT, fibrinogen, d-dimers and platelet count and administered heparin and blood products during ECMO were collected. Complications were defined according to the Extracorporeal Life Support Organization. The Mann-Whitney U test was used to compare groups.

Results: Eighty ECMO runs were performed in 35 neonates and 44 children (0-16.8 yrs). The median duration of ECMO runs was 120.5 hours (IQR 190.0). As shown in table 1, clotting (CC) or hemorrhagic complications (HC) occurred in 43 (53.8%) of all patients with 40 CC in 30 ECMO runs and 44 HC in 33 ECMO runs. Neonates and children did not significantly differ. Ten patients died after ECMO-treatment was considered futile due to HC. Patients who developed CC or HC were on ECMO for a longer duration (p=0.0003). As presented in table 2, patients with CC received more platelet transfusions. There were no significant differences in coagulation parameters or heparin between patients with and without complications and between survivors and non-survivors.

TABLE 1 Coagulation complications during ECMO

Variable	n (% ECMO runs)	Survival to discharge (n (% of variable))	Died due to complication (n (% of variable))	Neonate (n (% ECMO runs))	Children (n (% ECMO runs))	p-value
ECMO runs	80	46 (58.3%)		35	45	
ECMO runs with coagulation complications	43 (53.8%)	19 (44.2%)		22 (62.9%)	21 (46.7%)	0.152
ECMO runs with clotting complication(s)	30 (37.5%)	14 (46.7%)	0 (0%)	15 (42.9%)	15 (33.3%)	0.386
Amount of clotting complications	40			20	20	
Clotting in patient	12					
Clotting in ECMO system	28					
ECMO runs with hemorrhagic complication(s)	33 (41.3%)	13 (39.4%)	10 (30.3%)	15 (42.9%)	18 (40.0%)	0.798
Amount of hemorrhagic complications	44			17	27	
ECMO runs with clotting and hemorrhagic complications	20 (25%)	8 (40%)		8 (22.9%)	12 (26.7%)	0.698

TABLE 2 Laboratory tests and administered heparin and blood products during ECMO]

Variable	Clot (n=30) (median (IQR)	No clot (n=50) (median (IQR)	p-value	Hemorrhage (n=33) (median (IQR)	No hemorrhage (n=49) (median (IQR)	p-value
ACT (s)	181.00 (30.50)	176.00 (26.25)	0.631	182.00 (45.75)	176.00 (18.50)	0.150
APTT (s)	84.50 (35.75)	86.25 (48.25)	0.925	88.00 (35.50)	81.00 (43.00)	0.688
Fibrinogen (g/L)	2.200 (2.595)	2.450 (1.825)	0.754	2.600 (2.065)	2.400 (2.0623)	0.551
Maximum D-dimers (mg/L)	14.52 (85.49)	23.10 (41.12)	0.055	35.20 (71.72)	25.41 (44.25)	0.105
Platelet count (10^9/L)	108.50 (39.25)	112.5 (54.38)	0.429	108.00 (35.00)	115.00 (59.00)	0.102
Heparin infusion rate (IU/kg/hr)	31.14 (18.30)	34.74 (17.03)	0.297	35.25 (19.93)	33.58 (14.43)	0.664
Red blood cell transfusion (ml/kg/day)	12.93 (31.33)	13.52 (12.29)	0.193	11.24 (14.11)	15.30 (12.96)	0.811
Fresh frozen plasma transfusion (ml/kg/day)	2.75 (8.09)	2.03 (4.49)	0.261	1.56 (6.31)	2.65 (4.74)	0.811
Platelet transfusion (ml/kg/day)	23.55 (30.17)	11.95 (16.14)	0.005*	16.69 (21.78)	13.60 (21.67)	0.534

Conclusions: Coagulation complications are associated with longer ECMO runs and can be fatal. No coagulation parameter differed in relation to complications and survival. Management of coagulation on ECMO remains challenging and requires intensive cooperation between intensivists and hematologists and multi-international evaluation.

OC 64.3 | Intraoperative ROTEM Predicts Excessive Bleeding in Infants on Cardiopulmonary Bypass

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Background: Excessive perioperative bleeding (EB) in infants undergoing cardiopulmonary bypass (CPB) is associated with significant morbidity and mortality. Early and accurate diagnosis of platelet defects is essential for timely and targeted therapy. Rotational thromboelastometry (ROTEM) is a rapid test of hemostasis. ROTEM-guided platelet transfusion protocols have safely reduced transfusions in adults but have not been implemented for infants due to a lack of data correlating ROTEM values with perioperative EB.

Aims: Test the hypothesis that intraoperative ROTEM values predict EB risk.

Methods: This IRB-approved observational study consented infants (< 365 days) undergoing cardiac surgery with CPB at Children's Hospital

of Wisconsin from 2013-2016. ROTEM was performed 1) pre-surgery, 2) pre-separation from CPB, 3) post-CPB, and 4) at CICU admission. Platelet contribution to clot formation as determined by ROTEM (PLTEM A10) was calculated as EXTEM A10 - FIBTEM A10. EB was defined as ≥6 mL/kg/h chest tube output (CTO) for 2 consecutive hours in the first 6 hours, >4 mL/kg/h average CTO for the first 24 hours, and/or reexploration for bleeding or tamponade in the first 24 hours after surgery. Most patients were transfused platelets between samples 2 and 3. Logistic regression models performed with SAS Studio.

Results: 66 subjects (43 neonates, 23 infants) were included in this analysis; 17 (25.6%) had EB. Preoperative mean platelet volume (MPV), number of CPB runs, and PLTEM A10 at Time 2 predicted EB risk (Table 1). In patients with PLTEM < 32mm at Time 2, a platelet count of < $60x10^9$/L was associated with higher odds of EB (Fig 1) despite platelet transfusion.

TABLE 1 Risk factors that predict excessive perioperative bleeding.

Effect	Odds Ratio Estimate (95% Confidence Interval)	P value
Pre-Operative MPV		
< 9.5 fL (normal or low)	referent	
> 9.5 fL (high)	19.7 (1.8 - 205.9)	0.0128
CPB runs		
1	referent	
≥2	8.5 (1.6 - 45.0)	0.0115
Pre-CPB separation PLTEM A10		
<32 mm	10.1 (1.07 - 94.7)	0.0427
≥32 mm	referent	

Conclusions: Risk factors for EB were high baseline MPV, ≥2 CPB runs, and intraoperative PLTEM < 32mm. Patients with PLTEM < 32mm and < $60x10^9$/L were at highest risk of EB. Studies of the safety of avoiding platelet transfusion in patients with PLTEM >32mm and efficacy of increasing platelet transfusion in patients with platelet count < $60x10^9$/L are needed.

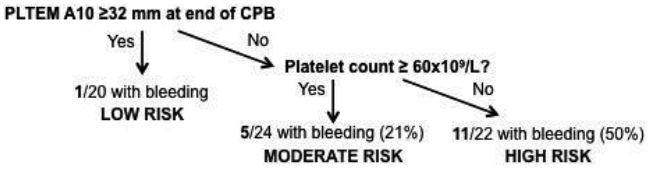

FIGURE 1 Risk of bleeding based on PLTEM A10 and platelet count prior to CPB discontinuation.

OC 64.4 | Antithrombotic Guideline for the PumpKIN Trial: Design and Rationale

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Background: The PumpKIN Trial will evaluate safety and probable benefit of the Jarvik 2015 continuous flow pediatric ventricular assist device (VAD) relative to the Berlin Heart EXCOR® pediatric pulsatile VAD, a device with a high early ischemic stroke risk.

Aims: To describe the antithrombotic (AT) guideline developed for the PumpKIN trial and its rationale.

Methods: Representatives from pediatric thrombosis, cardiology and cardiac surgery reviewed literature on pediatric VAD AT therapy and conducted a survey of 22 participating sites regarding local guidelines and laboratory practices. This information allowed development of a standard AT guideline to be used in each arm of the trial to minimize AT as a variable.

Results: Primary objectives were to develop a guideline that would be associated with few possible adverse events, reflect the most recent research, and be feasible for centers to implement. Challenges to guideline development included concern that the hemostatic "set point" of the original EXCOR® AT guideline was too low given the high stroke rate, uncertainty regarding thrombogenicity of the Jarvik 2015, Center variability in current EXCOR® AT practice, conflicting perceptions about the validity of using Platelet Mapping (PM) to guide platelet inhibition and inconsistent lab monitoring/availability across centers. Ultimately, the proposed guideline includes one anticoagulant plus dual high-dose antiplatelet therapy (with a third optional antiplatelet agent) with pre-specified antiplatelet dosing targets to supplement platelet-mapping data. Early surgical hemostasis is critical to enable timely up titration of AT therapy to address the early embolic stroke risk.

Conclusions: Despite uncertainty regarding the thrombogenicity of Jarvik 2015 pump, a higher-intensity antithrombotic guideline has been developed for the PumpKIN trial incorporating lessons learned from previous and emerging studies. Review of the guideline should be incorporated into routine data safety reviews as the clinical trial progresses.

OC 64.5 | Platelet Secretion Defects and Acquired von Willebrand Syndrome in Patients with Ventricular Assist Devices

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Background: Acquired Von Willebrand syndrome (AVWS) is caused by increased shear stress resulting from the contact of blood with

foreign surfaces in the ventricular assist device (VAD). Impaired binding of Von Willebrand factor (VWF) to platelets and to collagen due to AVWS may increase bleeding tendencies in VAD patients who show bleeding as most frequent complication. Loss of HMW multimers is characteristic of AVWS. The HeartMate III (HM III) is a novel left-ventricular VAD (LVAD) featuring improvements over its predecessor, HeartMate II (HM II).

Aims: We investigated prevalence, onset and decline of AVWS in the largest cohort (n=185) of VAD-patients whose longitudinal data on AVWS have been collected and analyzed. We also investigated whether AVWS is less severe in HM III-patients than in HM II-patients. Further, platelet function was analyzed because platelet dysfunction can contribute to bleeding risk.

Methods: Observation started at 2006. Patients received a Thoratec HM II or HM III LVAD-system. Patients with LVAD combined with temporary right ventricular assist device (RVAD) or isolated biventricular assist device (Thoratec-BVAD) were also included in the study. As controls 84 patients with heart transplants were included. We determined the ratio of Von Willebrand factor collagen binding capacity (VWF:CB) to VWF antigen (VWF:Ag) (VWF:CB/VWF:Ag) and analyzed multimers and platelet function (especially secretion of α- and δ-granules).

Results: All 185 VAD-patients developed AVWS. After the VAD explantation AVWS disappeared within hours. AVWS was less severe in HM III-patients than in HM II-patients. HM III-patients suffered from fewer bleeding symptoms. All investigated VAD patients exhibited a platelet α- and δ-granule secretion defect.

Conclusions: AVWS develops in VAD patients and can increase the bleeding risk. The HM III device causes less serious AVWS. Platelet secretion defects should be investigated in VAD patients.

PLATELETS – BASIC

OC 03.1 | Platelet Activation by CLEC-2-Podoplanin Interaction is Essential for Lung Development

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Background: We have previously identified CLEC-2 (*Clec1b*) as a platelet activation receptor and its endogenous ligand Podoplanin (*Pdpn*). Platelet CLEC-2 has a lot of physiological functions, such as blood-lymphatic vessel separation, inflammation and cancer metastasis. *Clec1b*$^{-/-}$ and *Pdpn*$^{-/-}$ mice die shortly after birth, but the mechanism has remained unclear. In the last ISTH congress, we reported that platelet CLEC-2 promotes alveolar myofibroblast (AMF) differentiation and normal alveolar formation.

Aims: The aim of this study is to elucidate how platelet CLEC-2 is involved in the AMF differentiation and to identify Pdpn-expressing cells required for lung development via binding to platelet CLEC-2.

Methods: Since AMFs are predominantly derived from lung mesothelial cells (LMCs), we histologically analyzed the LMC differentiation in *Clec1b*$^{-/-}$, *Syk*$^{-/-}$ and *Pdpn*$^{-/-}$ mice. We have found that Pdpn is expressed in LMCs, alveolar epithelial cells and lymphatic endothelial cells in the developing lung. We investigated AMF and PMC differentiation in those three tissue-specific *Pdpn*-deficient mice (*Wt1-Cre*, *Shh-Cre*, and *Tie2-Cre*, respectively). Neonatal lethality of all strains above was also examined. The effects of supernatants of activated platelets on AMF differentiation was examined in lung explant culture focusing on alpha-SMA expression.

Results: *Clec1b*$^{-/-}$, *Syk*$^{-/-}$, and *Pdpn*$^{-/-}$ mice showed overexpression of Wt1, a marker of LMC, hyperproliferation in LMCs and neonatal lethality. Of three tissue-specific *Pdpn*-deficient mice, only *Tie2-Cre*, *Pdpn*$^{fl/fl}$ mice exhibited similar phenotypes. In explant culture, the supernatants induced alpha-SMA expression in the interstitial cells of alveoli and the effect was canceled by TGF-beta antibody.

Conclusions: These results strongly suggest that TGF-beta released from activated platelets by interaction between CLEC-2 on platelets and Pdpn on lymphatic endothelial cells promotes AMF differentiation of LMCs, resulting in normal alveolar formation and acquisition of respiratory function.

OC 03.2 | Platelet Microparticles Infiltrating Solid Tumors Transfer miRNAs and Modulate Tumor Growth

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Background: Platelets, as well as other cells, release microparticles (microvesicles) into the plasma in response to receptor agonists and shear stress. The bulk of plasma-borne microparticles are platelet-derived microparticles (PMPs). PMPs are enriched in platelet-derived miRNA species, and can transfer platelet miRNAs to other cells including tumor cells following co-incubation *in vitro*. Tumor vasculature is highly permeable, allowing the possibility of PMP-tumor cell interaction.

Aims: The goal was to evaluate *in vivo* transfer of platelet-derived microRNAs (miRNAs) to tumor cells in solid tumors, via microparticles.

Methods: S.c. tumors.

Results: Here we report that PMPs infiltrate colon, lung, prostate, liver and breast solid tumors in humans, and PMPs infiltrated implanted tumors in mice. Infiltrating PMPs attached to tumor cells, and PMPs

transferred platelet-derived RNA, including microRNAs, to tumor cells *in vivo* and *in vitro*. MiR-24 was a major species in this transfer. We identified direct RNA targets of platelet-derived miR-24 in tumor cells, which included *mt-Nd2*, a mitochondrial mRNA, and *Snora75*, a non-coding small nucleolar RNA. These RNAs were depleted in PMP-treated lung and colon carcinoma cells, and PMP treatment resulted in mitochondrial dysfunction and tumor cell growth inhibition, in a miR-24-dependent manner. Tumor growth inhibition was the result of increased apoptosis but not blockade of cell cycle progression, *in vitro* and *in vivo*. PMP transfusion inhibited tumor growth in lung and colon carcinoma subcutaneous tumors, whereas blockade of miR-24 in tumor cells accelerated tumor growth *in vivo*, and prevented tumor growth inhibition by PMPs.

Conclusions: Thus, platelet-derived miRNAs transfer *in vivo* to tumor cells in solid tumors via infiltrating PMPs, regulate tumor cell gene expression, and modulate tumor progression. These findings shed novel insight onto mechanisms of horizontal RNA transfer and add multiple layers to the regulatory roles of miRNAs and PMPs in tumor progression.

OC 03.3 | Significance of Transforming Growth Factor-beta Signaling for Thrombofibrotic Remodeling in Murine Venous Thrombi and Human Chronic Thromboembolic Pulmonary Hypertension

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Background: Chronic thromboembolic pulmonary hypertension (CTEPH) develops as a result of thrombofibrotic remodeling and major pulmonary artery obstruction. Defects of transforming growth factor-beta (TGFβ) pathway have been implicated in pulmonary arterial hypertension, whereas its role in CTEPH is unknown.

Aims: To investigate if TGFβ released from activated platelets promotes fibrosis in CTEPH via endothelial-to-mesenchymal transition (EndMT) and the endothelial TGFβ II receptor.

Methods: CTEPH specimens were processed for immunohistochemistry and quantitative *real time* PCR (qPCR). Endothelial cells outgrown from CTEPH specimens were subjected to immunofluorescence and microarray analyses. Murine venous thrombi were induced by *inferior Vena cava* (IVC) ligation.

Results: Dual immunofluorescence staining of CTEPH specimens demonstrated cells simultaneously expressing endothelial and mesenchymal markers suggesting the presence of EndMT. QPCR analysis confirmed these findings and also revealed upregulated expression of transcription factors involved in EndMT. Microarray, qPCR and immunohistochemical expression analysis confirmed the expression of TGFβ ligands (TGFβ-1, -2), receptors (TGFBRII, endoglin, BMPRII) and activated TGFβ signaling (phospho-SMAD2 and -SMAD5) in CTEPH tissue. Furthermore, mice with platelet-specific TGFβ1 deletion or inducible endothelial-specific deletion of TGFBRII were subjected to IVC ligation followed by ultrasound over 3 weeks. Venous thrombi were smaller in mice lacking TGFβ in platelets (n=10). Conversely, endothelial-specific deletion of TGFβRII was associated with an increase in thrombus size at all points examined (n=6). Confocal microscopy analysis demonstrated that EndMT was only detected in the presence of TGFβ and the accumulation of fibrotic material was also significantly increased.

Conclusions: Using this combined approach we found that TGFβ signaling in endothelial cells contributes to chronic fibrotic remodeling and involves EndMT.

OC 03.4 | Aspirin Therapy Reduces the Ability of Platelets to Promote Colon and Pancreatic Cancer Cell Proliferation: Implications for the Oncoprotein c-MYC

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Background: Aspirin, an anti-inflammatory and anti-thrombotic drug, has become the focus of research as a potential anti-cancer agent owing to its ability to reduce tumor proliferation *in vitro* and to prevent tumorigenesis in patients. Studies have found an anti-cancer effect of aspirin when used in low, anti-platelet doses. However, the mechanism(s) through which low dose aspirin works is poorly understood.

Aims: To characterize the molecular mechanisms underlying the anti-cancer effect of an anti-platelet dose of aspirin.

Methods: We used 2 colon cancer cell lines, SW480 and SW620, and a pancreatic cancer cell line, PANC-1 and combined each with platelets and their releasates in direct and transwell co-culture platforms to understand routes of platelet-cancer communications. Platelet-induced changes in cancer-relevant oncoproteins and cell proliferation were detected by western blotting and immunofluorescence imaging, respectively. Moreover, aspirin and inhibitors of c-MYC, phosphatidylinositol 3-kinase (PI3K), platelet derived growth factor receptor (PDGF-R) and platelet integrin αIIbβ3 were used to identify the molecular signals that trigger oncoprotein overexpression and drive proliferative responses in cancer cells in the presence of intact human platelets or platelet releasates.

Results: We demonstrate that platelet-derived signals stimulate the expression of the oncoprotein c-MYC and induce proliferation of colon and pancreatic cancer cells. We establish that the ability of platelets to upregulate c-MYC and trigger proliferative responses in cancer cells can be reversed by an anti-platelet dose of aspirin.

Conclusions: In this study, we unveil, for the first time, the ability of platelets and their releasates to regulate the expression of oncoproteins in cancer cells. Moreover, we propose a novel anti-cancer mechanism of action of low-dose aspirin, namely through the inhibition of platelet-induced molecular signals that cause aberrant expression of the c-MYC oncoprotein in cancers.

OC 03.5 | Platelet Releasate Promotes Breast Cancer Growth and Angiogenesis via VEGF-integrin Cooperative Signaling

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Background: Platelets are an active player in angiogenesis. Selective platelet release of pro- or anti-angiogenic factors distinctly regulated angiogenesis.

Aims: To investigate if selective releases of platelet angiogenic factors differently regulate tumor growth.

Methods: *Breast cancer cell proliferation, cancer cell-induced endothelial tube formation in vitro, and tumor growth in vivo were studied in the presence of* protease-activated receptor 1-stimulated platelet releasate (PAR1-PR; rich in pro-angiogenic factors) or PAR4-PR (rich in anti-angiogenic factors).

Results: PAR1-PR and PAR4-PR supplementation (10%) similarly enhanced cell proliferation of MCF-7 and MDA-MB-231 breast cancer cells. Platelet releasates had, however, no influence on cancer cell apoptosis. MCF-7 and MDA-MB-231 cells triggered capillary-like tube formation of endothelial cells, and the effects were further enhanced by PAR1-PR, and tended to be enhanced by PAR4-PR. Further experiments showed that VEGF, but not SDF-1a blockade abolished PAR1-PR/PAR4-PR enhanced cell proliferation of both cancer cell lines, and that integrin blockade by RGDS had identical effects as VEGF inhibition. Intracellular signaling intervention of MCF-7 cells at Src and ERK diminished both PAR1-PR and PAR4-PR enhanced MCF-7 cell proliferation, while PI3K and PKC blockade inhibited platelet releasate-enhanced cell proliferation even more markedly. Similarly, the signaling blockade inhibited MDA-MB-231 cell proliferation. Using a model of subcutaneous implantation of MDA-MB-231 cells in nude mice, PAR1-PR demonstrated more marked enhancements on tumor growth than PAR4-PR. The exaggeration was linked to more profound tumor angiogenesis enhanced by PAR1-PR.

Conclusions: Platelet releasate increases breast cancer cell proliferation through VEGF-integrin cooperative signaling. Pro-angiogenic factor-rich platelet releasate enhances cancer cell-induced angiogenesis more markedly, and exaggerates tumor growth in vivo through promoting tumor angiogenesis.

OC 04.1 | Ultrastructural Characterization of the Cellular Contacts between Megakaryocytes and Endothelial Cells in the Bone Marrow

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Background: The final steps of platelet production require that megakaryocyte (MK) fragments are released into the circulation after crossing the sinusoids. The cellular mechanisms involved in this passage remain unclear.

Aims: The aim of this study is to characterize the cellular events associated to the interaction of MKs with endothelial cells (ECs) of mice bone marrow sinuses.

Methods: *In situ* observations are performed using transmission electron microscopy and 3D imaging with the focused ion beam-scanning electron microscope (FIB-SEM).

Results: We observe at least four types of MK/EC interactions, including 1) planar contacts (46%), 2) short MK extensions (35%), 3) invasive MK protrusions penetrating deeply into endothelial invaginations (10%), and 4) transmigrating MK fragments (9%) (n=118 MKs). The protrusions resemble the MK marginal zone known to be rich in actomyosin. Protrusions provoke endothelial squeezing, resulting in the apposition of apical and basal membranes. In addition, pores appear to be formed at these particular MK/EC contacts. In the endothelium, local enrichment and fusion of vesicles are detected at sites of MK protrusion suggesting that they may play a role as local membrane suppliers necessary for pore formation. 3D imaging demonstrated that transmigrating MKs form large intravascular fragments corresponding for two-third of the total volume of MK. Remarkably, they differ from the thin elongated MK extensions called “proplatelets” and cross the ECs at sites distant from interendothelial cell junctions indicating trans-endothelial passage.

Conclusions: Collectively, these observations suggest a model in which MKs extended invasive protrusions which trigger endothelial surface invaginations and which could facilitate progressively formation of transcellular pores through the endothelium.

OC 04.2 | Loss of the Hematopoietic Adaptor Protein ADAP Impairs Megakaryocyte Polarization and Induces Ectopic Platelet Release

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Background: Bone marrow megakaryocytes (MKs) produce platelets by extending proplatelets into sinusoidal blood vessels. Defects in thrombopoiesis can lead to thrombocytopenia associated with increased bleeding tendency. Recently, the platelet disorder congenital

autosomal recessive small-platelet thrombocytopenia (CARST) was described which is caused by mutations in the *ADAP* (Adhesion and degranulation promoting adaptor protein) gene, and characterized by a microthrombocytopenia and bleeding symptoms.

Aims: The aim of this study was to investigate the role of ADAP in thrombopoiesis.

Methods: We capitalized on constitutive as well as conditional ADAP knockout mice, studied platelet biogenesis in *in vitro* and *in vivo* assays, and used multiple microscopic techniques (electron, confocal, two-photon microscopy).

Results: Constitutive ADAP deficient mice reproduced the microthrombocytopenia observed in CARST patients, and platelet counts could not be increased after splenectomy. *Adap*$^{-/-}$ platelets had a shorter life span than control platelets. Whole sternum 3D confocal imaging and two-photon microscopic analysis revealed altered morphology of ADAP deficient MKs with signs of fragmentation and ectopic release of platelet-like particles as well as proplatelets into the bone marrow compartment. In addition, cultured bone marrow-derived MKs lacking ADAP showed a reduced capacity to spread on collagen and form podosomes, but displayed defective polarization of the demarcation membrane system *in vitro*. MK-/platelet-specific ADAP deficient mice also produced less and smaller-sized platelets and released platelets ectopically. These data suggest that the abnormal platelet production in the mutant mice is a MK intrinsic defect.

Conclusions: Our results point to a so far unidentified role of ADAP in the process of MK polarization and platelet biogenesis. Thus, we suggest that the analysis of ADAP deficient mice may help to understand the platelet production defect in CARST patients.

OC 04.3 | Centrosome Destabilization through the Ubiquitin-proteasome Pathway Regulates Proplatelet Production

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Background: Proteasome inhibitors such as bortezomib, a chemotherapeutic used to treat multiple myeloma, induce thrombocytopenia within days of initiation because proteasome activity is essential for platelet formation. The major pathway of selective protein degradation uses ubiquitin as a marker that targets proteins for proteolysis by the proteasome. This pathway is previously unexplored in megakaryocytes (MKs).

Aims: We aimed to define how the ubiquitin-proteasome pathway affects platelet production.

Methods: Pharmacologic inhibition and proteomic and polysome profiling analyses were used.

Results: To characterize how proteasomal protein degradation was occurring, we probed distinct ubiquitin pathways. Inhibition of the ubiquitin-activating enzyme E1 significantly inhibited proplatelet formation up to 73%. In addition, inhibition of the deubiquitinases UCHL5 and USP14 significantly inhibited proplatelet formation up to 83%. These data suggest the ubiquitin pathway is necessary for proplatelet formation.

Proteomic and polysome analyses revealed a subset of proteins decreased in proplatelet-producing megakaryocytes, consistent with data showing that protein degradation is necessary for proplatelet formation. Specifically, the centrosome stabilizing proteins Aurora kinase (Aurk) A/B, Tpx2, Cdk1, and Plk1 were decreased in proplatelet-producing MKs. Furthermore, inhibition of AurkA and Plk1, but not Cdk1, significantly inhibited proplatelet formation in vitro over 86%.

Conclusions: We hypothesize that proplatelet formation is triggered by centrosome disassembly, and that the ubiquitin-proteasome pathway plays a crucial role in this transformation. Specifically, the AurkA/Plk1/Tpx2 pathway may be key in maintaining centrosome integrity and initiating proplatelet formation. Determination of the mechanism by which the ubiquitin-proteasome pathway regulates the centrosome and facilitates proplatelet formation will allow us to design better strategies to target thrombocytopenia.

OC 04.4 | Scalable Production of Human Platelets in a Bioreactor

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Background: A low platelet count resulting from conditions such as cancer treatment, transplantation, and surgery, require platelet transfusions to prevent mortality due to uncontrolled bleeding. Platelet transfusion units are exclusively obtained from human volunteer donors. They must be stored at room temperature to prevent irreversible activation, which limits the shelf life of a unit to 5 days. However, storage at room temperature increases the risk of contamination. After 2 days of contaminant testing and 1 day for transportation, blood centers typically do not have more than 1.5 days of platelet transfusion units available, which are even further depleted during emergencies.

Aims: To address this major unmet need, we have developed a scalable, microfluidic bioreactor that reproduces the key features of the bone marrow to trigger human platelet production from megakaryocytes.

Methods: To optimize the design and operation of the bioreactor, megakaryocytes derived from primary peripheral blood CD34^{+} cells are utilized as a physiologically relevant cell line to produce platelets. We have developed a computation fluid model to understand how pressure and shear independently affect platelet production. This allows us to visualize pressure, flow and shear stresses experienced by megakaryocytes in the bioreactor and to optimize the conditions to increase platelet production.

Results: Computational fluid modeling shows that the megakaryocytes can be targeted to experience flow rates of 0-5 cm/s and shear stresses ranging from 500-3000 mPa, while platelets can experience a flow rate of 0-8 cm/s and shear stresses of 1000-6000 mPa, which

encompassing the physiological shear stress range of 912-936 mPa These bioreactor-derived platelets are comparable in morphology and biomarker expression to human platelets.

Conclusions: We have developed a scalable microfluidic device that can produce platelets from human megakaryocytes *ex vivo* at clinically relevant yield.

OC 04.5 | Platelet Release from Infused Megakaryocytes is Largely Limited to the Pulmonary Vasculature

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Background: Factors that control platelet (Plt) shedding from megakaryocytes (MKs) have not been fully defined. We previously showed that intravenously (IV) infused mouse or human MKs are entrapped in the pulmonary vasculature and release particles physically and functionally similar to donor-derived Plts. Whether Plts are uniquely shed in the lung or MKs would shed Plts at the first vascular bed encountered is unknown.

Aims: To examine whether the pulmonary bed differs from other vascular beds for Plt shedding from infused MKs and to begin to visualize infused MKs shedding Plts.

Methods: CD34+-derived human MKs were infused into NSG-mice via tail vein or left carotid artery. Plt release was monitored via flow cytometry and organs were analyzed histologically for trapped MKs. Confocal in situ lung microscopy was done to visualize infused and endogenous Mks within the lung vasculature in real time.

Results: In vitro-grown MKs were studied in parallel mice one receiving an arterial and the other a venous MK infusion. Infusion of MKs resulted in a rise in circulating human Plts after venous, but not arterial infusion (Fig. 1).

Histologic examination 10 min and 4 hrs after infusion showed predominant pulmonary entrapment of IV infused MKs but only minimal MKs presence in any organ after intra-arterial infusion. In situ lung microscopy show the rapid extension of long (up to 200μ) proplatelets in the lungs after infusing MKs that was complete by 10 mins (Fig. 2). No spontaneous MKs were noted to be entrapped during several hrs of observation.

Conclusions: The pulmonary bed might offer a unique environment to entrap MKs and promote thrombopoiesis. These features can include the unique physical features of the pulmonary bed or its endothelial lining or the sharp oxygen gradient encountered. In addition, in the mouse, spontaneous MK entrapment and shedding was not observed, suggesting that in mice Plt shedding normally may not involve the pulmonary bed.

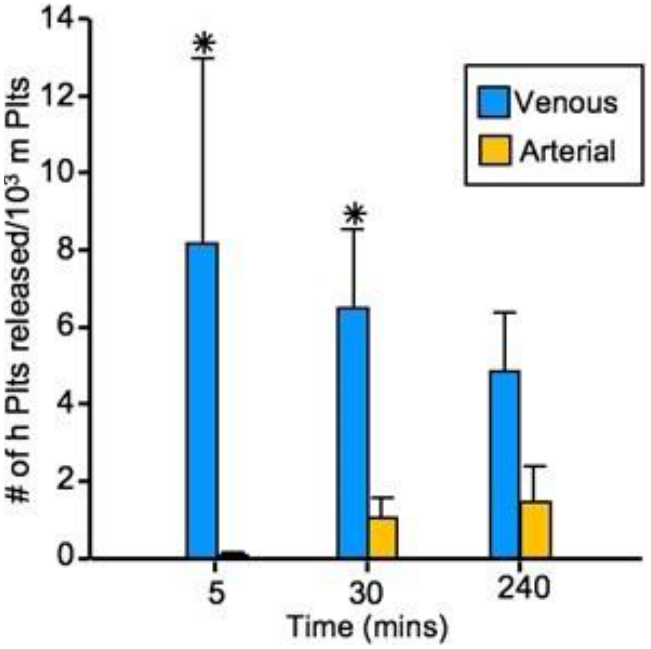

FIGURE 1 Arterial infused MKs produce significantly more plts than those infused venously (N=5; *=p<0.05)

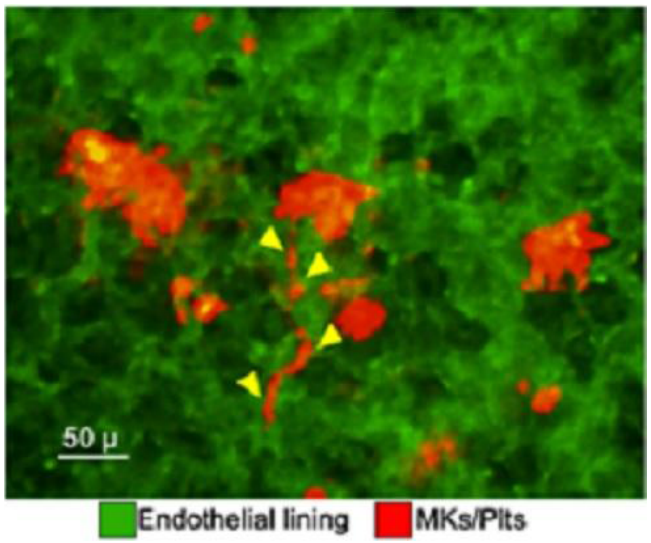

FIGURE 2 In situ lung microscopy shows calcein (red-orange) labeled human MK arrested in mouse lung with a sinuous extension after 8 mins.

OC 06.1 | Rap1A and Rap1B Functional Redundancy in Platelets and Megakaryocytes

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Background: RAP-GTPases are molecular switches that regulate various cellular functions, including integrin-mediated adhesion. Germline deletion of the RAP1B isoform causes elevated embryonic lethality in

mice. However, the platelet defects in the surviving *Rap1b-/-* mice are milder than in mice lacking the RAP activator, CalDAG-GEFI, suggesting that other RAP isoforms have important roles in platelet adhesion and hemostasis.

Aims: To determine the specific contribution of the most abundant RAP isoforms, RAP1A and RAP1B, to platelet activation and hemostasis.

Methods: Platelet functional responses were studied in mice where *Rap1a* and/or *Rap1b* were conditionally deleted in the megakaryocyte lineage (mKO). TALIN and integrin β3 colocalization was analysed by STORM imaging. Proplatelet formation was evaluated in bone marrow-derived megakaryocytes.

Results: *Rap1a/b-mKO* mice displayed a mild macrothrombocytopenia due to impaired proplatelet formation. *Rap1a-mKO* platelets had a significant and previously undetected defect in integrin activation, that was additive to that of *Rap1b-mKO platelets*. Combined deficiency in RAP1A and RAP1B impaired the ability of TALIN to colocalize with integrin β3 and led to an integrin activation defect comparable to that observed in *Tln1-mKO* mice. In contrast, granule secretion was only dependent on RAP1B. *In vivo, Rap1a/b-mKO mice, but not Rap1b-mKO or Rap1a-mKO* mice, were completely protected from arterial thrombosis and exhibited a severe defect in hemostasis after mechanical injury. Interestingly, at sites of inflammation, hemorrhage was observed in thrombocytopenic mice, but not in *Rap1a/b-mKO mice*.

Conclusions: Our studies demonstrate that RAP1A has a previously unrecognized role in platelet integrin activation, that the two RAP1 isoforms have both redundant and isoform-specific functions and that RAP1 signaling is critical for hemostatic plug formation, but not for other forms of hemostasis. Moreover, they provide the first direct evidence that RAP1 is important for platelet biogenesis.

OC 06.2 | The Kinase-phosphatase Pair Csk-CD148 is a Critical Regulator of Platelet Reactivity to Vascular Injury

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Background: Platelets contain high levels of Src family kinases (SFKs) that are essential for transmitting activation signals from a variety of receptors, including immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors, integrins and G protein-coupled receptors. However, it remains unclear how SFKs are regulated in platelets.

Aims: To determine the role of the kinase-phosphatase pair C-terminal Src kinase (Csk) and CD148 in regulating SFK activity in platelets and their response to vascular injury.

Methods: *Csk* and *CD148* conditional double-knockout (DKO) mice were generated by crossing *Csk*- and *CD148-floxed* mice with *Pf4-Cre* transgenic mice. Platelet function was measured *in vitro* and *in vivo* using standard assays. Platelet receptor expression and signal transduction was measured by flow cytometry, western blotting and capillary-based immunoassay.

Results: Deletion of *Csk-CD148* in the megakaryocyte lineage in mice resulted in increased platelet SFK activity, but reduced platelet reactivity to collagen due to down-regulation of the ITAM-containing collagen receptor complex GPVI-FcR g-chain. Interestingly, the immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B and Csk-homologous kinase (Chk) were concomitantly up-regulated, contributing to the reduced platelet response. *In vitro*, whole-blood thrombus formation under non-coagulant conditions on multiple surfaces was markedly impaired; however, tissue factor-mediated thrombus formation on collagen under flow with high fibrin formation was normal. Consequently, DKO mice exhibited increased bleeding in the tail bleeding assay and thrombus instability following laser injury of arterioles in the cremaster muscle.

Conclusions: Findings from this study establish the kinase-phosphatase pair Csk-CD148 as a critical regulator of platelet SFK activity and reveal novel cell intrinsic negative feedback mechanisms that prevent pathological thrombosis from occurring.

OC 06.3 | Major Role for Rac/Cdc42 in PI3Kβ-driven Platelet Function

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Background: Class I Phosphoinositide 3-kinase β (PI3Kβ) holds a key role in platelet activation and stable thrombus formation, attracting it considerable attention as a potential antithrombotic target. Despite this, the mechanisms underlying platelet PI3Kβ activation and it's predominance over the other Class I PI3K isoforms in these cells remain poorly understood.

Aims: Previous work in other cell types has defined a unique combination of activating inputs to PI3Kβ. These include the direct interaction of the Rho family GTPases Rac and Cdc42 with the ‚Ras-binding domain' (RBD) of the PI3Kβ catalytic subunit, p110β, and the association of p110β with the Gβγ subunits of activated heterotrimeric G proteins. We set out to define the importance of these inputs to PI3Kβ-driven platelet function.

Methods: We have used knock-in mice where p110β has been rendered unable to interact with Rac/Cdc42 (RBD mice) or with Gβγ subunits (GBG mice).

Results: Using RBD mice, we reveal that the Rac/Cdc42-binding domain of PI3Kβ is required for efficient agonist-induced platelet $\alpha_{IIb}\beta_3$ integrin activation and α-granule secretion. This was most notable

downstream of the collagen receptor glycoprotein VI (GPVI), with RBD mice also displaying a significant defect in GPVI-driven platelet aggregation and signalling to the PI3K effector, AKT. Strikingly, use of the PI3Kβ inhibitor AZD6482 suggests the Rac/Cdc42 input to p110β underlies much of PI3Kβ's role in these contexts. Surprisingly, results from the GBG mice suggest that Gβγ input to p110β does not hold a similar functional importance for PI3Kβ's role in platelet function.

Conclusions: This work identifies the Rac/Cdc42 input to p110β is an important requirement for PI3Kβ-driven platelet function, and provides important new mechanistic insights into PI3Kβ's dominant role in this cell type.

This work is funded by the British Heart Foundation.

OC 06.4 | CD40L Priming of Platelets and Activation of NF-κB are CD40-dependent

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Background: CD40 ligand (CD40L), a member of the Tumor Necrosis Factor superfamily, is released principally by activated platelets in the circulation and considered as a thrombo-inflammatory molecule that predicts cardiovascular events, related to enhanced platelet reactivity and thrombosis. We have shown that CD40L is a strong activator of NF-κB in platelets that primes and enhances platelet activation and aggregation in response to thrombotic stimuli. In addition to its main receptor CD40, platelets express two other CD40L receptors, αIIbβ3 and α5β1.

Aims: The present study was designed to identify the receptors of CD40L involved in platelet NF-κB activation and its implication in platelet aggregation.

Methods: Using washed human platelets, the presence of the different receptors of CD40L was determined by flow cytometry and WB. The release of CD40L was analyzed by ELISA. Activation of NF-κB was assessed by determining the phosphorylation of IκBα and P65 in human and mouse platelets. Platelet aggregation was measured optically.

Results: We showed that platelets release CD40L in response to thrombin and CD40L stimulation and express three CD40L receptors, CD40, αIIbβ3 and α5β1. CD40L, dose- and time- dependently, induced platelet NF-κB activation as revealed by IκBα and P65 phosphorylation. Activation of NF-κB by CD40L was absent in $CD40^{-/-}$ mouse platelets and inhibited by CD40 blockade in human platelets, but unaffected by αIIbβ3 or α5β1 blockade. CD40L alone had no effect on platelet aggregation but potentiated the aggregation response in the presence of priming doses of thrombin and collagen; an effect that was abolished by CD40 blockade.

Conclusions: This study demonstrates that CD40L triggers activation of NF-κB and primes platelets through the CD40 receptor, suggesting that CD40L is a platelet primer via signaling pathways involving CD40/NF-κB activation, which predisposes platelets to enhanced activation and aggregation in response to thrombotic stimuli.

OC 06.5 | Essential Role of GSK3α Phosphorylation in Restricting Platelet Activation and Thrombus Formation

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Background: Glycogen synthase kinase-3 (GSK3α) and GSK3β, are constituently active serine/threonine kinases whose activity is regulated by inhibitory phosphorylation at Ser21/Ser9 of GSK3α/β, respectively. Despite phosphorylation of GSK3α/β at Ser21/Ser9 being induced by a wide range of physiological platelet agonists, little is known about its role in platelet function and thrombosis.

Aims: To determine how loss of phosphorylation of GSK3α on Ser21 and GSK3β on Ser9 impacts on platelet function and thrombosis.

Methods: Platelet function and thrombosis was assessed using platelets from mice, where Ser21/9 of GSK3α/β is mutated to alanine. Rendering GSK3α (GSK3αKI) or GSK3β (GSK3βKI) or both GSK3α and β (GSK3α/βKI) resistant to inhibitory phosphorylation.

Results: Loss of GSK3β phosphorylation (GSK3βKI) resulted in reduced thrombin-mediated platelet aggregation and integrin $\alpha_{IIb}\beta_3$ activation, as well as reduced thrombin and CRP-mediated α-granule secretion, demonstrating that GSK3β phosphorylation contributes to both PAR and GPVI-mediated platelet function. Indeed, GSK3βKI mice showed a significant impairment of thrombus formation on a collagen-coated surface. In contrast, we found that loss of GSK3α phosphorylation had no effect on thrombin-mediated responses, but resulted in significantly enhanced responses to the GPVI agonist collagen-related peptide (CRP-XL). Integrin $\alpha_{IIb}\beta_3$ activation and P-selectin exposure were left-shifted with a decrease in the EC50 and a major increase in maximal binding. Furthermore, we show that GSK3α KI completely reversed the impaired thrombus formation observed in GSK3βKI mice, confirming that phosphorylation of these paralogs have opposing effects on thrombus formation. The enhanced platelet function in GSK3α KI platelets was blocked by wortmannin, suggesting that GSK3α phosphorylation may limit signalling through the PI3 kinase pathway.

Conclusions: Together these findings demonstrate that GSK3α phosphorylation restrains GPVI-mediated platelet activation and thrombosis.

OC 17.1 | Convection Denied: Platelet Packing Density is an Independent Regulator of the Hemostatic Response to Injury

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Background: The delivery and retention of coagulation factors and regulators is an important determinant of the hemostatic response to injury. Previous studies have suggested that the transport of thrombin and other platelet agonists can depend on the architecture of a nascent hemostatic thrombus and play a key role in determining its final size and the activation state of the platelets it contains.

Aims: Here we asked how the microenvironment within a hemostatic thrombus, including platelet packing density, affects the interplatelet plasma velocity and the thrombin concentration gradient in the gaps between platelets.

Methods: We have used a hybrid experimental and computational approach to reconstruct the microenvironment within a hemostatic thrombus, breaking the work into 3 stages. In the first, platelet aggregates formed under flow in a microfluidic chamber were analyzed using scanning electron microscopy to extract information about porosity and gap size distribution. In the second, a 3-dimensional model with single-platelet resolution was constructed with microenvironmental features matching the platelet aggregates formed *in vitro*. In the third, the 3D model was integrated with volume and morphology measurements of hemostatic thrombi formed *in vivo*.

Results: Our results show that over a broad range of thrombus sizes and gap sizes, the architecture of the hemostatic thrombus reduces interplatelet plasma velocity to near-stagnant levels. The internal environment of the thrombus is so sheltered that solute transport is governed by diffusion. This architecture helps to increase the local concentration of thrombin by preventing its escape.

Conclusions: Taken together, these findings demonstrate how the architectural features of the hemostatic thrombus microenvironment provide a basis to regulate chemical reactions by purely physical means.

OC 17.2 | Platelet Margination and Activation Are Essential for Thrombus Propagation in an in vitro Model of Venous Thrombosis

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Background: Platelets are essential for thrombus growth in murine models of venous thrombosis (VT). Yet, these models do not capture the vortical flows and geometry that are characteristic of human venous valves where VT initiates.

Aims: To develop an in vitro flow model of VT that mimics the hemodynamics of human venous valves and to investigate the role of platelets on thrombus propagation in this model.

Methods: The flow chamber consists of a 150 X 150 µm channel that undergoes a 1:3 expansion with a varying undercut angle (90-150°) to mimic different positions of a valve leaflet (Fig. 1). Liposomal tissue factor (TF) was adsorbed in the valve sinus. Human whole blood was separated into platelet rich plasma (PRP) and red blood cells (RBC) and reconstituted to hematocrits of 0-0.6. Platelets were labeled with $DiOC_6$ prior to and/or with Annexin V after an experiment and exogenous Alexa 555-fibrinogen was added to visualize fibrin. Thrombus formation was measured by confocal microscopy as plasma, PRP, or reconstituted blood was perfused at flow rates that yield primary and secondary vortices similar to those measured in humans.

Results:

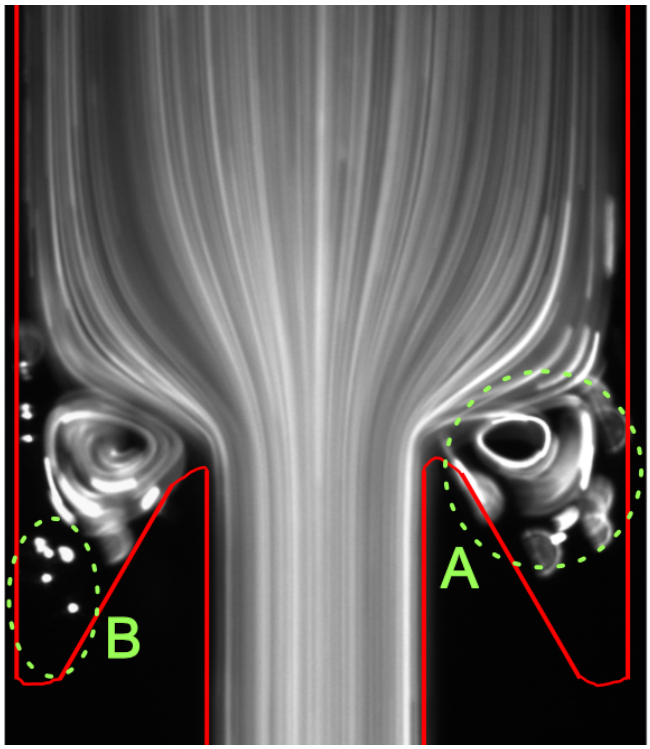

FIGURE 1 Particle tracing image of the model valve with a 150° undercut. A primary vortex (A) and a low flow zone (B) are created in the flow conditions

RBC were required for platelets to enter into the primary vortex suggesting the need for platelet margination. Platelets were required for the thrombus to grow out of the valve (Fig. 2). Plasma or RBC suspensions without platelets resulted in a fibrin gel that was confined to the deepest part of the pocket where flow is almost static. In reconstituted blood, platelets adhered to this initial fibrin gel, were activated and promoted coagulation as shown by Annexin V labeling, denser fibrin fibers, and robust thrombus growth.

Conclusions: We developed an in vitro model of VT that recreated essential features of hemodynamics in human valves. Thrombus propagation required RBC to marginate platelets so that they are delivered at a sufficient rate to adhere to fibrin and support further coagulation by PS exposure beyond TF-rich near wall regions.

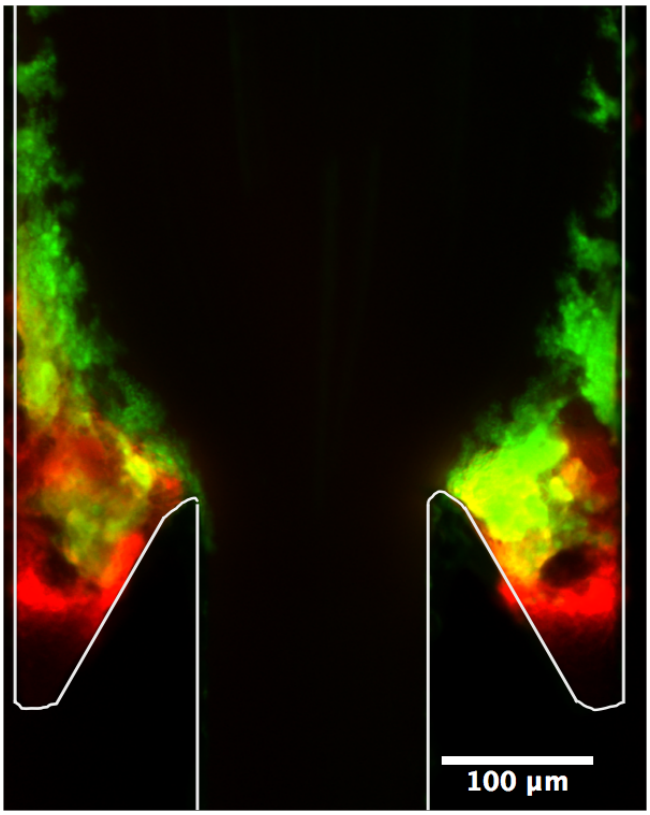

FIGURE 2 Thrombus formation in a model valve with a 150° undercut initiated by wall-bound TF after 25 min. Green: Platelets (DiOC6), Red: Fibrin(ogen)

OC 17.3 | Coactosin-like 1 is a Novel Regulator of Arterial Thrombosis and Hemostasis

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Background: Cytoskeletal rearrangements play an important role in platelet activation by facilitating platelet shape change and the formation of filo- and lamellipodia that are critical for normal hemostasis. Coactosin-like 1 (Cotl1) is a small filamentous actin-binding protein that consists of a single actin-depolymerizing factor homology (ADF-H) domain. Cotl1 was shown to prevent cofilin-mediated depolymerization of actin filaments and to regulate the activity of 5-lipoxygenase that is implicated in leukotriene synthesis.

Aims: We investigated the role of Cotl1 in platelet production and function *in vitro* and *in vivo*.

Methods: We generated the first MK-/platelet-specific Cotl1-deficient (*Cotl1*$^{fl/fl\text{-}Pf4Cre}$) mouse line. Platelet morphology and function was assessed *in vitro* under static and dynamic conditions, as well as *in vivo*.

Results: *Cotl1*$^{fl/fl\text{-}Pf4Cre}$ mice displayed unaltered platelet counts and size compared with controls, suggesting unaltered thrombopoiesis. In addition, platelet activation and aggregation responses to various platelet agonists were normal in Cotl1-deficient mice. Surprisingly, despite the proposed inhibitory role of Cotl1 in actin dynamics, actin polymerization and cytoskeletal organization were unaltered in platelets lacking Cotl1 compared with controls. Strikingly, however, *Cotl1*$^{fl/fl\text{-}Pf4Cre}$ platelets showed a significantly reduced adhesion and aggregate formation on collagen I in a flow adhesion assay indicating impaired cellular activation under conditions of shear flow. *In vivo* this defect translated into slightly prolonged bleeding times and a profound protection from occlusive arterial thrombus formation.

Conclusions: In summary, we show for the first time that Cotl1 is dispensable for actin dynamics in platelets, but we identified a central regulatory role of Cotl1 in hemostasis and arterial thrombosis.

OC 17.4 | Clathrin-mediated Endocytosis is Required for Normal Platelet Hemostatic Function

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Background: Clathrin-mediated endocytosis (CME), the process by which cells internalize specific extracellular material and plasma membrane proteins, contributes to a wide range of cellular functions, including receptor signaling, cell adhesion, and migration. In platelets and megakaryocytes (MKs), CME mediates the uptake of fibrinogen into α-granules, and the internalization of thrombopoietin (TPO), thereby regulating hemostasis, megakaryopoiesis and thrombopoiesis. CME requires cargo receptor-dependent formation of clathrin-coated vesicles (CCVs), and their subsequent release into the cytosol by dynamin 2 (DNM2)-dependent membrane fission. *Dnm2*$^{fl/fl}$ *Pf4-Cre* (*Dnm2*$^{Plt-/-}$) mice specifically lacking DNM2 in the MK/platelet lineage develop severe macrothrombocytopenia. Further, *Dnm2*$^{Plt-/-}$ platelets fail to internalize TPO. Consequently, *Dnm2*$^{Plt-/-}$ mice develop myelofibrosis.

Aims: Here we investigated the role of CME in platelet hemostatic function.

Methods: In vivo and in vitro platelet activation parameters were determined in *Dnm2*$^{Plt-/-}$ mice.

Results: *Dnm2*$^{Plt-/-}$ mice had a severe bleeding diathesis with a tail bleeding time greater than 10 min, compared to 1.16 min in control mice. Consistent with impaired CME, *Dnm2*$^{Plt-/-}$ platelets were depleted of fibrinogen, but normally contained other α-granule proteins

such as von Willebrand factor and CD62P. *Dnm2*$^{Plt-/-}$ platelets were severely dysfunctional, as evidenced by poor α-granule release (CD62P expression) and αIIbβ3 activation (fibrinogen binding) in response to thrombin and the GPVI agonists, collagen-related peptide (CRP) and convulxin. *Dnm2*$^{Plt-/-}$ platelets failed to phosphorylate proteins on tyrosine, including phospholipase C-γ2, in response to CRP. Further, *Dnm2*$^{Plt-/-}$ platelets failed to spread in response to thrombin, and instead accumulated arrested CCVs and filamentous actin (F-actin) clusters.

Conclusions: Together, the data show that CME plays a critical role in normal platelet hemostatic function in vivo, and platelet receptor signaling and spreading in vitro.

OC 17.5 | Platelet-mediated Activation of FasR and Subsequent Phosphatidylserine Exposure of Red Blood Cells is Substantial for Thrombus Formation and Hemostasis

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Background: A critical step in thrombus growth and stability is the contribution of platelets to coagulation and thrombin generation by providing a procoagulant surface. Recently, red blood cells (RBCs) were described to externalize phosphatidylserine (PS) on the membrane and might contribute to thrombin generation. First results point to a direct interaction of platelets and RBCs important for thrombin generation and stable thrombus formation suggesting an active role for RBCs in hemostasis and thrombosis beside their impact in rheology.

Aims: To analyze the impact of RBCs in platelet activation and stable thrombus formation.

Methods: Analysis of RBCs and RBC-platelet interactions upon thrombus formation were performed using platelets from different knock-out mice and patients.

Results: Platelet activation was enhanced by a small population of RBCs via direct cell-cell contact mediated by the FasL-FasR signaling pathway. Activated platelets externalized FasL on the membrane that activated FasR on RBCs leading to PS exposure on the RBC membrane. Accordingly, inhibition or genetic deletion of FasR on RBCs strongly reduced PS exposure followed by inhibition of three-dimensional thrombus formation *in vitro* and *in vivo*. Furthermore, decreased PS exposure of RBCs after treatment of platelets with ReoPro (Abciximab) suggests that integrin aIIbb3 serves as another ligand on the platelet membrane important for FasR activation on RBCs. Direct cell-cell contacts of platelets and RBCs have been observed in arterial thrombi derived from patients that underwent thrombectomy providing first evidence that FasL-FasR mediated cell contacts of platelets and RBCs reflect a pathophysiological mechanism as well.

Conclusions: RBCs play an active role in platelet thrombus formation. Thus, interfering with the interaction of platelets and RBCs by FasR inhibition might be a promising approach for a completely novel antithrombotic strategy.

OC 18.1 | Platelet GPIba Is Important for Liver Thrombopoietin (TPO) Production

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Background: TPO generation primarily occurs in liver parenchymal cells with minor amounts in other tissues. TPO clearance is mainly through platelets and megakaryocytes via c-Mpl-TPO interaction and its subsequent internalization and degradation. Although it was reported that platelets may play a role in TPO production, the mechanism and the key platelet receptor involved in this process are largely unknown.

Aims: To investigate the role of GPIbα in TPO generation.

Methods: Plasma TPO from wild type (WT), GPIbα$^{-/-}$ (KO), IL-4Rα/GPIbα transgenic (Tg) mice were measured with enzyme-linked immunosorbent assay. Clinical samples from Bernard Soulier Syndrome (BSS) patients were tested with chemiluminescentenzyme immunoassay.

Results: Interestingly, we observed a ~50% decrease in circulating TPO in KO mice compared with syngeneic WT and β3$^{-/-}$ mice. Similar results were also observed in GPIbα deficient BSS patients. We found TPO clearance was not enhanced but hepatic TPO mRNA was lower in KO mice compared to WT mice. Furthermore, transfusion of WT, but not KO platelets markedly increased TPO levels in circulation and mRNA transcription in the livers of KO mice. To determine whether the extracellular portion of GPIbα is required for platelet mediated TPO generation, we utilized Tg mice in which most of the extracellular domain of GPIbα is replaced by IL-4Rα. We found the TPO levels in these Tg mice were also low. Similarly, transfusion of Tg platelets did not affect TPO levels in KO, Tg or WT mice, but transfusion of WT but not KO or Tg platelets significantly improved TPO levels in Tg mice. In vitro studies further demonstrated that co-culturing GPIbα$^{-/-}$ platelets with hepatocytes resulted in less platelet uptake and lower mRNA transcription as compared to WT control platelets.

Conclusions: Our animal models and human studies clearly demonstrated that GPIbα is required for platelet mediated TPO generation from liver. These not only provide novel understandings of platelet homeostasis, but also the pathobiology of BSS.

OC 18.2 | Intravital Microscopy Reveals Key Role of Kupffer Cells in Clearance of Desialylated Platelets

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Background: The human body produces 10^{11} platelets every day by shedding from bone marrow megakaryocytes into the circulation. Platelets patrol the vasculature for about 10 days and at the end of their lifetime get cleared through a process that was shown to depend on the loss of sialic acid residues on the platelet surface receptor GPIb. Recent studies have shown that desialylation causes platelet clearance via the Ashwell-Morell receptor on hepatocytes. While there have been several advances in the field, it is still unclear how exactly platelets are cleared and how this process is regulated.

Aims: To investigate the process of platelet clearance *in vivo* using intravital microscopy of the liver.

Methods: Spinning-disk intravital confocal microscopy was performed on the liver of anesthetized mice. Mice were either treated with sialidase to induce *in vivo* desialylation of platelets or *ex vivo* desialylated platelets were labeled with CellTracker dye and transfused into mice. Kupffer cells and endogenous platelets were labeled using fluorescent anti-F4/80 and anti-CD49b antibodies, respectively.

Results: After application of sialidase, *in vivo* desialylated platelets accumulate on Kupffer cells within minutes. To rule out side-effects of *in vivo* administration of sialidase, *ex vivo* desialylated platelets were transfused into mice. *Ex vivo* desialylated platelets rapidly adhered to Kupffer cells and this adherence was specific for sialidase-treatment, since untreated transfused platelets continued circulating in the liver vasculature. Interestingly, the adhesion of desialylated platelets to Kupffer cells was independent of integrin alpha-M, since platelets adhered to the same extent in both wildtype and Mac-1-deficient mice. Of note, we did not observe uptake of platelets by hepatocytes.

Conclusions: Our data indicates that Kupffer cells play an important role in the clearance of desialylated platelets that is independent of integrin alpha-M.

OC 18.3 | Twinfilin 2a Is a Central Regulator of Platelet Reactivity and Turnover in Mice

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Background: Controlled reorganization of the actin cytoskeleton is a prerequisite for proper platelet production and function and defects in actin-regulating proteins have been associated with platelet disorders in humans and mice. The small actin monomer-binding protein Twinfilin 2a (Twf2a) is thought to play an ambivalent role in actin dynamics. Twf2a inhibits actin filament assembly on barbed ends by capping and pointed end growth by sequestration of G-actin monomers. On the other hand, it favors filament assembly by localizing actin monomers to places of rapid actin turnover. Even though Twf2a has been implicated as a key molecule in actin dynamics, only little is known about its precise molecular function *in vivo*.

Aims: We aimed to elucidate the precise role of Twinfilins in platelet production and function.

Methods: Platelet production and function was studied in a broad range of *in vitro* assays and *in vivo* models of hemostasis and arterial thrombus formation.

Results: Here we report that constitutive Twf2a-deficient mice (*Twf2a*$^{-/-}$) display a mild macrothrombocytopenia due to a markedly reduced platelet half-life, and an increased number of proplatelet-forming megakaryocytes in the bone marrow. Twf2a-deficient platelets showed enhanced integrin activation and α-granule release in response to stimulation of (hem)ITAM and G-protein-coupled receptors, increased adhesion and aggregate formation on collagen I under flow and accelerated spreading on fibrinogen. In vivo, Twf2a deficiency resulted in shortened tail bleeding times and accelerated occlusive arterial thrombus formation. The hyper-reactivity of *Twf2a*$^{-/-}$ platelets was attributed to enhanced actin dynamics leading to sustained integrin activation, which collectively may explain the accelerated platelet turnover in *Twf2a*$^{-/-}$ mice.

Conclusions: In summary, our results demonstrate that Twf2a-controlled actin rearrangements dampen platelet activation responses and thereby indirectly regulate platelet survival in mice.

OC 18.4 | Investigating the Mechanisms of Clearance of Pre-activated Platelets in Mice Lacking Function of the Rap1 GAP Rasa3

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Background: Recently, our lab has shown that loss of the Rap GTPase-activating protein (GAP) Rasa3 (Rasa3$^{hlb/hlb}$, "hlb") leads to pre-activation and premature clearance of circulating platelets due to increased Rap1 activation. However, the mechanism of clearance of these platelets remains undetermined.

Aims: The objective of these studies was to determine the mechanism(s) underlying increased platelet clearance in hlb mice.

Methods: Mice used for these studies were at least 8 weeks old and were bred onto a C57BL/6 background. Platelet lifespan was determined by labeling platelets in vivo with an anti-GPIX antibody. Spleens and livers were perfusion fixed and frozen sections collected

for immunofluorescence using anti-GPIX and anti-F4/80 antibodies to stain platelets and macrophages in tissue, respectively.

Results: Mice lacking Rasa3 function (hlb) are thrombocytopenic primarily due to a markedly reduced platelet half-life in circulation. The increased clearance is intrinsic to platelets as hlb platelets transfused into wild-type mice are also rapidly cleared. While radioactive tracer studies identify the spleen and liver as sites of clearance, splenectomy and/or clodronate treatment had no significant effect on platelet counts in hlb mice. When we crossed hlb mice with mice lacking CalDAG-GEFI or the integrin adaptor Talin1, platelet survival and peripheral counts were significantly rescued, suggesting that enhanced platelet clearance in hlb mice is the result of uncontrolled Rap1-αIIbβ3 signaling. In ongoing work, we are using conventional immunohistochemistry approaches as well as intravital microscopy imaging of the liver microcirculation to identify mechanisms driving platelet clearance.

Conclusions: The Rap-GAP, Rasa3, is a critical inhibitor of Rap1-dependent platelet activation in circulation. Platelets lacking functional Rasa3 are seemingly cleared in a phagocyte-independent manner by a mechanism that involves talin-dependent integrin signaling.

OC 18.5 | TNF-α is an Important Factor for Platelet Hyperreactivity in Aging

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Background: Chronic inflammation due to elevated levels of IL-1β and TNF-α is a major component of the aging process. In addition, aging is an independent risk factor for thrombosis. The contribution of platelets to thrombosis during aging or states of inflammation and the mechanism promoting hyperreactivity needs to be further elucidated.

Aims: To investigate the mechanism(s) by which pro-inflammatory cytokines involved in aging may promote platelet hyperreactivity.

Methods: We analyzed megakaryocyte maturation, platelet number, and activation by FACS and functional assays using the following murine models of aging and inflammation: 1) aged C57BL/6J (>16 months old); 2) mice with constitutive expression of TNF-α ($TNF^{\Delta ARE}$) and; 3) C57BL/6J mice (2 months old) injected with IL-1β or TNF-α (1,5 or 20 days). In addition, to gain a better understanding of the molecular changes that occur in megakaryocytes and platelets during aging and inflammation, we sorted native megakaryocytes for transcriptome analysis by RNA-seq.

Results: Platelets from aged, $TNF^{\Delta ARE}$, and mice injected with TNF-α, have significantly higher phosphatidylserine exposure and active αIIbβ3 upon activation with thrombin. This hyperreactivity was also evident in a microfluidic assay when measuring platelet adhesion and aggregation on collagen surfaces. Interestingly, mice exposed to either IL-1β or TNF-α exhibited thrombocytosis and accelerated megakaryocyte maturation. However, only mice exposed to TNF-α developed the platelet hyperreactive phenotype.

Conclusions: Our data suggest that the platelet hyperreactive phenotype of aging could be directly associated with elevated TNF-α levels given that mice treated with IL-1β developed thrombocytosis but not the hyperreactive phenotype. We are undertaking transcriptome analysis of native megakaryocytes to provide significant insight into the molecular pathways governing platelet hyperreactivity, therefore, opening doors to possible new therapeutic interventions.

OC 29.1 | Platelet-dependent Leukocyte Adhesion and Fate in Whole Blood Thrombus Formation

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Background: Leukocytes attract to platelet thrombi in late stages of thrombus formation. How this leads to leukocyte activation is unknown.

Aims: Characterization of leukocyte functions attracted to a thrombus.

Methods: Human platelet thrombi with increasing extent of platelet activation (type I< II< III) were formed by whole blood perfusion over collagen microspots. Leukocyte adhesion, differentiation and responses were assessed by multi-colour confocal microscopy. Interactions of activated platelets and neutrophils were confirmed in static systems and by flow cytometry.

Results: Type-III thrombi formed on collagen/tissue factor, supporting thrombin generation, were most attractive for leukocytes (>95% neutrophils CD15+). Platelets in type-III thrombi displayed highest secretion markers. Neutrophils attracted to these thrombi stayed attached for up to 6 h, and expressed the following functional markers/responses:

1. expressed CD66b and MPO;
2. production of reactive oxygen species (ROS);
3. repetitive, transient rises in cytosolic Ca^{2+} during movement at and between thrombi;
4. proteolytic degradation and endocytosis of collagen. Remarkably, the neutrophils were highly reluctant to form neutrophil extracellular traps (NETs) even after 16 h of incubation (citrulline histone H3; < 15%).

Post-silencing of platelets in thrombi with stable prostacyclin marked impaired neutrophil responsiveness, pointing to persistent platelet activity. Surface-located CCL5 (RANTES) stimulated neutrophil attachment. Blockage of CXCL7 (NAP2) but not of CXCL4 (PF4) silenced Ca^{2+} rises of those neutrophils nearby thrombi. Platelet silencing and CXCL7 blockage diminished the majority of neutrophil responses, but not ROS production.

Conclusions: Persistent secretion of chemokines CXCL7 and CCL5 from platelet thrombi attracts and activates neutrophils, induces Ca^{2+} rises, secretion and proteolytic collagen degradation, with no more than limited NETs formation. Thrombus silencing suppresses this neutrophil activation.

OC 29.2 | The Contribution of Platelet Adhesion Receptors to Vascular Integrity during Inflammation Is Stimulus and Organ Dependent

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Background: Platelets maintain vascular integrity in various inflamed organs. Results from previous studies have indicated that platelet GPVI and CLEC-2 could be central to this function. Notably, GPVI was shown to support repairing of neutrophil-inflicted vascular injury in the inflamed skin. Whether the same mechanism also operates in other inflamed organs remains to be determined, as well as how CLEC-2 would contribute to it.

Aims: We investigated and compared the roles of GPVI, CLEC-2, and GPIb to the prevention of inflammatory bleeding in the inflamed skin and lungs.

Methods: The cutaneous reverse passive Arthus reaction (rpA) and intranasal lipopolysaccharide (LPS) instillation were induced in mice with a deficiency in CLEC-2 and/or GPVI, in IL4R-GPIbα transgenic mice, which lack the extracellular domain of GPIb, and in mice immunodepleted for platelets. Neutrophil recruitment and bleeding following rpa and LPS-induced lung inflammation were assessed by analysis of skin homogenates and of bronchoalveolar lavage fluid (BALF).

Results: Mice with deletion of CLEC-2 on platelets did not bleed in the rpa. This was in contrast to the cutaneous bleeding of $GPVI^{-/-}$ mice. RpA-induced bleeding in mice deficient in both GPVI and platelet CLEC-2 was more severe than in $GPVI^{-/-}$ mice, and approached that of thrombocytopenic mice. In LPS-inflamed lungs, neither GPVI- nor platelet CLEC-2-deficient mice bled, which was in contrast to the bleeding phenotype of IL4R-GPIbα-tg mice. Quantification of neutrophils in BALF indicated that the inflammatory reaction developed normally in both GPVI- and platelet CLEC-2-deficient mice.

Conclusions: GPVI plays the major role in preventing bleeding in the rpa, with CLEC-2 being involved only in the absence of GPVI. In contrast, neither receptor plays a prominent part in preventing bleeding in LPS-inflamed lungs, which relies on GPIb. Our results demonstrate that the contribution of platelet adhesion receptors to vascular integrity varies between vascular beds and inflammatory challenges.

OC 29.3 | Human Endoglin as a Potential New Partner Involved in Platelet-Endothelium Interactions

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Background: Hereditary hemorrhagic telangiectasia type 1 (HHT1) is characterized by a bleeding tendency that is postulated to be a consequence of telangiectasia fragility rather than a platelet defect. HHT1 patients present heterozygous mutations in the endoglin gene resulting in a loss of expression of membrane endoglin (Eng) on endothelial cells (EC).

Aims: We reported that endothelial Eng is involved in inflammation via its RGD motif, through integrin-mediated leukocyte adhesion and transmigration. These data prompted us to hypothesize that Eng may act as an adhesion molecule involved in the interaction between EC and platelets through integrin recognition.

Methods: Blood samples were obtained from healthy donors, HHT1 and Glanzmann´s thrombasthenia patients. Bleeding time in Eng-haplodeficient (Eng+/-) and wildtype (Eng+/+) mice was studied. Generation of stable cell transfectants in L6E9 rat myoblasts, expressing human Eng, and in Chinese hamster ovary (CHO) cells, expressing human αIIbβ3, were carry out. Microfluidic devices were used to evaluate shear-resistant platelet adhesion.

Results: We find that the extracellular domain of human Eng promotes platelet adhesion under static conditions and confers resistance of adherent platelets to detachment upon exposure to flow. Also, platelets adhere to confluent EC in an Eng-mediated process. CHO cells ectopically expressing the human αIIbβ3 integrin acquire the capacity to adhere to myoblast transfectants expressing human Eng, whereas platelets from Glanzmann´s thrombasthenia patients lacking the αIIbβ3 integrin are defective for Eng-dependent adhesion to EC. Furthermore, the bleeding time, but not the prothrombin time, is significantly prolonged in Eng+/- mice compared to Eng+/+ mice.

Conclusions: These results suggest a new and critical role for Eng in αIIbβ3 integrin-mediated adhesion of platelets to the endothelium and may provide a better understanding on the basic cellular mechanisms in thrombo-inflammatory events.

OC 29.4 | VWF-Mediated Platelet ´Priming´ Potentiates Novel Leukocyte Interactions under Flow

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Background: Platelet-leukocyte interactions are important in diverse conditions, such as atherogenesis, infection and DVT. All previously characterised interactions between platelets and leukocytes, require at least one of the cells to be fully activated before they can interact (e.g. via P-selectin,-PSGL, Mac1, CD40-CD40L). We hypothesised that the VWF-GPIbα interaction ‚primes' (but does not activate) platelets under flow leading to leukocyte binding.

Aims: To characterise novel platelet-leukocyte interactions under flow.

Methods: VWF A1 domain was purified and captured onto flow channels. Labelled whole or plasma-free blood was perfused at defined shear rates ($50s^{-1}$ to $1000s^{-1}$). Platelet and leukocyte binding were quantified.

Results: Binding of GPIbα to VWF A1 under flow ‚primed' platelets causing rapid and repeated release of intracellular Ca^{2+}, activation of Src kinase and activation of $\alpha_{IIb}\beta_3$ that promoted platelet aggregation. VWF-primed platelets captured neutrophils and T cells (but not monocytes or B cells) under low shear ($50s^{-1}$). Leukocyte binding was not influenced by blocking P-selectin, but was completely inhibited by $\alpha_{IIb}\beta_3$ blockade. Leukocyte binding was increased 3- to 5-fold in the absence of fibrinogen. Addition of fibrinogen to plasma-free blood induced a concentration-dependent decrease in leukocyte adhesion, suggesting that leukocytes and fibrinogen compete for binding activated $\alpha_{IIb}\beta_3$. In support of this, channels directly coated with activated $\alpha_{IIb}\beta_3$ captured neutrophils and T cells leading to phenotypic changes within neutrophils.

Conclusions: VWF A1-GPIbα binding under flow ‚primes' platelets, leading to the activation of $\alpha_{IIb}\beta_3$. For the first time, we show that $\alpha_{IIb}\beta_3$ can directly interact with neutrophils and T cells, providing a novel mechanism that drives platelet-leukocyte cross-talk. Work is now underway to identify $\alpha_{IIb}\beta_3$-binding receptors, and to characterise the functional response of leukocyte tethering to primed platelets.

OC 29.5 | Platelets Protect Cardiomyocytes from Ischaemic Damage

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Background: Upon activation, platelets secrete their granule cargo causing occlusive thrombosis and ischaemic injury, while also exerting heterogeneous effects on numerous cells in the vascular niche. It is unknown if this cargo can directly modulate ischaemic injury to cardiomyocytes.

Aims: To investigate if cargo secreted from activated platelets can exert protective or deleterious effects on cardiomyocytes during ischaemia.

Methods: Primary ventricular cardiomyocytes were isolated by Langendorff perfusion from hearts of adult mice and subjected to ischaemic injury using an *in vitro* pelleting method.

Results: Pretreating cardiomyocytes with releasates from collagen related peptide (CRP)-treated murine platelets delayed the rate of cardiomyocyte death during ischaemia. This cardioprotective effect was completely lost using platelets from *Unc13d*^{Jinx}mice, which have a major granule secretion defect. Similarly, the protective effect was abrogated when the releasate was separated into a < 3 kDa fraction, excluding a role for small molecules from platelet dense granules. Inhibitor studies targeting receptors for stromal cell-derived factor (SDF)-1α and transforming growth factor (TGF)-β1 on cardiomyocytes identified important roles for these α-granule-derived proteins in mediating cardioprotection. Mechanistically, platelet releasates enhanced protein kinase C (PKC) activity in cardiomyocytes during ischaemia, while inhibiting PKC activity in cardiomyocytes effectively blocked the protective signals from platelet cargo. Importantly, pretreating platelets with a $P2Y_{12}$ antagonist, but not the cyclooxygenase inhibitor aspirin, substantially attenuated the protective effect on cardiomyocytes.

Conclusions: These findings therefore reveal a paradoxically protective role for platelet activation during cardiac ischaemia, but also have important implications for the use of anti-platelet therapeutics in the management of myocardial infarction.

OC 30.1 | NOX2 as an Anti-thrombosis Target: Small Molecule Targeting of Rac1-p67phox Signaling Prevents NOX2 Mediated GPVI- and Non-GPVI Dependent ROS Generation and Platelet Activation

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Background: Platelets from mice deficient in NADPH oxidase isoform NOX2 exhibit diminished ROS generation and platelet activation (ATVB 36:846, 2016). Binding of Rac1 GTPase to $p67^{phox}$ plays a critical role in NOX2 activation by facilitating the assembly of the NOX2 enzyme complex (Physiol Rev 87:245, 2007).

Aims: In this study we tested our hypothesis that Rac1-$p67^{phox}$ interaction is critical for ROS generation by NOX2 and that small molecules interfering with this interaction may constitute anti-thrombosis agents.

Methods: We investigated the effects of Phox-I, a rationally designed small molecule inhibitor of Rac1-$P67^{phox}$ interaction (Chem & Biol

19:228, 2012), to determine if inhibition of Rac1-p67^{phox} interaction prevents ROS generation and platelet activation. ROS generation was determined by flow cytometry in dcf-da loaded washed platelets.

Results: Collagen-related peptide (CRP) or thrombin induced ROS generation in a time- and a dose-dependent manner. Treatment of platelets with Phox-I (1-10 μM) inhibited CRP induced:

(a) ROS generation;
(b) binding of PAC1 antibody to platelets;
(c) release of p-selectin;
(d) secretion of ATP; and
(e) Akt phosphorylation. Platelets from Rac1$^{-/-}$ mice or human platelets treated with NSC23766, a specific Rac inhibitor, produced significantly less ROS in response to CRP, but a combination of NSC23766 and Phox-I did not show an additive effect on ROS inhibition. Incubation of platelets with Phox-I inhibited thrombin induced:

(a) ROS generation;
(b) secretion of ATP;
(c) platelet aggregation;
(d) phosphorylation of Akt; and
(e) the rise in cytosolic calcium.

Conclusions: These data, taken together with earlier reports that Rac1 is required for thrombin induced ROS production (PloS One 11(9): e0163227, 2016) and NOX2 complex is genetically essential for thrombus formation (ATVB 36:846, 2016), suggest that small molecules targeting the Rac1-p67phox interaction may serve as anti-thrombosis agents by preventing GPVI- and non-GPVI mediated ROS generation and platelet activation.

OC 30.2 | Evidence from Protein Kinase C δ Y155F Knock-in Mice Reveal Positive Regulatory Role of Y155 in GPVI-mediated Platelet Activation

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Background: Protein kinase C delta (PKCδ) is a serine/threonine kinase involved in a vast array of cellular functions and is differentially tyrosine phosphorylated depending on the agonist. In platelets, PKCδ positively and negatively regulates dense granule secretion in response to protease-activated receptors (PAR) and Glycoprotein VI (GPVI) agonists, respectively however how PKCδ regulates these functions is not understood. Hence, we speculate that the differential regulation by PKCδ in platelets is due to differences in its tyrosine phosphorylation state.

Aims: We investigated several phosphorylation sites on PKCδ and found that Y155 was phosphorylated in response to GPVI agonist but not PAR agonist. Hence, the focus of this study is to characterize the function of PKCδY155 in platelets.

Methods: We generated PKCδY155F mice to characterize the function of Y155 phosphorylation in platelets using *ex vivo* and *in vivo* methods.

Results: PKCδ Y311 and Y332 were phosphorylated in response to both convulxin (GPVI) and thrombin (PAR), however Y155 was only phosphorylated in response to convulxin (Figure 1A). AYPGKF (PAR4) stimulated PKCδY155F platelets did not show any difference in platelet aggregation and dense granule secretion. However, CRP (GPVI) stimulated PKCδY155F platelets showed decreased platelet aggregation and dense granule secretion (Figure 1 B & C) despite normal surface GPVI expression, suggesting that PKCδ Y155 phosphorylation is important for GPVI-mediated signaling. Whole blood from PKCδY155F mice perfused over collagen under arterial shear conditions showed decreased thrombus formation. Similarly, we observed that PKCδY155F mice survive longer than control following pulmonary embolism. Furthermore, PKCδY155F mice also exhibited longer time to occlusion using the ferric-chloride injury model. These data indicate that phosphorylation of Y155 on PKCδ is pro-thrombotic.

Conclusions: PKCδY155 is phosphorylated by GPVI agonist and Y155 phosphorylation positively regulates GPVI-mediated thrombus formation.

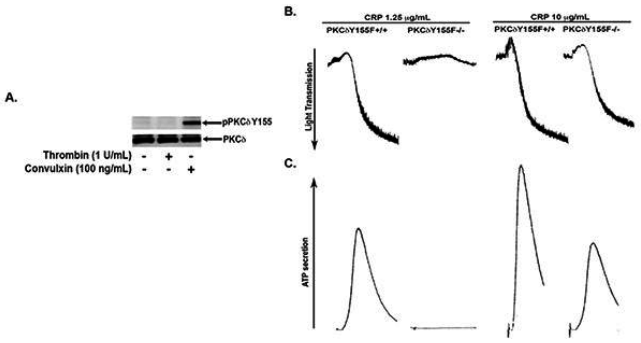

FIGURE 1 (A) Western blot of human platelets stimulated with indicated agonists for 1 minute. (B) Platelet aggregation. (C) Dense granule secretion

OC 30.3 | Implications of eNOS-signaling Heterogeneity within Human Platelets on Adhesion and Aggregation

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Background: Despite a vast number of studies, molecular mechanisms regulating formation of platelet thrombus still remain poorly understood. In 1990 Radomski et al. proposed that human platelets have

endothelial nitric oxide synthase (eNOS)-signaling pathway present, providing an endogenous negative-feedback mechanism that prevents platelet adhesion and aggregation. Currently a great deal of controversy exists with regards to whether this pathway is actually present in platelets.

Aims: To investigate if platelet subpopulations exist based on the presence (eNOS+ve) and absence (eNOS-ve) of eNOS-signaling and whether this may contribute to platelet differential roles in hemostatic reactions.

Methods: Prostacyclin-washed human platelets were used to measure nitric oxide (NO) production by DAF-FM using flow-cytometry and fluorescence microscopy. Levels of eNOS, soluble guanylyl cyclase (sGC) and platelet aggregation-mediating receptor (GPIIb/IIIa) were measured using flow-cytometry. Platelet functionality was assessed using light-aggregometry and flow-chamber confocal microscopy.

Results: Based on DAF-FM fluorescence we identified a platelet subpopulation that produced no/low-levels of NO (17.9% ± 2.4%) and a platelet subpopulation which produced high-levels of NO (82.1%±2.4%). Intracellular flow-cytometry showed platelets that lacked eNOS (17.7%±5.0% Fig 1) and platelets that had eNOS present (82.3%±5.2% Fig 1.). Furthermore, eNOS+ve platelets had higher levels of sGC vs. eNOS-ve platelets (1.8±0.2 vs.1.2±0.1 MFI, P< 0.05, Fig 1). Additionally, eNOS-ve vs.eNOS+ve platelets activated a greater portion of their GPIIb/IIIa in response to collagen (78.0%±8.5% vs.21.4%±7.2%). Finally, under flow conditions, eNOS-ve platelets adhered to collagen prior to their eNOS+ve counterparts (Fig 2).

Conclusions: Human platelet subpopulations exist based on the presence or absence of a functional eNOS-PKG-signaling pathway. eNOS-ve platelets are less abundant and more reactive than eNOS+ve platelets and initiate thrombus formation.

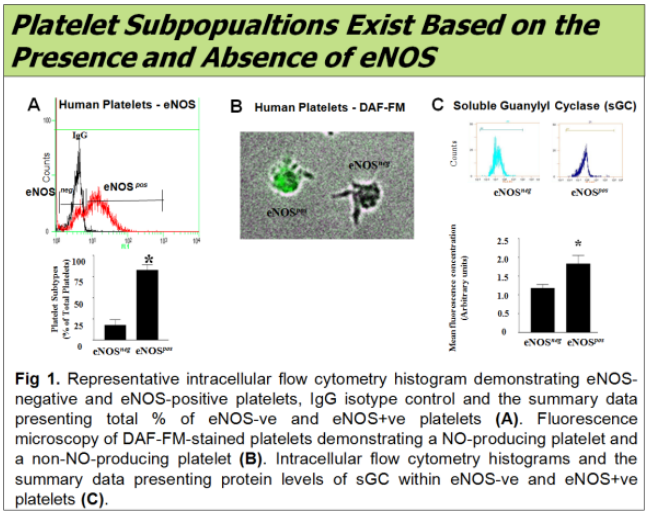

Fig 1. Representative intracellular flow cytometry histogram demonstrating eNOS-negative and eNOS-positive platelets, IgG isotype control and the summary data presenting total % of eNOS-ve and eNOS+ve platelets **(A)**. Fluorescence microscopy of DAF-FM-stained platelets demonstrating a NO-producing platelet and a non-NO-producing platelet **(B)**. Intracellular flow cytometry histograms and the summary data presenting protein levels of sGC within eNOS-ve and eNOS+ve platelets **(C)**.

FIGURE 1 eNOS-signaling heterogeneity within human platelets

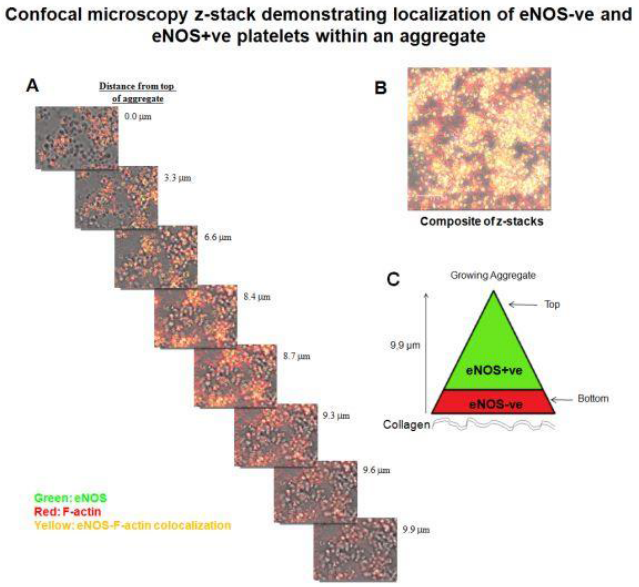

Fig 2. Representative merged immunofluorescence-brightfield confocal microscopy z-planes of an aggregate demonstrating eNOS localization in 3D **(A)**. Composite image of all z-planes **(B)**. Red fluorescence (Alexa 568 Phalloidan: F-actin, green fluorescence (Alexa 488): eNOS. Cartoon demonstrating 3-dimensional structure of an aggregate **(C)**.

FIGURE 2 Confocal microscopy z-stack demonstrating localization of eNOS-ve and eNOS+ve platelets within an aggregate

OC 30.4 | Differential Roles of Paraoxonase-2 (PON2) and NADPH Oxidase-2 (NOX2) in Murine Platelets

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Background: Paraoxonase-2 (PON2), an anti-oxidative protein with anti-inflammatory properties, has an emergent role in cardiovascular diseases, as it counter-acts atherosclerosis. As counterpart for anti-oxidative PON2, the NADPH oxidase 2 (NOX2) has been proposed as major pro-oxidative enzyme. Platelet activation is regulated by reactive oxygen species (ROS) and NOX2 has been suggested to modulate platelet activation via ROS generation.

Aims: To determine the effect of PON2 and NOX2 on platelet phenotype, activation and redox control using PON2- and NOX2-deficient mice.

Methods: PON2 was analyzed by immunoblotting and its localization by confocal microscopy. Platelet activation and ROS were measured by flow cytometry after stimulation with different concentrations of thrombin, convulxin, ADP or calcimycin. Platelet count and mean platelet volume were analyzed by an automated cell counter.

Results: Murine platelets expressed PON2 which was distributed in clusters intracellularly. PON2-deficient mice had unaltered

platelet count but significantly increased mean platelet volume whereas NOX2-deficient mice had slightly increased platelet count and decreased mean platelet volume compared to wildtypes. PON2 (-/-) platelets showed increased basal and agonist-induced ROS levels ex vivo. However, agonist-induced αIIbβ3 integrin activation and P-selectin surface expression were decreased in PON2 (-/-) mice. NOX2 (-/-) platelets showed decreased basal and agonist-induced ROS levels ex vivo as expected, but increased αIIbβ3 integrin activation and P-selectin activation in response to convulxin whereas thrombin stimulation resulted in unaltered responses.

Conclusions: Our data indicate that PON2 and NOX2 regulate platelet ROS generation and platelet activation differentially in a reverse dependent manner.

OC 30.5 | Alox12 Regulates Syk in FcγRIIA and Integrin αIIbβ3 Signaling in Platelets

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Background: Heparin induced thrombocytopenia (HIT) is mediated by FcγRIIA and has a complex pathophysiology with high morbidity in affected individuals. We previously reported that Alox12 inhibitor-ML355 reduced FcγRIIA platelet activation but the signaling remains to be fully elucidated.

Aims: In the present study, we hypothesized that Alox12 regulated Syk activity upstream of PLCγ2 and also contributed to ‚outside-in' signaling.

Methods: Alox12 action was assessed in two ways:

1. Pharmacological intervention using ML355 in human and FcγRIIA transgenic mouse platelets, and

2. alox12 gene KO in mouse platelets expressing FcγRIIA. FcγRIIA stimulation (IV.3 antibody + goat-anti mouse) of washed platelets was done in the presence or absence of the inhibitor, and signaling was assessed. Platelet spreading on fibrinogen was evaluated for human platelets treated with ML355 and for alox12-null FcγRIIA transgenic mouse platelets.

Results: In the presence of ML355 or with alox12 KO, phosphorylation of Syk Y352/Y346 (mouse) decreased significantly within 30s post-stimulation of FcγRIIA. A reduction in phosphorylation of activation loop Tyr525/Y519 (m) within 30s was also observed. The phosphorylation of PLCγ2 was reduced within 30s of activation in mouse platelets, upon attenuation of Alox12 activity. Platelet spreading decreased by 62% in human platelets treated with ML355 and by 44% in alox12$^{-/-}$ FcγRIIA transgenic mouse platelets, on immobilized fibrinogen.

Conclusions: The results provide direct evidence of Alox12 activity in early activation of FcγRIIA signaling by regulating Syk tyrosine kinase. Alox12 activity is also required for ‚outside-in' signal transduction via integrin αIIbβ3 signaling, which involves FcγRIIA and Syk. Inhibition of platelet activation by the Alox12 inhibitor will impinge on both ‚inside-out' and ‚outside-in' signaling in thrombosis.

OC 44.1 | Thrombopoiesis is Spatially Regulated by the Bone Marrow Vasculature

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Background: Thrombopoietin acts on hematopoietic stem cells and induces their differentiation into the megakaryocytic lineage. During their maturation, megakaryocytes (MKs) are thought to migrate towards the vascular sinusoids in the bone marrow (BM) to release proplatelets into the bloodstream. The concept of blood precursor migration is less supported by direct evidence, but mostly based on evaluation of cell populations present at distinct spatiotemporal niches. Interestingly, contrary to this MK maturation model, two-photon intra-vital microscopy (2P-IVM) data indicated that MKs barely migrate and are mostly found in close proximity to blood vessels.

Aims: We aimed to reconcile the discrepancy between the current model of megakaryopoiesis and 2P-IVM data.

Methods: MK localization in intact BM of mice was studied by combining 2P-IVM and *in situ* light-sheet fluorescence microscopy (LSFM) supplemented by computational simulations.

Results: Using 3D reconstruction of murine femora or sterna, we show the vascularization of the entire BM and hence the absence of vessel-distant niches. Immunohistochemical analysis of cryo-sections and LSFM of intact bones revealed that MKs are homogeneously distributed within a dense vascular network. Likewise, two-photon intra-vital MK tracking under steady-state condition as well as under conditions of increased platelet-demand revealed that even vessel-distant MKs are essentially sessile. Using computational modeling based on ‚real' segmented BM structures, we tested whether MKs are randomly distributed. Indeed, while 79% of the MKs were found at the vessels, the remaining non-vessel associated MKs were randomly distributed, clearly arguing against MK migration.

Conclusions: Our data challenge the current thrombopoiesis model of MK migration and support a modified model where MKs at sinusoids are replenished by sinusoidal precursors rather than cells from a distant periostic niche.

OC 44.2 | Rap1a/b Isoforms Differentially Regulate Megakaryocyte Biology

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Background: Thrombocytopenia, or low circulating platelet count, is often caused by a defect in megakaryocyte (MK) development. MKs reside primarily in the bone marrow, where they develop from undifferentiated multipotent cells into highly specialized, polyploidy cells that undergo the process of proplatelet formation (PPF). It is currently not clear if and how Rap1, a member of the Ras small GTPase superfamily, contributes to platelet generation.

Aims: We hypothesize that the two Rap1 isoforms, Rap1a and Rap1b, are critical in MK development and/or PPF.

Methods: We used two different cell models:

(1) primary murine MKs isolated from conditional knockout mice lacking Rap1a (Rap1a-mKO), Rap1b (Rap1b-mKO), or both isoforms (Rap1a,b-mKO) in megakaryocytes and platelets and

(2) an immortalized human MK cell line derived from pluripotent stem cells (imMKCLs). Flow cytometry, immunohistochemistry and confocal microscopy imaging were used to analyze MKs ex vivo, in situ, and in vitro.

Results: Rap1a-mKO mice exhibited a normal peripheral platelet count (PPC) but significantly increased MK numbers in the bone marrow and spleen. MKs from Rap1a-mKO mice were smaller and less differentiated than controls, and proplatelet extensions were shorter than in MKs isolated from control mice. Rap1b-mKO mice exhibited a mild macrothrombocytopenia and increased MK numbers. While MK maturation was only minimally affected, PPF was markedly altered (few but very large proplatelets). Consistently, Rap1a,b-mKO mice exhibited a marked macrothrombocytopenia, impaired MK endomitosis and a dramatic defect in PPF. Studies in imMKCL cells confirmed an important role for Rap1a and Rap1b in endomitosis and PPF, respectively.

Conclusions: These results provide the first definitive proof for a critical role of Rap1 GTPases in MK biology. Future studies will have to show whether some inherited or acquired thrombocytopenias are caused, at least in part, by altered Rap1 signaling in MKs.

OC 44.3 | A Cdc42/RhoA Regulatory Circuit Downstream of Glycoprotein Ib Guides Transendothelial Platelet Biogenesis

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Background: During the process of platelet biogenesis, megakaryocytes (MKs) extend long cytoplasmic protrusions (proplatelets) into bone marrow (BM) sinusoids from which platelets are released. The molecular cues that control MK polarization towards sinusoids and limit transendothelial passage to proplatelets remain incompletely understood.

Aims: We have previously shown that deficiency of the small GTPases RhoA or Cdc42 in MKs results in macrothrombocytopenia. Here, we investigated the roles of these GTPases in MK polarization-dependent platelet biogenesis.

Methods: Conditional Rho GTPase knock-out (KO) mice, GPIbα transgenic mice (*Gp1ba-Tg*), where the ectodomain of GPIbα is replaced by that of the human interleukin-4 receptor α, as well as inhibitors of MK/platelet signaling pathways were used in this study. MK localization was investigated by confocal immunofluorescence microscopy of native BM sections. MK demarcation membrane system (DMS) polarization was assessed in vitro and platelet biogenesis studied in vivo by two photon microscopy.

Results: We found a dramatic MK mislocalization in the BM of RhoA KO mice with a large population of whole MKs being present *inside* BM sinusoids. This led to perturbed platelet biogenesis in vivo, explaining the reduced platelet counts in RhoA KO animals. By contrast, in Cdc42 KO mice, no MKs were found in sinusoids and the number of MKs with direct contact to blood vessels was reduced. Our previous studies indicate that Cdc42 confers signaling downstream of GPIbα. Consistently, MKs also had less contact to sinusoids in the BM from *Gp1ba-Tg* mice exhibiting defective GPIbα ectodomain signaling. While *Gp1ba-Tg* MKs showed decreased Cdc42 activity and MK polarization, these processes were markedly increased in RhoA KO MKs. Finally, combined deficiency in RhoA and Cdc42 reverted the MK mislocalization seen in RhoA KO mice.

Conclusions: Our results reveal a Cdc42/RhoA regulatory circuit that controls GPIb-dependent transendothelial platelet biogenesis by mutual „go“/”stop” signals.

OC 44.4 | Megakaryocyte-Specific Sin1 Deficiency Leads to Thrombocytopenia and Defective Platelet Activation in Thrombosis and Myocardial Infarction

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Background: Mammalian target of rapamycin complex 2 (mTORC2), consisting of mTOR, Sin1, Rictor and mLST8, has been proven to be present and intact in platelets. To date, it is still not clear whether loss of the integrity of mTORC2 complex has effects on platelet function.

Aims: The aims of this research were to identify the role and molecular mechanism of Sin1 in regulating platelet aggregation, spreading, clot retraction, thrombosis and myocardial infarction.

Methods: Platelet aggregation test; spreading; clot retraction; western blot; immunofluorescence; FecI3 artery thrombosis mouse model; LAD-ligation mouse model; flow cytometry.

Results: Here we reported that deficiency of Sin1, an essential component of mTORC2, caused a defect in platelet aggregation stimulated by thrombin, ADP, U46619 and collagen respectively. Interestingly, megakaryocyte/platelet-specific Sin1-deficient ($Sin1^{-/-}$) mice developed a severe thrombocytopenia, which was due to enhanced platelet apoptosis and decreased reticulated platelet count. Compared with wildtype platelets, Sin1 deficient platelets showed impaired clot retraction and incomplete platelet spreading on immobilized fibrinogen. Surprisingly, Sin1 deficiency resulted in significant decreased the phosphorylation and protein levels of Rictor and mTOR. Furthermore, ablation of Sin1 decreased the phosphorylation of AKT on Thr308, Ser473 and Thr450, which were all hyperphosphorylated in the platelets from myocardial infarction patients, indicating a potential role of Sin1 in myocardial infarction disease. Moreover, Sin1 was significantly phosphorylated on Thr86 in platelets treated with thrombin, and the levels of Sin1 Thr86 phosphorylation were correlated with the dose of thrombin. Importantly, Sin1 deficiency protected the heart against myocardial infarction-induced heart failure.

Conclusions: Together, our findings revealed that Sin1 deficiency caused a severe thrombocytopenia and defective platelet activation in thrombosis and myocardial infarction by ablating AKT phosphorylation.

OC 44.5 | CK2β Deficiency Results in Severe Macrothrombocytopenia due to Premature Megakaryocyte Fragmentation

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Background: Platelets are anucleated cells originated from polyploid megakaryocytes (MKs). MKs form proplatelets within the bone marrow (BM) and release platelets in BM sinusoids. The tetrameric serine/threonine kinase casein kinase 2 (CK2) is highly expressed in platelets. Although thrombopoiesis is strictly regulated by a wide variety of kinases less is known about the role of CK2 in the process of platelet biogenesis and proplatelet formation.

Aims: The present study explored the impact of the CK2 regulatory β-subunit on proplatelet formation and thrombopoiesis in MK/platelet-specific CK2β-deficient mice ($ck2\beta^{-/-}$).

Methods: megakaryocyte/platelet specific KO mice, flow cytometry, in vivo and in vitro proplatelet formation assay.

Results: As demonstrated by blood counts, CK2β deficiency resulted in a severe macrothrombocytopenia whereas platelet survival was only mildly affected. However, megakaryocytes from $ck2\beta^{-/-}$ mice exhibited increased plasma levels of thrombopoietin (TPO) and an increased extramedullar megakaryopoiesis correlating with enhanced ratio of premature platelets as shown by thiazole orange (TO) measurements. Megakaryocytes within BM of $ck2\beta^{-/-}$displayed drastic increased fragmentation resulting in significantly impaired proplatelet formation *in vitro* and *in vivo* as shown by means of multiphoton intravital microscopy. Interestingly, megakaryocyte fragmentation came along with decreased ploidy and less contact to bone marrow sinusoids in *ck2β*-deficient mice compared to wildtype littermates as investigated by flow cytometry or immunofluorescence stainings. At least in part, megakaryocyte fragmentation occurs due to abolished microtubule stability as megakaryocytes from $ck2\beta^{-/-}$ mice were unable to stabilize tubulin under taxol treatment leading to disrupted tubulin polymerization and incorrect distribution of microtubule-associated protein RP/EB family member 3 (EB3).

Conclusions: Finally, the present observations disclose CK2 as pivotal regulator in thrombopoiesis and proplatelet formation.

OC 45.1 | A Small Molecule Ligand for CLEC-2 Blocking Podoplanin Binding Inhibits Experimental Tumor Metastasis and Thrombus Formation in Mice

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Background: A platelet activation receptor, C-type lectin-like receptor 2 (CLEC-2) interacts with podoplanin, which expresses on the surface of certain types of tumor cells or lymphatic endothelial cells, and facilitates tumor metastasis and blood/lymphatic vessel separation during development. CLEC-2 stabilizes thrombus under flow condition by unidentified mechanisms. Since CLEC-2-dificient mice do not show a significant increase in bleeding tendency, CLEC-2 may be a good target for anti-metastasis or anti-thrombotic drug.

Aims: The aim of this study is to identify a small molecule ligand for CLEC-2 that inhibits podoplanin binding.

Methods: We screened 6720 small molecules for ability to inhibit CLEC-2/podoplanin binding using ELISA assay. Hit molecules by the 1st screening were analyzed its blocking ability using the CLEC-2-expressing cell line and recombinant podoplanin by flow cytometry.

Aggregation of human or mouse washed platelets was measured by Born's aggregometry. In vivo thrombus formation stimulated with FeCl3 was evaluated by femoral blood flow monitored using a Doppler blood flow velocimeter. For the hematogenous metastasis model, podoplanin-expressing melanoma cell line, B16F10 were injected intravenously into mouse tail veins. After 14 days, the weight of the lungs was measured.

Results: Sixty-three hit-molecules in the first screening were further screened for the ability to inhibit podoplanin binding to CLEC-2 expressing cells. The second screening resulted in 2 hit-molecules. We optimized one of the hit-molecules, X to make X complexed with cobalt (Co-X). Co-X specifically inhibited rhodocytin-induced platelet aggregation. Intravenous administration of Co-X to mice significantly inhibited hematogenous tumor metastasis to the lung and in vivo thrombus formation. On the other hand, Co-X did not significantly increase time of bleeding from the tail vein.

Conclusions: Co-X may be a good leading molecule for anti-metastatic and anti-platelet drug without bleeding tendency as an adverse event.

OC 45.2 | Pharmacological Characterization of the Functional Role of Calcium-activated Potassium Channels in Platelets

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Background: In arteries, stimulation of endothelial cell small (SK_{Ca}) and intermediate (IK_{Ca}) conductance calcium-activated potassium channels provides a negative-feedback mechanism to limit agonist-induced vasoconstriction. Additionally, endothelial cell K_{Ca} channels in conjunction with nitric oxide (NO) mediate vasodilation in response to agonists and physical stimuli. Platelets, like endothelial cells, possess K_{Ca} channels and generate NO via endothelial nitric oxide synthase (eNOS). NO is known to limit platelet aggregation but the role of K_{Ca} channels in platelet function and NO-generation has not been explored. Our hypothesis was that activation of K_{Ca} channels would inhibit platelet aggregation and enhance platelet NO production.

Aims: Our objective was to pharmacologically characterize SK_{Ca} and IK_{Ca} channel function in platelets, and investigate their role in platelet NO production.

Methods: Platelets were isolated from the blood of healthy volunteers and aggregometry performed in the presence of SK_{Ca} (CyPPA) and IK_{Ca} (SKA-31) channel activators. Dense granule secretion was measured by ATP chemiluminesence. DAF-FM flow cytometry was used to measure NO generation.

Results: CyPPA and SKA-31 inhibited collagen-induced aggregation in a concentration dependent manner. IK_{Ca} selective channel blocker reversed the anti-aggregatory effects of 10μM SKA-31 but not CyPPA. SK_{Ca} channel-selective blocker did not reverse the effect of either CyPPA or SKA-31. CyPPA and SKA-31 inhibited NO generation back to basal resting platelet levels. CyPPA and SKA-31 demonstrated similar inhibitory effects on platelet dense granule secretion, whereas only SKA-31 significantly inhibited alpha granule secretion.

Conclusions: Activation of SK_{Ca} and IK_{Ca} channels inhibits both platelet aggregation and platelet NO generation. Furthermore, the use of selective blockers suggest that IK_{Ca} is the dominant K_{Ca} channel within platelets. These data indicate that K_{Ca} channels may provide novel targets for therapeutics to inhibit platelet aggregation.

OC 45.3 | Pharmacological Inhibition of the Class II PI3K, PI3KC2α, Provides Marked Anti-thrombotic Effects in Mice and Humans via a Unique Anti-platelet Mechanism

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Background: The Class II PI3K, PI3KC2α, is a broadly expressed lipid kinase with few known roles. We recently used a mouse genetic approach to show that PI3KC2α is important for platelet function; PI3KC2α-deficient mice have a dysregulated open canalicular system structure and exhibit an impaired thrombotic response (Mountford, Nat Comm, 2015). These observations suggest PI3KC2α may represent a viable target for novel anti-platelet therapy. However, given the lack of PI3KC2α inhibitors, whether or not a similar function occurs in human platelets remains unknown.

Aims: To develop pharmacological inhibitors of PI3KC2α and use these to examine whether PI3KC2α similarly regulates the structure and function of human platelets as in PI3KC2α-deficient mouse platelets.

Methods: A rational drug design approach generated first generation PI3KC2α inhibitors. The effects of these inhibitors were examined on platelet structure (via SEM and TEM) and prothrombotic function (aggregation of isolated platelets, ex vivo thrombosis in human whole blood perfused over collagen-coated microslides, and in vivo thrombosis in mouse models).

Results: We developed X151, a competitive active site inhibitor of PI3KC2α with an IC_{50} of 13nM against purified enzyme. Pharmacological inhibition of PI3KC2α with X-151 closely mimicked the changes in platelet structure and function observed in PI3KC2α-deficient mice. Specifically, X151 (1 μM) dilated the open canalicular system of platelets isolated from both mice and humans to a similar extent to that observed in PI3KC2α-deficient mouse platelets (~40% increase in surface area). Furthermore, X151 nearly abolished thrombus formation in an ex vivo human whole blood model and two distinct in vivo mouse models.

Conclusions: These findings demonstrate PI3KC2α regulates human platelet structure and function and suggest that PI3KC2α may be a suitable target for novel anti-thrombotic drug therapies.

OC 45.4 | Quercetin and its Metabolites Inhibit Platelet Function and Thrombus Formation both in vitro and in vivo, and Combine with Aspirin to Increase Anti-platelet Effects

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Background: Flavonoids, a class of plant secondary metabolites, are found in diets globally. Cohort studies have found one such flavonoid, quercetin, to possess anti-CVD (Cardiovascular Disease) effects. However, its extensive metabolism by the small intestine and liver has resulted in difficulty elucidating precise mechanisms of action.

Aims: To investigate the mechanisms through which quercetin and its methylated metabolites tamarixetin and isorhamnetin inhibit platelet function and thrombus formation *in vitro* and *in vivo*, and their interaction with the anti-platelet effect of aspirin.

Methods: Flavonoid:aspirin combinatorial effects on platelet aggregation were investigated using a 96-well aggregation assay. Inhibition of thrombus formation *in vitro* under physiological shear rate was investigated using microfluidic blood perfusion channels. A laser-injury model in C57/BL6 mice was used to assess thrombosis in mice.

Results: Quercetin, tamarixetin and isorhamnetin inhibited significantly thrombus formation *in vitro* at 10μM. Tamarixetin was most potent, indicating a 4´-methyl group in increased potency. Treatment of mice with isoquercetin over 48 and 72-hours resulted in significantly reduced thrombus size *in vivo*. The concentration of aspirin required for 50% inhibition of aggregation was lowered significantly by flavonoid administration, with higher (10μM) concentrations reducing IC_{50} values by an order of magnitude.

Conclusions: Thrombus formation is inhibited in whole blood at physiologically achievable concentrations, demonstrating the ability of flavonoids to inhibit platelet function despite high plasma-binding properties. This effect was maintained in a murine model of thrombosis. This may offer insight into the ability of a diet high in flavonoids, such as quercetin, to reduce CVD risk, and with their combinatorial effects with aspirin, offer potential to lower therapeutic aspirin doses and alleviate associated symptoms such as gastric bleeding.

OC 45.5 | Putative, Novel Mechanisms of Action for Citalopram-induced Platelet Inhibition

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Background: Citalopram, a selective serotonin reuptake inhibitor (SSRI), suppresses platelet function via a serotonin reuptake transporter (SERT)-independent mechanism. We report evidence for two putative, distinct mechanisms of action for citalopram-induced platelet inhibition.

Aims: To identify novel pharmacological mechanisms of action for citalopram-induced platelet inhibition.

Methods: Washed platelets (WP) were prepared from citrated blood from healthy volunteers and stimulated with either the GPVI agonist, collagen-related peptide (CRPXL, 0.5 μg/ml) or the thromboxane analogue, U46619 (0.2 μM). HPLC was used to quantify nucleotide release from platelet dense granules. Tyrosine phosphorylation of signalling proteins was determined by Western Blot. Rap1-GTP was isolated from platelet lysates using a GST-RalGDS-RBD fusion protein pull-down assay prior to Western Blot. Intracellular calcium ($[Ca^{2+}]_i$) mobilisation was measured in fura2/AM-loaded WPs.

Results: Citalopram inhibited CRPXL-induced dense granule release ($pIC_{50} \approx 4.5$), and tyrosine phosphorylation of FcRγ chain, Src-family kinases ($pIC_{50} \approx 4.2$), LAT ($pIC_{50} \approx 4.0$) and PLCγ2 ($pIC_{50} \approx 4.4$). Rap1 activation was also blocked by citalopram (200 μM) in both CRPXL- and U46619-stimulated platelets. CRPXL-induced $[Ca^{2+}]_i$ mobilisation was abolished by citalopram ($pIC_{50} \approx 4.4$). By contrast, U46619-induced $[Ca^{2+}]_i$ mobilisation was unaffected by citalopram up to 200 μM.

Conclusions: We have identified two putative distinct mechanisms of action for platelet inhibition by citalopram.

1) Citalopram suppressed protein phosphorylation proximally in the GPVI pathway, resulting in the inhibition of $[Ca^{2+}]_i$ mobilisation and dense granule release.

2) Despite blocking U46619-induced Rap1 activation, citalopram failed to inhibit $[Ca^{2+}]_i$ mobilisation induced by U46619. A likely target is the regulator of Rap1 activation, the Guanine Nucleotide Exchange Factor, CalDAG-GEFI.

OC 53.1 | Defective Arterial Thrombosis and Thrombo-inflammation in Mice Lacking Bridging Integrator 2 (Bin2)

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Background: Store-operated calcium entry (SOCE) is the principal route of Ca^{2+} influx in platelets, critical for platelet activation. The calcium sensor molecule stromal interaction molecule 1 (STIM1) regulates SOCE by activation of the membrane calcium channel protein Orai1, but the exact mechanism of this interaction remains elusive. We used affinity chromatography to screen for STIM1 interacting

proteins in platelets and identified bridging integrator 2 (BIN2, mainly expressed in the hematopoietic system), a protein of the family of N-terminal Bin-Amphiphysin-Rvs (BAR) proteins.

Aims: We investigated the role of BIN2 in SOCE in platelets and its pathophysiological role in thrombotic and thrombo-inflammatory activity of the cells.

Methods: We generated a megakaryocyte-/ platelet-specific Bin2 KO mouse line (*Bin2*$^{fl/fl\text{-}Pf4Cre}$) and assessed platelet function *in vitro* using flow cytometry, biochemistry, calcium fluorimetry, aggregometry and flow adhesion assays. In addition, *in vivo* models of thrombosis and hemostasis, as well as the transient middle cerebral artery occlusion (tMCAO) model of ischemic stroke were performed.

Results: Bin2 KO platelets displayed markedly impaired SOCE in response to thapsigargin as well as CRP, convulxin and rhodocytin as well as thrombin, ADP and U46619, agonists acting on immunoreceptor tyrosine-based activation motif (ITAM) or G protein-coupled receptors, respectively. This SOCE defect resulted in impaired (hem) ITAM induced platelet activation, aggregate formation under flow and procoagulant activity, as assessed by measuring PS exposure. Similarly, mice lacking BIN2 in platelets were protected from occlusive arterial thrombus formation and thrombo-inflammatory cerebral infarct progression in a model of experimental stroke.

Conclusions: Our results show that the adapter protein BIN2 is an important regulator of SOCE in platelets, critically required for occlusive arterial thrombosis and thrombo-inflammatory brain infarction.

OC 53.2 | SNAP23 is Critical for Platelet Granule Secretion

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Background: Secretion of platelet granule cargo is critical for efficient platelet function. Previous evidence from *in vitro* exocytosis assays using permeabilized platelets has suggested a role for SNAP23 in the regulation of granule secretion. A definitive assessment of its role in the regulation of all platelet granule types, in response to platelet agonists, and its role in mediating haemostasis and thrombosis *in vivo*, is currently lacking.

Aims: To characterize the role of SNAP23 in platelet secretion and function.

Methods: A SNAP23 conditional knockout was generated by crossing floxed allele mice with Cre-PF4. A range of functional assays *in vitro* and *in vivo* were used to determine the role of SNAP23.

Results: We have found that platelet-specific deletion of SNAP23 results in complete ablation of P-selectin surface exposure and PF4, ATP and beta hexaminidase release in response to thrombin or collagen-related peptide (CRP), indicating a complete absence of alpha granule, dense granule and lysosome secretion, respectively. Activation of the $\alpha_{IIb}\beta_3$ integrin was attenuated in these mice, but was rescued upon exogenous addition of ADP; however, exogenous addition of ADP did not rescue P-selectin exposure, demonstrating that the alpha granule defect observed is not due to absent dense granule release. Strikingly, these mice also presented as macrothrombocytopenic, with a 60% reduction in platelet count and 30% increase in mean platelet volume.

Conclusions: SNAP23 has long been suspected as playing a critical role in platelet secretion. Here we provide the first definitive evidence using a genetic approach that SNAP23 is essential for secretion from all granule types. Unexpectedly, we also observed a macrothrombocytopenia, which may indicate an additional role for SNAP23 in platelet biogenesis.

OC 53.3 | 14-3-3ζ Regulates the Mitochondrial Respiratory Reserve Linked to Platelet Phosphatidylserine Exposure and Procoagulant Function

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Background: The 14-3-3 family of adaptor proteins are ubiquitously expressed in eukaryotic cells and were the first identified phosphoserine-and phosphothreonine-binding proteins. They play a key role in regulating cell-cycle progression, apoptosis, metabolism, intracellular trafficking and regulating cell stress responses. Six 14-3-3 isoforms are present in human platelets including β, ε, ζ, γ, η and τ, with ζ and γ expressed at high levels. The first identified 14-3-3 binding partner in platelets was the VWF receptor GPIb/V/IX. 14-3-3 ζ binds to the cytoplasmic tails of GPIb α/β and numerous studies focussed on the relevance of this interaction for platelet activation and signalling, with varying views on its significance.

Aims: In the current study, we investigated the importance of 14-3-3ζ in regulating platelet function.

Methods: This was achieved by using mice genetically deficient in this specific 14-3-3ζ isoform.

Results: We demonstrated that mice specifically deficient in platelet 14-3-3ζ were protected against thrombosis, in the absence of a bleeding phenotype. Interestingly, the thrombotic defect in 14-3-3ζ$^{-/-}$ mice was not due to abnormal VWF-GPIb adhesive function, nor defective platelet activation induced by soluble platelet agonists. Instead, we identified an important role for 14-3-3ζ in regulating platelet phosphatidylserine (PS) exposure and procoagulant function associated with the generation of thrombin. We found evidence for a role for 14-3-3z in modulating levels of metabolic ATP and mitochondrial

respiratory reserve, potentially through the interaction of 14-3-3ζ with the mitochondrial F1-F0 ATP synthase.

Conclusions: Our studies have identified an important role for 14-3-3ζ in regulating platelet bioenergetics by modulating the mitochondrial respiratory reserve, leading to decreased platelet PS exposure and procoagulant function. Therapeutic targeting of 14-3-3ζ signalling may represent a new approach to reduce platelet procoagulant function and thrombosis without increasing bleeding.

OC 53.4 | Kinesin-1 Regulates Platelet Secretion and Thrombus Stability Through the Interaction with the Granular Slp4/Rab27b Effector Complex

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Background: Even if several regulators of fusion machinery for granule exocytosis have been identified in platelets, the exact molecular mechanisms regulating their trafficking on cytoskeleton are still poorly understood.

Aims: To define the role of the molecular motor, kinesin-1, in platelet secretion.

Methods: Using kinesin-1-deficient mice (cKO^{Kif5b}) in platelets, platelet functions were assessed (i) *in vivo* in a tail bleeding assay and in a thrombosis model, (ii) *in vitro* in thrombus formation under flow conditions and in platelet aggregation. Dense and α granule secretion were evaluated by measuring P-selectin exposure and ATP released. Rab27b, Slp4 and kinesin-1 interactions were studied by co-immunoprecipitation assays.

Results:

cKO^{Kif5b} mice display features of unstable hemostasis: in a tail clip-bleeding assay, a rebleeding tendency was observed (29% cKO^{Kif5b} mice versus 0% in WT mice). In a FeCl3-induced in vivo thrombosis model, thrombi formed normally; nevertheless they appeared less compact and stable over time. This instability was confirmed in vitro in a whole-blood perfusion assay. Aggregations induced by thrombin and collagen were impaired in cKO^{Kif5b} platelets. These results indicate that kinesin-1 plays a role in platelet functions and thrombus stability.

Kinesin-1 regulates dense and α granule secretion: ATP released and P-selectin exposure after thrombin stimulation were impaired in cKO^{Kif5b} platelets. As both granules appeared normal upon electron microscopy, total serotonin content was normal in dense granules and the defect of ATP release was overcome by high thrombin concentration, altogether, these results ruled out defects of granule cargo.

Kinesin-1-dependent transport machinery: We demonstrated that kinesin-1 links microtubules to α and dense granules through the molecular machinery composed of granule-associated Rab27b and the Slp4 adaptor protein.

Conclusions: Kinesin-1 regulates platelet granule secretion, and therefore thrombus stability, in collaboration with Rab27b and Slp4.

OC 53.5 | Secretory Granules and Molecular Interaction of DOCK7, VAC14 and SEC16A with NBEAL2

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Background: Variants in *NBEAL2* are causal of Grey Platelet Syndrome (GPS), a rare bleeding disorder characterized by absence of alpha- and specific- granules in platelets and neutrophils, respectively. The role of the scaffolding multidomain NBEAL2 protein in cell biology and granule homeostasis is unknown.

Aims: To investigate the biochemical role of NBEAL2 in cell biology.

Methods: We have performed proteomics to identify NBEAL2's binding partners followed by different layers of validation including biochemical, cellular and functional analysis *in vitroand in vivo*.

Results: HEK293T cells overexpressing the Pleckstrin homology (PH), BEACH and the WD40 repeat domains of NBEAL2 fused with a Tandem Affinity Purification (TAP) tag were used. Baits were immune-purified and 164 proteins were identified by mass spectrometry but were unobserved in a control precipitation with a GFP-TAP tag. Reverse immunoprecipitation confirmed the binding of DOCK7 and VAC14 to NBEAL2's BEACH domain, while SEC16A specifically binds to the WD40 domain. Similar patterns of interactions were replicated in the megakaryocytic cells (CHRF) overexpressing PH-BEACH-WD40-TAP. Proximity ligation assays on endogenous proteins carried out in stem cell derived megakaryocytes (MKs) revealed significant interactions of NBEAL2 with DOCK7 and VAC14. Insertion of GPS-causing variants in the BEACH domain significantly impacted on its interaction with DOCK7 (P2100L, R2172H, G2290W) and VAC14 (M2080K, P2100L, G2290W), respectively. DOCK7 was significantly reduced in platelets from GPS mice and the phosphorylation pattern of coffilin and LIMK-2, effectors downstream the DOCK7 pathway, was also altered.

Conclusions: Our study shows the first NBEAL2 protein interactions and places DOCK7, SEC16A and VAC14 as binding partners of this uncharacterized multidomain protein. Further validation of new

proteins from the network will allow us to understand and define the biochemical and functional role of NBEAL2 in cell biology.

OC 57.1 | Blocking the b´x Domain of PDI Interferes with its Interaction with αIIbβ3 and Prevents Thrombus Formation

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Background: Protein disulfide isomerase (PDI) serves an essential role in platelet activation and thrombus formation. αIIbβ3 has been proposed as an important substrate of PDI, but whether PDI mediates disulfide bond rearrangements in αIIbβ3 and contributes to the activation of the receptor is unknown.

Aims: To evaluate the mechanism by which PDI facilitates αIIbβ3 activation.

Methods: We identified PDI inhibitors using a high throughput approach. Several compounds with submicromolar potency were screened for their ability to inhibit platelet aggregation, identifying three structurally unrelated platelet antagonists (termed bepristats 3-5). We used these compounds to study how PDI influences αIIbβ3 activation.

Results: Studies using isolated PDI fragments showed that bepristats 3-5 all bound PDI at the substrate-binding domain (**b´x**) and did not block activity at the catalytic domains (**a** and **a´**). These compounds potently inhibited αIIbβ3 activation as determined by PAC-1 binding. Incubation of PDI with purified αIIbβ3 stimulated incorporation of maleimide polyglycol biotin (MPB) into αIIbβ3, demonstrating PDI-dependent exposure of free thiols. Unexpectedly, MPB was robustly incorporated into PDI upon exposure to αIIbβ3, indicating reciprocal disulfide exchange between αIIbβ3 and PDI. Activation of platelets with thrombin increased MPB incorporation into both endogenous αIIbβ3 and PDI. Thrombin-dependent disulfide exchange was inhibited by bepristat 4, showing involvement of the **b´x** domain of PDI. Conversely, the **b´xa´** fragment had substantially enhanced ability to reduce αIIbβ3 compared with the isolated **a´** domain. Comparison of different bepristats in a laser-induced model of thrombosis showed that bepristats that potently blocked PDI-αIIbβ3 interactions were better inhibitors of thrombosis than those that did not.

Conclusions: These studies show that **b´x** is required for disulfide exchange between PDI and αIIbβ3 and identifies improved strategies for developing antithrombotic PDI inhibitors.

OC 57.2 | Profilin 1 is a Central Regulator of Integrin Turnover and Function in Mouse Platelets

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Background: Firm platelet adhesion at sites of vascular injury is mainly mediated by heterodimeric receptors of the β1- and β3-integrin families and is crucial to prevent excessive blood loss, but may also lead to thrombosis. Recruitment of talin-1 to β-integrin tails is essential for integrin activation and connects these adhesion receptors to the actin cytoskeleton. The small actin-binding protein Profilin 1 (Pfn1) is critical for actin rearrangements and platelet biogenesis. However, its role in platelet function and integrin activation is unknown.

Aims: We aimed to elucidate the precise role Pfn1 in platelet function and particularly integrin activation.

Methods: We generated mice with a platelet/ megakaryocyte-specific Pfn1-deficiency (*Pfn1*$^{fl/fl\ Pf4\text{-}cre}$). Platelet function was studied in a broad range of *in vitro* assays and *in vivo* models of hemostasis and arterial thrombus formation.

Results: Pfn1-deficient platelets showed a pronounced β1- and β3-integrin activation defect characterized by impaired platelet aggregation, adhesion to collagen under flow and clot retraction. *In vivo*, Pfn1 deficiency compromised hemostasis and resulted in a marked protection form arterial occlusive thrombus formation. The impaired integrin function in Pfn1-deficient platelets was characterized by an altered recruitment and localization of talin-1 and β3-integrins to the cell cortex of spreading platelets, as well as accelerated, calpain-mediated integrin inactivation.

Conclusions: These results reveal Pfn1 as a critical regulator of platelet integrin turnover with implications for hemostasis and thrombosis.

OC 57.3 | The Platelet Oxidoreductase ERp5 Modulates Fibrinogen Binding to A2bβ3 Integrin by Cleaving a βI Domain Disulphide Bond

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Background: The oxidoreductase, endoplasmic reticulum protein 5 (ERp5), is released at the site of thrombus formation in mice and ERp5 blocking antibodies (Passam et al. Blood, 2015) or morpholinos reduce platelet accumulation, fibrin generation and thrombus formation. ERp5 is secreted by platelets and endothelial cells and binds to surface

β3 integrin receptor. The ERp5 binding site has been localised to the βI domain of the β3 subunit of the major platelet integrin, α2bβ3. The oxidoreductase activity of ERp5 suggests that the enzyme cleaves one or more allosteric disulphide bonds in the integrin.

Aims: To determine the disulphide bonds in α2bβ3 targeted by ERp5 and the functional effect of their oxidoreduction.

Methods: Native α2bβ3 was incubated with RGD or RGE peptide in the presence or absence of reduced ERp5 or mutant inactive ERp5. The redox state of disulphide bonds in α2bβ3 was measured by differential cysteine alkylation and mass spectrometry. Molecular dynamics studies and atomic force microscopy were employed to determine the effect of reduction of disulphide bonds by ERp5 on α2bβ3 structure and ligand binding.

Results: We have measured the redox state of 31 of the 37 disulphide bonds in α2bβ3 integrin using differential cysteine alkylation and mass spectrometry. Incubation of RGD-extended α2bβ3 integrin with ERp5 resulted in significant cleavage of only one of the 31 disulphide bonds - the Cys177-Cys184 disulphide bond in the βI domain where ERp5 binds. The Cys177-Cys184 bond lies at the rim of the fibrinogen binding pocket of the integrin. Molecular dynamics studies reveal that cleavage of the βI domain Cys177-Cys184 bond changes the ligand binding pocket and the nature of the fibrinogen interaction. This has been confirmed in atomic force microscopy measurements of fibrinogen binding to α2bβ3 in the presence of ERp5.

Conclusions: Based on these findings, we suggest that ERp5 released during thrombus formation modulates fibrinogen binding to α2bβ integrin by changing the conformation of the binding site.

OC 57.4 | Synergistic Inside-out and Outside-in Activation of Integrin αIIbβ3

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Background: Integrin $\alpha_{IIb}\beta_3$ can be activated by both inside-out and outside-in signaling. ADP and thrombin result in its chemical inside-out activation. Binding of GPIb to immobilized VWF under force gives rise to mechanical inside-out activation. Binding of $\alpha_{IIb}\beta_3$ to fibronectin (FN) triggers outside-in activation. It is unclear how these bi-directional signaling pathways cooperate to generate $\alpha_{IIb}\beta_3$ activation.

Aims: To elucidate the synergy among mechanical/chemical inside-out with mechanical outside-in activation of $\alpha_{IIb}\beta_3$.

Methods: We used a dual biomembrane force probe to sequentially stimulate/interrogate two receptors on a single platelet. To trigger mechanical inside-out activation, a micropipette aspirated platelet repeatedly contacted a VWF probe to exert force on GPIb, then switched to the FN probe to measure adhesion frequency. The above first step was replaced by inserting the platelet into a large micropipette pre-filled with ADP or thrombin to trigger chemical inside-out activation. For mechanical outside-in activation, the platelet was interrogated with the FN probe over a large number of repeated contacts to observe adhesion frequency change, with or without pre-conditioning by mechanical/chemical inside-out activation.

Results: GPIb-mediated mechanical inside-out activation activates $\alpha_{IIb}\beta_3$ to an intermediate level between the resting and ADP-activated states (Fig. 1A). Sequential stimulations by mechanical inside-out and outside-in activation activates $\alpha_{IIb}\beta_3$ to the level of the ADP-activated state (Fig. 1B). Mechanical outside-in activation further increases the affinity of ADP-activated $\alpha_{IIb}\beta_3$ by another 19-fold (Fig. 1C). Substituting ADP with low- but not high-dose thrombin resulted in a similar result (Fig. 1D). Without pre-conditioning by inside-out activation, mechanical outside-in activation was prevented (Fig. 1B).

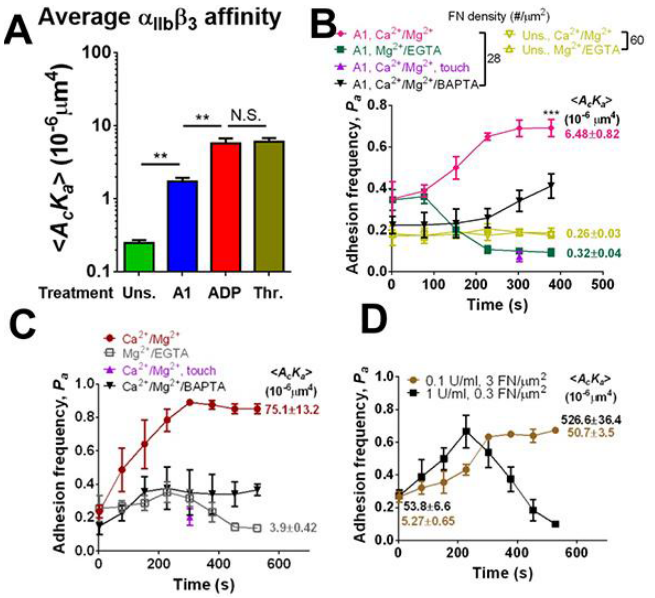

FIGURE 1 The discovery of two new states in integrin αIIbβ3, which has an intermediate and hyperactive affinity in binding to its ligand

Conclusions: Activation of $\alpha_{IIb}\beta_3$ is finely tuned by synergistic mechanical and chemical bi-directional signaling pathways.

OC 57.5 | IκB Kinase 2 impairs GPIIb/IIIa Activation in Platelets

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Background: Megakaryocytes can sense inflammatory signals, but little is known how this might change platelet function. Most inflammatory signalling pathways converge at the kinase IKK2 (IκB kinase 2) activating the transcription factor NF-κB. Our aim was to determine the effect of chronic inflammation on platelets by using a conditional transgenic mouse model that alters NF-κB activity in megakaryocytes.

Aims: The aim of this study was to determine the effect of persistent inflammation on platelet function, by altering NF-κB activity in megakaryocytes with a constitutively active IKK2.

Methods: Mice with a megakaryocyte-specific constitutively active IKK2 were compared to littermate controls. Platelet count and lifespan was determined and function was tested *in vitro* by agonist-induced degranulation and aggregation and *in vivo* by tail bleeding and *intra vital* microscopy of ferric chloride induced thrombus formation.

Results: Platelet count and lifespan is unaltered, however GPIIb/IIIa activation was decreased in platelets with constitutively active IKK2 upon stimulation with ADP and PAR4 receptor agonist peptide. Consistently, *in vitro* platelet aggregation is reduced, *in vivo* thrombus formation delayed and bleeding time increased.

Conclusions: Platelets of mice with megakaryocyte-specific constitutive active IKK2 exhibit decreased GPIIb/IIIa activation and aggregation *in vitro*, while degranulation is only slightly affected. This specific reduction of GPIIb/IIIa activation is further supported *in vivo* by *intra vital* microscopy of ferric chloride induced thrombus formation, which is impaired in mice with constitutively active platelet IKK2, explaining the increased bleeding time. Taken together our data indicates that active IKK2 or NF-κB interferes with GPIIb/IIIa activation, either directly through kinase activity in platelets or via constitutively active NF-κB signaling in megakaryocytes.

OC 58.1 | Platelets Kill Bacteria after Bridging Innate and Adaptive Immunity via PF4 and FcγRIIA

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Background: Chemokine platelet factor 4 (PF4) bound to bacteria is recognized by anti-PF4/Heparin(H) IgG. Human platelets specifically interact with these opsonized bacteria through Fc gamma receptor 2A (FcγRIIA) resulting in bacterial killing.

Aims: (i) How platelets respond to PF4 and anti-PF4/H IgG complexes? (ii) What is the role of human platelet FcγRIIA PF4 anti-bacterial activity?

Methods: Platelet activation on planar and biomimetic bacteria-like micropatterns, or *E. coli* (wild type BW30270 and mutants expressing truncated LPS-KPM53 and KPM121 showing enhanced PF4 binding) coated with IgG, PF4, or anti-PF4/H IgG complexes was quantified by live imaging, in the presence/absence of platelet receptor/cytoskeletal function inhibitors. FcγRIIA and antibody-dependent bacterial killing by platelets was assessed by quantiative microscopy and co-culture experiments.

Results: Platelets adhered and spread on planar micropatterned arrays functionalized with IgG [% micropattern area covered 60.23% ±13.1 (mean ± SD)] and aggregated IgG [83.41%±11.36]. FcγRIIA blocking mab IV.3 reduced ($P < 0.0001$) platelet spreading on IgG [3.39% ±3.35] and aggregated IgG [11.41%±3.96], as did inhibitors of αIIbβ3, cytochalasin D and blebbistatin. Similar outcomes were obtained with biomimetic bacteria-like microbead arrays functionalized with IgG, aggregated IgG, PF4 or anti-PF4/H IgG complexes. In the presence of anti-PF4/H-IgG, platelets were able to kill *E. coli* strains within two hours, directly dependent on the bacterial PF4 binding capacity (up to 75.4 ±6.3% killing rate), which was inhibited by mab IV.3.

Conclusions: PF4 binds to polyanions on bacteria, enabling opsonization by anti-PF4/H IgG, and FcγRIIA mediated killing of *E.coli*. As PF4 binds to many gram positive and negative bacteria, preformed anti-PF4/H IgG rapidly recognizes even bacteria the organism has not seen before. Potentially, this IgG mediated innate immune defense mechanism is also relevant for control of Gram-positive bacteria such as staphylococci or pneumococci.

OC 58.2 | Aging-Associated Increases in Platelet Granzyme A Regulates Pro-inflammatory Gene Synthesis by Target Monocytes

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Background: Platelets govern signal-dependent inflammation by leukocytes. While dysregulated inflammation is common in older adults, platelet-leukocyte signaling events and inflammatory gene synthesis in aging is unknown.

Aims: We hypothesized that aging would alter the platelet molecular signature, leading to dysregulated platelet-leukocyte interactions and inflammatory cytokine synthesis.

Methods: Platelets and monocytes were isolated from healthy older (age>60, n=27) and younger (age< 45, n=36) adults. Inflammatory gene synthesis by monocytes in the presence or absence of activated platelets was examined. RNA-sequencing profiled the platelet transcriptome in older and younger adults. Differentially expressed candidates were validated (mRNA and protein), and inhibitors were used to identify putative receptors.

Results: Basal MCP-1 and IL-8 synthesis by monocytes alone did not differ in older and younger adults. However, when co-incubated with platelets, monocytes from older adults synthesized greater MCP-1 (867±150 vs. 216±36 ng/mL, $p< 0.0001$) and IL-8 (41±5 vs. 9±2 ng/mL, $p< 0.0001$) than younger adults. Switch experiments confirmed that aged platelets were sufficient for upregulating MCP-1 and IL-8. Platelet adhesion and signaling proteins that induce MCP-1 synthesis (p-selectin, RANTES) were not increased in aged platelets. Rather,

using RNA-seq followed by RT-PCR and immunoblot, we identified that granzyme A (GrmA), a serine protease not previously studied in human platelets, is significantly increased in aging (~9-fold vs. young). GrmA is secreted by aged platelets in signal-dependent fashion. Blocking GrmA inhibited MCP-1 and IL-8 synthesis through TLR4 and Caspase-1.

Conclusions: Inflammatory gene synthesis stimulated by platelet-leukocyte interactions is exaggerated in aging in a platelet-dependent manner. We identify a previously unrecognized protein in human platelets, GrmA, and demonstrate that increased platelet GrmA in aging contributes to exaggerated inflammation through TLR4 and Caspase-1.

OC 58.3 | Mature Murine Megakaryocytes Process and Present Both Exogenous and Endogenous Antigens to CD8+ T Cells and Transfer this Ability to Platelets

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Background: Megakaryocytes (MKs) are bone marrow cells that are responsible for releasing platelets into the blood circulation. Although MKs can express immune molecules such as Major Histocompatibility Complex (MHC) class I and II molecules along their differentiation pathway, little is known about their ability to interact with T cells.

Aims: We analyzed whether MKs can cross-present an exogenous Ovalbumin (OVA) antigen as well as endogenous GPIIIa antigens to activate CD8+ T cells and whether they can transfer this ability to platelets.

Methods: We used cultured murine MKs to analyze OVA endocytosis and test their capacity to cross-present antigens, i.e. proteolytically generate immunogenic peptide ligands which are presented on their surface with MHC class I molecules as well as their ability to transfer this process to platelets. Co-culture experiments with antigen-specific T cells and an in vivo murine model of immune thrombocytopenia (ITP) were used to test if the antigen-pulsed MKs could induce CD8+ T cell activation.

Results: Murine $CD34^-$ MHC class II^- $CD41^+$ MKs endocytosed exogenous OVA and processed it into its immunogenic peptide ligand that was loaded into the antigen binding groove of MHC class I molecules and re-expressed on the MKs surface. This re-expression event was correlated with activation of OVA-specific CD8+ T cells (OT-I) in vitro and when the OVA-pulsed MK were injected into in OT-I transgenic mice. Furthermore, these MK MHC/peptide complexes were packaged into α-granules and transferred to platelets during thrombopoiesis. MKs were also able to mediate GPIIIa-specific CD8+ T cell activation and thrombocytopenia in an in vivo murine model of T cell-mediated ITP.

Conclusions: These results suggest that bone MKs have the ability to interact with and activate CD8+
T cells in vitro and in vivo and transfer this ability to their platelet progeny. MKs also have the ability to present their endogenous protein antigens to T cells that then mediate thrombocytopenia in vivo.

OC 58.4 | Platelet Desialylation: Novel Mechanism of Immune Tolerance

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Background: GPIbα and GPIIbIIIa are two abundant platelet (PLTs) surface receptors commonly targeted by auto- and allo-antiplatelet antibodies in thrombocytopenias. Interestingly, we found GPIbα was significantly less immunogenic than GPIIbIIIa, unless there is a co-infection.

Aims: Given that GPIbα is the most heavily sialylated protein, we hypothesized removal of charged terminal sialic residues will unmask and alter the glycosylation profile to enhance antigen presentation and antibody production.

Methods: N/A

Results: However, unexpectedly, we found desialylated platelets (dPLTs) led to suppressed antibody generation. This was true in both iso (WT (wild-type) PLTs into β3-/- or GPIbα-/- mice) and allo (C57BL/6 WT PLTs into BALB/c mice) anti-platelet responses. To assess whether dPLTs had immunomodulatory function beyond decreased immunogenicity, we transfused WT BALB/c mice with WT BALB/c dPLTs or PLTs during the course of immunization with allogeneic C57BL/6 WT PLTs. We found a significant decreased antibody response against alloantigen $H\text{-}2K^b$ in mice that were transfused with dPLTs but not WT PLTs. The suppressed antibody response was specific to platelet antigens, as there was no significant difference between the two groups following challenge with sheep red blood cells. Utilizing state-of-the-art non-invasive, in vivo Multispectral Optoacoustic Tomography imaging, we tracked, in real-time, increased pooling of ICG labeled dPLTs to the liver and surprisingly the gut in mice following transfusion. In contrast, ICG labeled PLTs remained in circulation. Correspondingly, intravital microscopy revealed increased adherence and arrest of dPLTs in mesenteric veins compared with PLTs.

Conclusions: Thus increased dPLTs sequestration and clearance in the liver/gut may be immunosuppressive against platelet associated antigens. These findings may be exploited as a therapeutic target to decrease alloantigenicity in transfusions/transplants (e.g. desialylated platelets carrying coagulation factors for hemophilia therapy).

OC 58.5 | Platelets Promote Monocyte Polarization to a M1 Phenotype upon LPS Stimulation

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Background: Platelets and monocyte/macrophages interaction appears to play a relevant role in the pathophysiology of sepsis. However, it has been reported that platelets can positively or negatively regulate monocytes/macrophages responses. Although the reasons for these discrepancies are not clear, we hypothesize that LPS concentrations and stimulation times are critical determinants.

Aims: To clarify the role of platelets in the biology of monocytes/macrophages upon LPS challenge, we analyzed the monocyte inflammatory response at day 1 and 2 and their polarization at day 5 in the presence of platelets and broad range of LPS concentrations.

Methods: Human monocytes were purified by positive selection, incubated with autologous platelets and stimulated with LPS.

Results: LPS induced the release of pro (TNFα/IL-6)- and anti (IL-10/IL-4)-inflammatory cytokines (ELISA) in a LPS concentration-dependent manner after 1 and 2 days, respectively. Platelets decreased the release of all cytokines at all LPS concentrations (Fig.1).

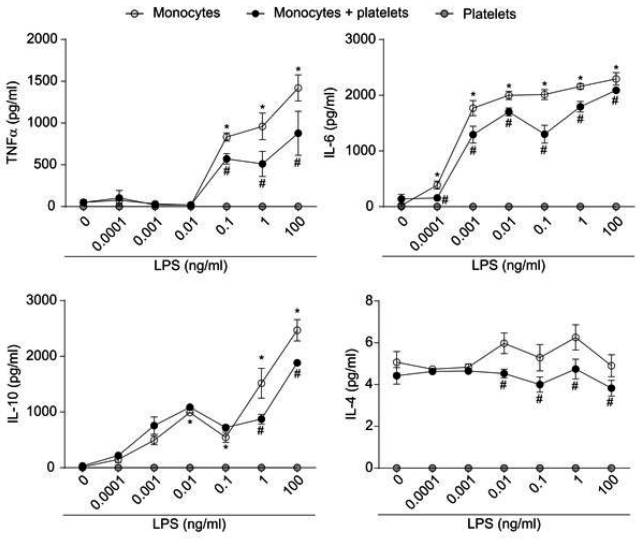

FIGURE 1 Cytokine release from platelet/monocytes co-cultures (n=5, ANOVA)

While low LPS concentrations differentiated monocytes into M2 macrophages by decreasing CD64 and augmenting CD206 and CD163 (flow cytometry), a more pro-inflammatory phenotype was observed with increasing LPS concentrations (Fig.2). Platelets polarized monocytes predominantly towards M1 phenotype at all LPS concentrations. Accordingly, macrophages derived from platelet/monocyte co-cultures had an increase capacity to produce TNFα and exerted a higher bacterial phagocytic activity (flow cytometry) (Fig.2). Moreover, endothelial cells presented a reduced capability of healing when incubated with supernatants from platelet/monocyte co-culture-derived macrophages (scratch assay) (Fig.2). The skewing effect of platelets on monocyte polarization was abrogated in a transwell assay.

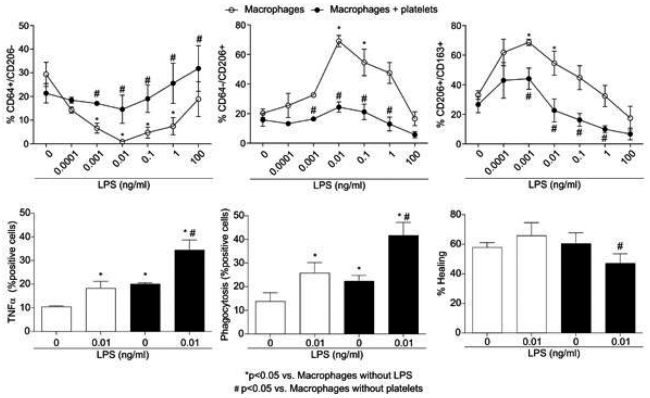

FIGURE 2 Phenotype and functionality of monocyte-derived macrophages in the presence of platelets and LPS stimulation (n=3, ANOVA)

Conclusions: Our results demonstrated that, upon LPS stimulation, platelets decrease the release of both pro and anti-inflammatory cytokines from monocytes but polarize them to M1 phenotype in a cell-contact dependent manner.

OC 66.1 | Platelet Collagen Receptor GPVI-dimer Binds to Fibrinogen D-fragment and D-dimer and May Contribute to Platelet Activation and Adhesion during Thrombus Formation

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Background: Platelet collagen receptor GPVI-dimer initiates signalling leading to thrombus formation. Recently it has also been reported to stabilize thrombi through binding to fibrin.

Aims: Due to its possible emerging role as a fibrin receptor, we investigated the interaction between monomeric and dimeric GPVI with fibrinogen substrates to further explicate this interaction.

Methods: Binding of recombinant GPVI monomeric extracellular domain ($GPVI_{ex}$) or dimeric Fc fusion protein [$(GPVI\text{-}Fc)_2$] to immoblized fibrinogen derivatives was measured by ELISA (1G5 pan GPVI antibody)/IRDye®800CW anti-Mouse IgG; Licor Odyssey). Flow adhesion was performed with DiOC6 labelled normal or Glanzmann thrombasthenia (GT) platelets over fibrinogen substrate-coated slides.

Results: Under static conditions, $GPVI_{ex}$ did not bind fibrinogen D-fragment or D-dimer, but $(GPVI\text{-}Fc)_2$ exhibited specific, saturable

binding to both fragments. $(GPVI\text{-}Fc)_2$ showed only very low binding to fibrinogen, monomeric fibrin, and polymerized fibrin, contrary to published reports; $GPVI_{ex}$ bound none of them. Binding of $(GPVI\text{-}Fc)_2$ to D-fragment or D-dimer was abrogated by collagen type III, Horm collagen, or CRP-XL, suggesting proximity between the D-fragment-, D-dimer-, and collagen binding sites on GPVI-dimer. Neither $(GPVI\text{-}Fc)_2$ nor $GPVI_{ex}$ bound E-fragment. Under arterial shear rates, adhesion of normal platelets to fibrinogen, polymerized fibrin, D-fragment and D-dimer was inhibited by anti-GPVI-dimer mFab-F or Eptifibatide (inhibits αIIbβ3) used alone, and further inhibited when they were used in combination, suggesting that both receptors are involved in adhesion to these substrates under flow. GT platelets showed limited adhesion to fibrinogen substrates, which was further reduced by mFab-F, supporting involvement of GPVI-dimer in this interaction.

Conclusions: GPVI-dimers bind to fibrinogen D-fragment, suggesting that only GPVI-dimers contribute to platelet adhesion on fibrinogen and polymerized fibrin.

OC 66.2 | Gp6 Signaling is Compromised in Early Young Platelets in Mice

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Background: Platelets are derived from the cytoplasm of megakaryocytes (MKs) by extension of proplatelets into bone marrow sinusoids and ensuing release into the bloodstream. In humans, platelets circulate for 8-10 days before clearance by the reticuloendothelial system. It has been proposed that circulating young platelets are more reactive than aged platelets.

Aims: We investigated the reactivity of the circulating young platelets produced under high demand by using a mouse model of platelet recovery after induction of acute thrombocytopenia.

Methods: Mice were rendered thrombocytopenic by intravenous injection of polyclonal rat anti-mouse GPIba antibody and newly produced platelets in circulation were tested *in vivo* and *in vitro*.

Results: Newly produced circulating platelets on day 4-5 post-depletion showed an increased size and were called Early Young Platelets (EYPs). The surface expression on EYPs of major receptors including GPVI as well as integrins αIIb and β1 were largely unaltered. Remarkably, however, EYPs displayed a pronounced activation defect in response to GPVI agonists as detected by reduced aIIbb3 integrin activation and a-granule release. The GPVI defect in EYPs was linked to hypo-phosphorylation of downstream signaling proteins translating into reduced aggregation and thrombus formation in *in vitro* and *in vivo* platelet assays. A similar GPVI signaling defect was observed when mice were rendered thrombocytopenic using an anti-integrin aIIbb3 antibody, thus indicating that GPVI signaling is diminished in newly produced platelets independently of the exact mechanism underlying the preceding thrombocytopenia. The GPVI signaling defect was overcome with establishment of normal platelet counts in circulation.

Conclusions: Our results show for the first time that young platelets produced by MK rupture in response to acute thrombocytopenia display a severe and selective GPVI-ITAM signaling defect that is linked to hypo-phosphorylation of the GPVI signalosome.

OC 66.3 | Dissecting PAR1 Specific Signaling in Platelets Using Quantitative Mass Spectrometry Based Phosphoproteomics

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Background: Protease-activated receptor 1 (PAR1) is a key platelet (PLT) receptor which is activated by thrombin and is a target for antiplatelet therapy. In clinical trials, PAR1 inhibitors are associated with increased risk of bleeding, likely due to PAR1's role in other cell types, notably the endothelium. Further understanding of PLT specific PAR1 signaling may help in the design of better antiplatelet therapies.

Aims: To unravel PLT specific PAR1 signaling and identify new targets for intervention.

Methods: PLT PAR1 signaling was assessed using mass spectrometry-based quantitative phosphoproteomics. Washed PLTs from transfusion-ready PLT pools were activated with PAR1 activating peptide ($SFLLRN\text{-}NH_2$) for 60s. PLT lysates were processed to peptides using trypsin digestion, and phosphopeptides enriched using TiO_2 beads and labelled using isobaric TMT labels. Phosphopeptides were identified and quantified on an Orbitrap Fusion Tribrid mass spectrometer.

Results: We reliably quantified >1600 phosphopeptides of which 110 changed upon PAR1 stimulation, corresponding to 98 proteins. Of the identified proteins, many have established roles in PLT biology but have not been previously associated with thrombin signaling (I.E. WIPF1, ITGA6, TLN1). A large proportion of the proteins are receptors or membrane bound (n=55), indicative of the formation of receptor complexes (I.E. PAR1 & PAR4). This is supported by STRING analysis, revealing that most regulated proteins form an interaction network, eluding to fast signal transduction at the PLT membrane.

Conclusions: This study provides novel phosphoproteomic data on PAR1 signaling in PLTs. Comparison with previously published phosphoproteomic studies on signaling of PAR1 in endothelial cells and ADP in PLTs revealed strong overlap with our dataset and identified shared but also specific PAR1/PLT signaling events, revealing potential new targets for antiplatelet therapy. These results pave the way for intimate understanding of converging and diverging platelet signaling pathways.

OC 66.4 | Perlecan: a Physiological Ligand for the Inhibitory Platelet Receptor G6b-B

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Background: The immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B is an essential regulator of platelet production and activation, loss of which results in severe macrothrombocytopenia and aberrant platelet function in mice and man.
Aims: To identify the physiological ligand of G6b-B and determine its biological effects on platelets.
Methods: Recombinant dimeric Fc-tagged mouse G6b-B ectodomain (mG6b-B-Fc) was used to identify tissues expressing the putative ligand of G6b-B by immunohistochemistry and to pull-down the ligand from tissue lysates. Following identification by mass spectrometry, the interaction between G6b-B and its binding partner was characterised *in vitro*. Effects of the ligand on platelets were assessed using standard functional and biochemical assays.
Results: The most abundant protein identified in mG6b-B-Fc pull-downs from vena cava lysates was the heparan sulfate (HS) proteoglycan perlecan. This result was confirmed by *in vitro* binding assays, where mG6b-B-Fc bound robustly to purified perlecan, but not to other extracellular matrix proteins. Heparinase III treatment of immobilised perlecan almost completely abolished its interaction with mG6b-B-Fc, strongly suggesting that G6b-B binds to the HS side-chains of perlecan. This was supported by surface plasmon resonance measurements, revealing binding affinities of mG6b-B-Fc to perlecan, HS and heparin in the low nanomolar range. Intriguingly, heparin induced G6b-B phosphorylation in washed human platelets. Adhesion of platelets to perlecan was observed only after removal of the HS side-chains, pointing to their inhibitory function. However, soluble HS and heparin enhanced aggregation of human platelets to subthreshold concentrations of collagen in platelet rich plasma.
Conclusions: Findings from this study establish the HS side-chains of perlecan as physiological ligands of G6b-B. Ongoing studies are aimed at determining the optimal binding sequence of HS and analysing the biological consequences of this interaction.

OC 66.5 | Structural Analysis of Protease Activated Receptor 4 (PAR4) with Histidine Hydrogen Deuterium Exchange

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Background: Protease activated receptors (PARs) are GPCRs that have central roles in the cardiovascular system. The signaling pathways mediated by PARs are well-studied. However, the structural basis for PAR activation is unknown. PARs are activated by a tethered ligand that interacts with a binding pocket on the receptor. Since the ligand is attached to the receptor, there are likely substantial conformational changes that occur during PAR activation. We now describe the first biophysical studies examining this unique mode of activation using PAR4.
Aims: The goal of this study is to test the hypothesis proteolytic activation PAR4 results in a specific structural rearrangement of the receptor.
Methods: We have expressed and purified PAR4 from Sf9 cells for histidine-hydrogen deuterium exchange (His-HDX). The changes in overall stability of PAR4 were evaluated by proteolytic digestion.
Results: PAR4 has 9 histidine residues that are spaced throughout the protein allowing a global view of solvent accessible and non-accessible regions. Peptides containing each of the 9 His residues were used to determine the $t_{1/2}$ for each His residue in full length or thrombin cleaved PAR4. The thrombin cleaved PAR4 had a 2-fold increase ($p > 0.01$) in $t_{1/2}$ values observed for four histidine residues (His^{180}, His^{229}, His^{240}, and His^{380}) demonstrating that these regions have a decrease in solvent accessibility upon thrombin treatment. In agreement, thrombin cleaved PAR4 was resistant to thermolysis digestion. In contrast, activation with the PAR4 agonist peptide was digested at the same rate as uncleaved PAR4. Further analysis showed the C-terminus is protected in the thrombin cleaved PAR4 compared to uncleaved or agonist peptide treated PAR4.
Conclusions: The studies described here are the first to examine the tethered ligand activation mechanism for a PAR family member using biophysical approaches. These studies shed light on the overall conformational changes that follow activation of PARs by a protease.

OC 68.1 | Platelet Transcriptome Analysis of Mice with Altered Thrombin Regulation

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Background: Although mature platelets do not synthesize RNA, existing RNA can undergo splicing and translation. The RNA profile of platelets may also reflect changes in megakaryocyte development or alterations of the bone marrow (BM). Circulating platelets are therefore sentinels for pathological changes in BM niches, blood and acute or chronic diseases that could be detected by RNA profiling of platelets.

Aims: To identify platelet signatures associated with prothrombotic states.

Methods: We determined the platelet transcriptome in mice with altered thrombin regulation, specifically PAR4$^{-/-}$ mice and GP1ba-deficicent mice with defective platelet thrombin binding and TM$^{Pro/Pro}$ mice with diminished thrombin neutralization in the circulation. Total RNA of leukocyte-depleted platelets was sequenced yielding at least 35 Million reads per sample. Reads were mapped to GRCm38.76.

Results: GPIba-deficient, PAR4$^{-/-}$, and TM$^{Pro/Pro}$ mice showed highly reproducible NGS profiles with a distinct clustering of repeats from each receptor mutant in principle component analysis. Differential expression analysis identified transcripts that were changed selectively in TM$^{Pro/Pro}$ (190 transcripts), PAR4$^{-/-}$ (563) and GP1ba-deficient (609) platelets. Pathway analysis indicated distinct functional alterations for each strain. Furthermore, platelet transcript profiles were changed in opposite direction between TM$^{Pro/Pro}$ and PAR4$^{-/-}$ (323 transcripts) or GP1ba-deficient and PAR4$^{-/-}$ (574) mice, indicating that diminished thrombin neutralization by endothelial cell-expressed TM or by platelet-expressed GP1ba causes increased PAR4 signaling to alter platelet RNA profiles.

Conclusions: The transcriptome analysis provides insights into common and distinct pathways regulated by thrombin and its receptors expressed by the endothelium and platelets/megakaryocytes. These transcript profiles can be used to evaluate chronically elevated thrombin levels and coagulation activation in diverse clinical settings predisposing to thrombo-embolic complications.

OC 68.2 | Identification of eQTLs for Platelet and Hemostasis Related Genes in Platelets and Leukocytes within the Framingham Heart Study

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Background: Platelets and hemostatic factors are important in thrombosis, inflammation, and wound healing. Their functions and signaling pathways interact in the tight control of these processes. Consequently, platelets and hemostatic factor measures have been associated with cardiovascular disease, myocardial infarction, stroke, and venous thrombosis. Identifying key genetic regulators of platelets and hemostatic factors and their mechanisms are important to understanding their contribution to disease.

Aims: To identify expression quantitative trait loci (eQTLs) that influence the expression of key platelet and hemostatic factor associated genes.

Methods: We selected platelet and/or hemostasis related transcripts based on known biological function or past genetic associations in the literature. We then measured their RNA expression levels by qRT-PCR in leukocytes and platelets from ~1,600 Framingham Heart Study participants. We identified cis-eQTLs (±1 Mb of gene) and trans-eQTLs of expression levels corrected for age, sex, and other technical covariates. Conditional analyses were performed to identify independent loci and disease associations identified using GRASP and GWAS Catalog.

Results: We identified many *cis*- and *trans*-eQTLs in both leukocytes and platelets (Table 1). Of the *trans*-eQTLs, SNPs within the well-characterized *ARHGEF3* region were associated with expression of 14 transcripts (e.g. *VWF* and *FADS2*). Additionally, we observed intriguing associations of: 1) SNPs near *GP1BA*, a receptor for vWF, with platelet *CCL5* expression and 2) SNPs in *AP2B1*, a clathrin-dependent endocytotic factor associated with platelet count, with platelet *ITGA2B* expression.

TABLE 1 Summary of RNA Transcripts Investigated

	Leukocytes	Platelets	Overlapping
Total Number of Transcripts	84	140	79
Number with cis-eQTLs	39	67	22
Number with trans-eQTLs	8	41	6

Conclusions: We identified numerous *cis*- and *trans*-eQTLs of platelet and hemostatic factor related transcripts. Mapping these eQTLs enable the determination of whether genetic regulation of gene expression influences thrombosis, hemostasis, and ultimately human disease.

OC 68.3 | The First Quantitative Phosphoproteome after Selective cGMP/PKG Stimulation by Riociguat In Human Platelets and Comparison to the cAMP/PKA Phosphoproteome

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Background: Human platelets are tightly regulated by multiple vasoactive substances or drugs including endothelial-/drug-derived NO and prostacyclin (Iloprost). NO activates the soluble guanylyl cyclase (sGC)/cGMP pathway and inhibits platelets, often in synergy with prostacyclin. cGMP effects in platelets are poorly understood and sometimes controversial. Recently, we characterized the human platelet proteome (Burkhart et al. Blood 2012), and dynamic changes of the phosphoproteome upon Iloprost and ADP treatment (Beck et al. Blood 2014; Beck et al. Blood 2017).

Aims: Elucidation of the Riociguat/ sGC/ cGMP affected phosphoproteome in human platelets.

Methods: Human platelets were either stimulated with Iloprost (cAMP/PKA) or Riociguat, a robust, cAMP-independent cGMP-stimulator. Changes in protein phosphorylation were studied using established quantitative phosphoproteomics workflows.

Results: In total we quantified 8181 phosphorylation sites from 2249 proteins across three biological replicates. Riociguat increased (>1.5-fold up) and decreased (>1.5-fold down) phosphorylation levels in 345 and 94 proteins, respectively. These covered many platelet functions, including 24 protein kinases (e.g. MYLK, CAMKK1/2, CDK16/17/18, BRAF) with increased and 4 protein kinases (KALRN, KSR2, PAK2, WNK1) with reduced levels. Comparison of the Riociguat- and Iloprost phosphoproteomes showed a significant overlap but also specific Riociguat/cGMP effects. The top-list of Riociguat affected phosphoproteins includes known PKG substrates (e.g. VASP, ITPR1, MRVI1/IRAG) but also novel candidates including the PP2A inhibitor ENSA.

Conclusions: This first human platelet phosphoproteome of the Riociguat/sGC/cGMP pathway demonstrates an unexpected magnitude, diversity and involved protein kinase network. The Riociguat/sGC/cGMP and Iloprost/cAMP substrates show a remarkable overlap but also some specific events. Our data also provide a number of novel PKG substrates including the PP2A inhibitor ENSA which require further study.

OC 68.4 | Reversible Protein Lysine Acetylation as a Novel Regulatory Modality Orchestrating Platelet Function

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Background: The reversible acetylation of protein lysine ε-amino groups - catalyzed by lysine acetyltransferases (KATs) and deacetylases (KDACs) - impacts diverse cellular and physiological activities. We recently demonstrated that KATs such as p300 regulate platelet cytoskeletal dynamics and platelet hemostatic function (Aslan et al., *JTH* 2015). To date, our proteomics efforts have identified >2,000 acetyllysine (acK) modifications on >1,000 platelet proteins that serve as potential nodes in the regulation of platelet function by lysine acetylation.

Aims: Here, we aim to determine mechanistic roles for protein lysine acetylation in platelet function.

Methods: We have developed a multi-dimensional targeted proteomics workflow using Tandem Mass Tags (TMT) and ultrahigh resolution mass spectrometry to quantify dynamic changes in protein lysine acetylation in the platelet activation program. Using a combination of bioinformatics, molecular modeling and cell physiological methods, we interrogate roles for protein lysine acetylation in platelet function.

Results: We find that >200 lysine residues on >100 platelet proteins undergo a >1.5 fold-change in site-specific acetylation following activation with the platelet glycoprotein GPVI agonist collagen-related peptide (CRP). A number of platelet cytoskeletal-associated proteins, including myosin IIA heavy chain (MYH9) show specific, several fold-change increases in lysine acetylation following stimulation with CRP. Molecular modeling, *in vitro* and functional studies point to MYH9 acetylation as a key regulatory step, similar to and concurrent with myosin light chain phosphorylation, critical to committing platelets to an activated state.

Conclusions: Our proteomics-based investigation supports specific roles for lysine acetylation in key molecular events of platelet activation and offers novel insights into potential molecular targets for the analysis and manipulation of platelet function in normal physiology and disease.

OC 68.5 | The Platelet Releasate is a ‚Barcode' for the Health Status of an Individual

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Background: Upon activation, platelets release a multitude of soluble and vesicular signals, collectively termed the ‚platelet releasate' (PR). This PR plays an important role in haemostasis, wound healing and the inflammatory response. We and others have used qualitative/quantitative proteomic approaches to characterise the PR; however, with reports of marked inter-individual variability, confident and reliable insights have been hindered.

Aims:

1. Provide a reproducible and quantifiable proteomic analysis and establish a ‚core' human PR.
2. Assess the variability of the PR in healthy human pregnancy where increased platelet activation is observed.
3. Characterise the pathologic proinflammatory changes in patients with early-onset preeclampsia (EOP), a disease associated with potentially life-threatening consequences for both mother and baby.

Methods: PR from 32 healthy donors, 22 EOP patients (onset < 34 gestational weeks) and matched healthy pregnant controls, was obtained with informed consent. After trypsin/lys-C double digest, samples were subjected to label-free quantitative proteomic analysis.

Results: 897 proteins from thrombin-induced PRs were quantified across 32 healthy donors, with 278 proteins forming a ‚core' PR. The variability & origin of these proteins was assessed. In healthy pregnancy, 173 proteins were differentially found in PR, including proteins produced only in pregnancy such as pregnancy-specific glycoproteins

and CSH2. Moreover, an additional 36 PR proteins were modified in EOP, with 6/36 proteins previously associated with the pathogenesis of EOP. Strikingly, several platelet transmembrane and signalling proteins could differentiate between a moderate and severe maternal outcome in EOP.

Conclusions: The PR comprises a ‚core' set of proteins between healthy individuals. This dynamic proteome significantly changes in physiologic and pathologic conditions. Thus, the PR is a barcode for the health status of an individual at a given time.

PLATELETS – CLINICAL

OC 09.1 | Conformation of ADAMTS13 is Altered in Acquired TTP Patients

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Background: Autoantibodies (autoAbs) targeting ADAMTS13 cause acquired thrombotic thrombocytopenic purpura (aTTP). ADAMTS13 has a ‚closed' conformation by interaction between its spacer and CUB domains. Disruption of this interaction by addition of VWF or an activating anti-CUB Ab changes the ADAMTS13 conformation, resulting in exposure of cryptic epitopes.

Aims: Determine if the conformation of ADAMTS13 is altered in aTTP patients compared to healthy donors (HD).

Methods: Abs recognizing cryptic epitopes in the cysteine/spacer (C/S) domain (hence recognizing ADAMTS13 with an altered conformation) were selected via ELISA. The conformation of ADAMTS13 in HD, acute aTTP patients and sepsis patients was determined via ELISA, in which plasma was added to the Ab that recognizes a cryptic epitope in ADAMTS13. As a control, plasma was also incubated with the activating anti-CUB Ab 17G2 which changes the conformation of ADAMTS13 and allows binding to the Ab recognizing a cryptic epitope.

Results: From our panel of Abs, we could identify one anti-C/S domain Ab (1C4) that recognizes a cryptic epitope in ADAMTS13. While Ab 1C4 could readily capture MDTCS, it only captured full length ADAMTS13 when its conformation was changed by addition of 17G2. Interestingly, ADAMTS13 in HD (n=33) adopts a closed conformation where the spacer and CUB domains interact and the epitope of 1C4 is inaccessible. In HD, changing the conformation of ADAMTS13 by addition of 17G2 did expose the cryptic epitope of 1C4. As expected, similar results were obtained for sepsis patients (n=60). Intriguingly, the conformation of ADAMTS13 is spontaneously altered in aTTP patients (n=57) as the cryptic epitope of 1C4 was readily available. Addition of 17G2 did not further change the conformation of ADAMTS13 in these aTTP patients.

Conclusions: The conformation of ADAMTS13 in aTTP patients is altered compared to the one in HD and sepsis patients. Exposure of cryptic epitopes in the C/S domain might induce immune responses and explain autoAb development in aTTP.

OC 09.2 | PAD4 Citrullination of ADAMTS13: A New Link between NETosis and Thrombosis

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Background: Neutrophils and neutrophil extracellular traps (NETs) are considered important for immunothrombosis and have been shown to promote coagulation. A driving force in NETosis is peptidyl arginine deiminase type IV (PAD4), an enzyme released with the decondensed chromatin, after it citrullinates arginine residues on histones. Citrullination of proteins is significant in many disorders. However, the effect in thrombosis is still unknown. ADAMTS13, cleaving VWF, is essential in regulating thrombosis and reduced activity or absence leads to microvascular thrombosis. Interestingly, plasmatic reduction of ADAMTS13 is observed in many thrombo-inflammatory disorders where NETs have also been associated, suggesting a link between NETs and reduction in ADAMTS13 activity.

Aims: ***To determine whether PAD4 interacts with and citrullinates ADAMTS13 and evaluate the effect of this post-translational modification on ADAMTS13 function.***

Methods: Recombinant human PAD4 (r-huPAD4) was incubated with recombinant human ADAMTS13 (r-huADAMTS13; provided by Shire), and citrullination of the protein was examined by western blot using an anti-citrulline antibody. Subsequently, we analyzed r-huADAMTS13 activity by VWF-FRETS assay and evaluated the function of citrullinated-r-huADAMTS13 upon injection into ADAMTS13$^{-/-}$ mice by intravital microscopy.

Results: We observed that r-huPAD4 citrullinated r-huADAMTS13 and that citrullination dramatically reduced its capacity to cleave VWF-substrate. This effect could be reversed by pre-incubating r-huPAD4 with a PAD inhibitor. Additionally, we observed in vivo, that VWF/platelet strings, seen on venules of ADAMTS13$^{-/-}$ mice, were cleared after infusion of r-huADAMTS13, while they remarkably persisted in vessels of mice treated with citrullinated-r-huADAMTS13.

Conclusions: Our results indicate that citrullination by PAD4 inactivates ADAMTS13 reducing its capacity to cleave VWF/platelet strings. This suggests a new way in which NETs are pro-thrombotic and support pathological thrombosis.

[ADAMTS13 was incubated with PAD4 with or without the PAD inhibitor Cl-Amidine. Then, ADAMTS13 activity was determined by VWF73-FRETS assay.]

OC 09.3 | The Role of ADAMTS13 Exosites in VWF Recognition and Proteolysis

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Background: ADAMTS13 cleaves VWF with unprecedented specificity, owing to multiple exosite interactions between ADAMTS13 Spacer, Cysteine-rich (Cys), Disintegrin-like (Dis) and Metalloprotease (MP) domains, and binding sites in the unravelled VWF A2 domain.

Aims: To kinetically characterise the role of each ADAMTS13 exosite in coordinating VWF proteolysis.

Methods: A VWF A2 domain fragment (VWF96) was expressed and purified. VWF96 mutants, in which each exosite binding site was entirely ablated, were generated (Table 1). VWF96 variants were used as substrates in activity and binding assays with ADAMTS13.

TABLE 1

VWF A2 domain fragment	Sequence
VWF96	VWF1573-1668
VWF96-MP	VWF1573-1668 (L1603N, P3 residue)
VWF96-Dis	VWF1573-1668 (DIKRD1614/6/7/8/1622AQEET)
VWF96-Cys	VWF1573-1668 (IWAILI1642/4/7/9/1650/1QYSQQQ)
VWF96-Spacer	VWF1573-1668 (LVL1664/5/6TNQ)
VWF87ΔSpacer	VWF1573-1659

Results: VWF96 was proteolysed efficiently (k_{cat}/K_m=14x10^{5}M^{-1}s^{-1}). Deleting either the entire Spacer binding site (VWF87ΔSpacer) or mutating the 3 hydrophobic residues LVL1664-6 in that region (VWF96-Spacer) caused the same 17-fold reduction in proteolysis due to corresponding increases in K_m and K_D values. Mutating the Cys binding site (VWF96-Cys) reduced proteolysis 30-fold, again manifest through reduced substrate binding (higher K_m). Conversely, proteolysis of VWF96-MP was 130-fold reduced, characterised by normal substrate binding but reduced k_{cat} for proteolysis. Remarkably, mutation of the Dis binding site (VWF96-Dis) reduced proteolysis by 1000-fold, caused by a combination of reduced binding (26-fold higher K_m) and a 45-fold reduction in the k_{cat}.

Conclusions: We demonstrate the importance of VWF A2 domain residues LVL1664-6 in ADAMTS13 Spacer exosite binding. We also show that ADAMTS13 Spacer, Cys and Dis exosites form distinct interactions with VWF that influence the K_m for proteolysis. The low affinity interaction between ADAMTS13 MP and VWF P3 influences the k_{cat}, likely due to its proximity to the scissile bond. The ADAMTS13 Dis exosite interaction is the most important. The influence of this remote exosite on the k_{cat} suggests a potential allosteric mechanism that confers enzyme specificity.

OC 09.4 | Autoantibody Binding to 'Open' and 'Closed' ADAMTS13 in Patients with Acquired Immune Thrombotic Thrombocytopenic Purpura

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Background: Patients with an ADAMTS13-deficiency develop thrombotic thrombocytopenic purpura (TTP). Acquired, immune-mediated TTP (iTTP) accounts for ~95% of cases, with patients varying in terms of clinical severity. These patients develop anti-ADAMTS13 IgG autoantibodies that most frequently bind to the ADAMTS13 Spacer domain. ADAMTS13 can exist in two distinct conformations, a ‚closed' form in which the CUB domains fold back upon the Spacer domain, and a more extended ‚open' form.

Aims: To investigate whether iTTP patient autoantibody binding to ADAMTS13 is dependent upon ADAMTS13 conformation.

Methods: ELISAs were developed to specifically detect autoantibody binding to either ‚open' or ‚closed' forms of ADAMTS13 in 17 iTTP patients with medium/high titre anti-ADAMTS13 IgG. Recombinant ADAMTS13 was either coated directly onto ELISA wells (‚open') or captured via its 6xHis tag to the surface of Ni^{2+} coated plates (‚closed'). After iTTP plasma incubation, bound patient antibodies were detected using an anti-human antibody.

Results: For each patient there was higher autoantibody binding to ‚open' compared to ‚closed' ADAMTS13. In 3/17, autoantibody binding could only be detected towards the ‚open' form. In 11/17 5%-40% of those antibodies that bound ‚open' also bound ‚closed' ADAMTS13. Whereas in 3/17, 40-80% of those autoantibodies that could bind to the ‚open' form could also bind to ‚closed' ADAMTS13.

Conclusions: iTTP patient autoantibody binding to ADAMTS13 is highly dependent upon its conformation. Whilst ADAMTS13 is in its ‚closed' conformation, many frequently targeted epitopes recognised by autoantibodies are concealed, preventing antibody binding. It remains to be determined whether these differences effect how readily antibodies can bind to ADAMTS13 within the circulation and subsequently exert their pathogenicity either through inhibition and/or clearance of ADAMTS13.

OC 09.5 | Parallel Profiling of ADAMTS13-derived Peptide Repertoires on HLA-DR and HLA-DQ

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Medical Center, University of Amsterdam, Department of Immune Pathology, Amsterdam, The Netherlands, [5]KU Leuven Campus Kulak Kortrijk, Laboratory for Thrombosis Research, Kortrijk, Belgium, [6]Academic Medical Center, Department of Experimental Vascular Medicine, Amsterdam, The Netherlands, [7]Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Department of Pharmaceutics, Amsterdam, The Netherlands

Background: Thrombotic thrombocytopenic purpura (TTP) is a severe life-threatening disorder resulting from the formation of autoantibodies against the von Willebrand Factor cleaving protease ADAMTS13. Several studies have identified HLA-DRB1*11, HLA-DQB1*03 and HLA-DQB1*02:02 as risk factors for acquired TTP. Previous research in our department identified ADAMTS13 CUB2 domain-derived peptides "FINVAPHAR" and "ASYILIRDTHSLR" to be presented on HLA-DRB1*11 and HLA-DRB1*03, respectively. We also showed that $CD4^+$ T cells reactive with these peptides were present in patients with acquired TTP.

Aims: We previously identified ADAMTS13-derived peptides presented on HLA-DR. The aim of this study was to identify peptides that are presented on HLA-DQ.

Methods: Monocyte-derived dendritic cells (DCs) of 11 HLA-typed healthy donors were pulsed with 100 nM of ADAMTS13. MHC-II:peptide complexes were purified using the HLA-DR specific antibody L243 and the HLA-DQ specific antibody SPV-L3. Peptides were separated by nanoscale C18 reverse phase chromatography connected to an Orbitrap Fusion Tribid mass spectrometer. All data was acquired with Xcalibur software.

Results: In 5 out of 11 donors ADAMTS13-derived peptides presented on HLA-DQ were identified. One peptide with the core sequence "CAVAIGRPL" derived from the CUB-1 domain was presented by DCs from 3 different HLA-DQB1*06 positive donors. Peptides "SFLDGTRCM" and "IRDTHSLRT" derived from the cysteine-rich and CUB-2 domain were presented by 2 different donors. Four HLA-DQ presented peptides were only identified on DCs derived from a single donor. In total, 7 different ADAMTS13-derived core peptides were identified on HLA-DQ; 4 core peptides were exclusively identified on HLA-DQ and not HLA-DR.

Conclusions: Our study shows that HLA-DQ contributes significantly to the MHC-II presentation of ADAMTS13 derived peptides. We propose that $CD4^+$ T cells recognizing these peptides may contribute to the onset of and/or relapses in acquired TTP.

OC 34.1 | Platelet Factor 4: von Willebrand Factor Complexes: An Important Antigenic Target in Heparin-induced Thrombocytopenia?

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Background: Heparin-induced thrombocytopenia (HIT) is driven by the binding of pathogenic HIT antibodies (Abs) to platelet factor 4 (PF4) complexed to cell surface glycosaminoglycans on vascular cells, including endothelial cells (ECs). However, in studies of HIT in an EC-lined microfluidic system, post hematoporphyrin-induced photochemical damage, we noted that infused PF4 bound specifically in the form of extended linear strands along the surface of ECs, a pattern described previously for von Willebrand Factor (vWF) under flow.

Aims: To evaluate the formation of HIT antigenic complexes of PF4 with extruded vWF and to explore their contribution to thrombosis in HIT.

Methods: Microfluidic channels coated with human umbilical vein ECs were exposed to blue light while cell media containing hematoporphyrin was infused. PF4, labeled HIT-like monoclonal Ab KKO, and labeled polyclonal anti-vWF Ab were then infused to identify HIT complexes. To examine if PF4-KKO-vWF complexes contribute to thrombosis, recalcified-human WB (WB) labeled with calcein AM was then flowed through the channels.

Results: PF4 co-localized with labeled vWF along the surface of injured EC (Fig. 1A). KKO bound PF4-vWF complexes (Fig. 1B).

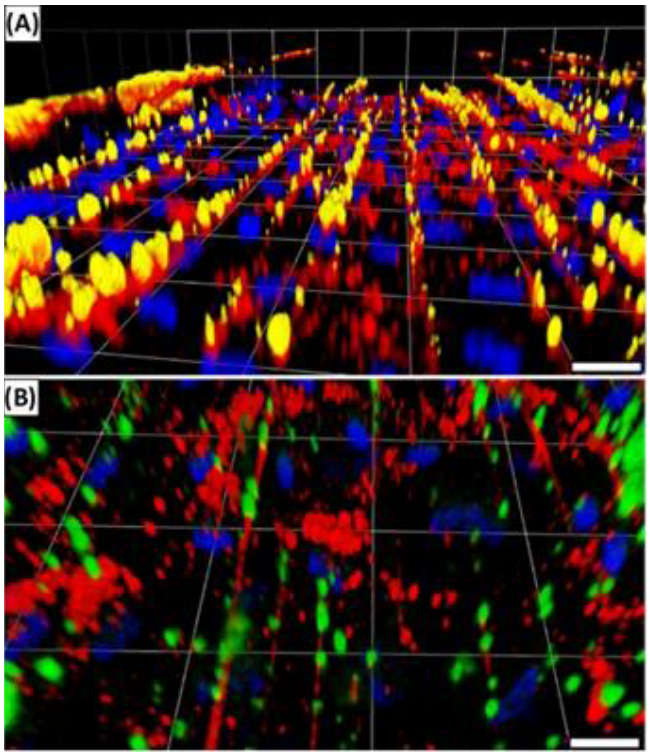

FIGURE 1 3D image (A) showing PF4 (yellow) bound along vWF (red) and KKO (green) binding in a similar linear fashion along vWF

When WB was flowed through channels containing PF4+KKO, large platelet aggregates developed within 10 mins along the extended strands of vWF which did not occur with KKO alone or PF4+ TRA, an isotype control. By 60 mins, aggregates in the PF4+KKO had grown significantly, but had largely disappeared in the other settings (Fig. 2).

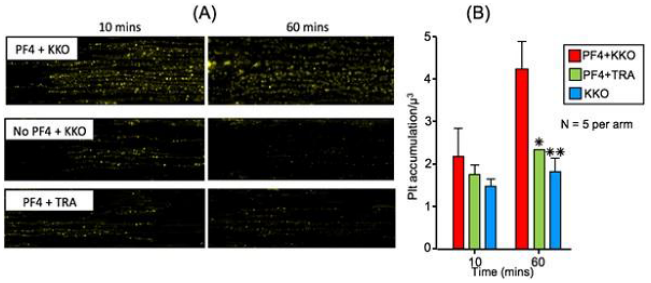

FIGURE 2 vWF-PF4-KKO are prothrombotic complexes that accumulate significantly more platelets than non-complexed vWF

Conclusions: These studies identify vWF as a PF4 binding site. PF4-vWF complexes bind a model HIT antibody. The ability of PF4-vWF-KKO complexes to form large platelets aggregates suggests that PF4-vWF complexes may contribute to thrombotic complications and provides a potential new therapeutic target. Studies to define the mechanistic details of PF4-vWF binding and its biological implications in HIT and other thrombotic disorders are underway.

OC 34.2 | A New Mechanism of Autoimmune HIT Caused by a Subset of Antibodies

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Background: Antibodies recognizing complexes of the chemokine platelet factor 4 (PF4/CXCL4) and polyanions (P) cause the prothrombotic adverse drug reaction heparin-induced thrombocytopenia (HIT). In patients with autoimmune-HIT, antibodies activate platelets even in the absence of polyanions. The binding mechanism of these antibodies remains unclear.

Aims: We used multiple analytical techniques to elucidate the binding mechanism of these antibodies.

Methods: Multiple analytical techniques including ELISA, HIPA, single-molecule force spectroscopy, scanning electron microscopy, isothermal titration calorimetry, and dynamic light scattering were used.

Results: Antibodies with binding forces of ~60-100pN activate platelets in the presence of polyanions, while a subset of antibodies from patients with autoimmune-HIT with binding forces ≥100pN and low thermal off-rates self-clusters PF4 in the absence of polyanions. Their binding to PF4 releases more energy than heparin binding ($\Delta H = -3.5 \pm 0.86 \times 10^7$ cal/mol *vs* $\Delta H = -7.26 \pm 1.36 \times 10^3$ cal/mol). The PF4/antibody complexes subsequently allow binding of polyanion-dependent antibodies resulting of large immunocomplexes, which finally induce massive platelet activation.

Conclusions: We have discovered a new binding mechanism in autoimmune HIT caused by a subset of antibodies from patients with autoimmune-HIT. Antibody-mediated changes in endogenous proteins triggering binding of otherwise non-pathogenic (or cofactor-dependent) antibodies may also be relevant in other antibody-mediated autoimmune disorders.

OC 34.3 | A Standardized Functional Assay for Routine Reliable HIT Diagnosis: A Potential Alternative to the Serotonin Release Assay

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Background: Reliable diagnosis of HIT remains a major clinical concern. Screening tests are highly sensitive but lack specificity, while confirmatory tests lack standardization and are not widely available.

Aims: Our objective was to evaluate the performance of a novel functional flow cytometric assay (FCA) vs. clinical expert opinion and the serotonin release assay (SRA).

Methods: Plasmas of 228 patients included (after signing the informed consent) in a multicenter study „HIT Score" (NCT00748839) were randomly selected: 106 with a positive HIT diagnosis and 122 with a negative HIT diagnosis, as defined by the expert´ opinion adjudication. The experts expressing the opinions used in this study were blinded to SRA results. SRA and FCA were centrally performed. Here, we present a modified FCA based on a method previously described by B.Tardy-Poncet during 24th congress ISTH (2015), with a time to results of about 90 min, simplified reagent handling, and a standardized procedure for analyzing and interpreting the results.

Results: The FCA performed as well as the gold standard SRA when both tests were compared to clinical expert opinion. The sensitivity and specificity of the FCA vs. expert´ opinion were respectively 83% (95% CI: 75-90) and 97% (95% CI: 93-100). The sensitivity and specificity of the SRA vs. expert´ opinion were respectively 88% (95% CI: 81-94) and 97% (95% CI: 93-100). The sensitivity and specificity of the FCA vs. the SRA were respectively 85% (95% CI: 78-93) and 94 % (95% CI: 89-98).

Conclusions: This new FCA gave results similar to those of the SRA for HIT diagnosis, without requiring either radioactivity, or profound expertise in flow cytometry. Based on these results, the new FCA may be conveniently used in routine practice as a reliable test for HIT diagnosis.

OC 34.4 | Soluble Glycoprotein VI (sGPVI) Measurement Is a Useful Biomarker of Platelet Activation in Heparin-induced Thrombocytopenia (HIT) and Correlates with Thrombotic Events

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Background: The receptor FcγRIIA plays a key role in the pathogenesis of HIT. The platelet-specific collagen receptor GPVI releases a soluble ectodomain fragment (sGPVI) in hypercoagulable states and by FcγRIIA-mediated signaling upon engagement by HIT (H-PF4) antibodies. Confirming HIT diagnosis is challenging. sGPVI could serve as a surrogate marker of pathological HIT antibodies and as a unique readout of patient platelet activation *in vivo*, in contrast to using donor platelets.

Aims: To assess the utility of measuring sGPVI in concert with standard HIT testing methods and a new automated test, and to correlate sGPVI with thrombotic events.

Methods: sGPVI measurements by ELISA were obtained from stored plasma of 65 patients with clinical suspicion of HIT from 2008-2014 who underwent assessment for HIT, including 4T score, ELISA for HIT H-PF4 antibodies(GTI IgG), serotonin release assay (SRA), platelet aggregation (PAT) and a new chemiluminescent test, HemosIL HIT-Ab assay (Acustar,Werfen).

Results: Patients with moderate/strong positivity for ELISA and positivity for PAT had higher sGPVI than patients with negative results ($p< 0.05$). sGPVI showed 90% agreement with negative PAT and Acustar with ELISA results. With SRA as the gold standard, NPV was 100% and PPV 76% with a combination of sGPVI and ELISA GTI IgG. Patients with thrombosis (scored 2 on the 4T score "thrombosis" parameter) had higher levels of both sGPVI and HIT antibody than patients who scored 0 ($p< 0.05$).

Conclusions: sGPVI testing is easy to perform, compares well with existing and new HIT testing, and is a useful tool in the assessment of patients with clinical suspicion of HIT. Patients negative for both sGPVI and ELISA for HIT antibodies are unlikely to have HIT and further testing can be avoided. Importantly, sGPVI levels are associated with the clinical outcome of thrombosis. These findings support the measurement of sGPVI in HIT, and provides novel insights into the mechanism of pathological platelet response to HIT antibodies *in vivo*.

OC 34.5 | Identification of Clinical Parameters for Heparin Induced Thrombocytopenia: An International Multicenter Prospective Study

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Background: The accuracy of clinical scores currently proposed for the diagnosis of heparin-induced thrombocytopenia (HIT) varies greatly in clinical practice. In addition, their criteria were not established and/nor validated prospectively in a large group of patients.

Aims: To identify clinical and laboratory parameters associated with HIT in patients with suspected HIT included in a prospective multicenter study.

Methods: Consecutive patients with suspected HIT leading to an ELISA test were included in 31 centres in France, Belgium and Switzerland. Serotonin release assay (SRA) was centrally performed for each patient. The final diagnosis of HIT was reached by 2 independent experts (3 in case of discrepancy) based on all the clinical and laboratory data. To identify the parameters, 70 % of the total population was selected at random. Univariate analysis was performed to screen for potential variables associated with HIT diagnosis. Variables with a p value ≤0.15 were entered in the final multiple regression analysis.

Results: 1597 patients were selected among a total of 2280 included patients (HIT prevalence: 14.7%). The following parameters were identified as independent factors associated with HIT diagnosis (Hazard Ratio, 95% CI) : unfractionated heparin (1,53 [0,97-2,42]), therapeutic dose of heparin (1,6 [1,16-2,22]), cardiopulmonary bypass (2,09 [1,50-2,93]), platelet decrease occurring after 5 to 21 days (2,09 [1,50-2,93]), platelet decrease > 40 % occurring within the previous 6 days before suspicion (3,22 [1,89-5,50]), arterial thrombosis (3,62 [1,97-6,64]), venous thromboembolism (3,89 [2,45-6,20]), major trauma (3,66 [1,46-9,17]).

Conclusions: This study identifies clinical and laboratory parameters associated with HIT in a large cohort of patients. These parameters may improve identification of patients with a high likelihood for HIT. These parameters will be evaluated on the 30 % resting patients.

OC 59.1 | Phenotyping and Genotyping Inherited Platelet Disorders: The Iberian Peninsula Multicenter Project Experience

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Background: Diagnosis of inherited platelet disorders (IPDs), rare genetic defects of platelet function/production associated to bleeding diathesis, is challenging due to a heterogeneous clinical and laboratory presentation, low specificity of platelet function tests and large number of possible genetic culprits. Multicentre projects on IPDs could help to overcome this drawback.

Aims: To establish a multicentric project facilitating specialized functional and molecular characterization of patients with suspicion of IPD.

Methods: In 8 years, we studied 139 patients (47 M, 92 F; median age 29yr;125 unrelated families; 69 thrombocytopenia; 70 thrombocytopathy), referred from different hospitals. Clinical records were reviewed and bleeding scored with ISTH-BAT (median 5[1-25]). Centralized platelet phenotyping was done in fresh or delivered (18-24h) blood samples from patients and healthy volunteers. Basic studies included: Blood count/film; PFA-100, platelet aggregation, GPs expression and granule secretion, ^{14}C-5HT uptake, protein immunoblotting, whole mount or full electron microscopy. Patients DNAs were analyzed by Sanger sequencing of target genes (37 cases), high-throughput sequencing (HTS) using a 71 gene panel (Lozano ML. Blood 2016)(102 cases) or whole exome sequencing (WES) (7 cases).

Results: Clinical information and centralized platelet phenotyping supported an IPD in 94% of cases. Molecular analysis identified a candidate gene leading to IPDs in 108 cases (77.7%). Three patients bear a possible harmful variant in a plasma coagulation protein. Sanger sequencing of selected candidate gene was successful in all but one case. HTS with our panel revealed candidate causative variants in 73% of the assessed cases. WES permitted molecular diagnosis in 3 out of 7 cases. The diagnosis of these patients is shown in Fig 1

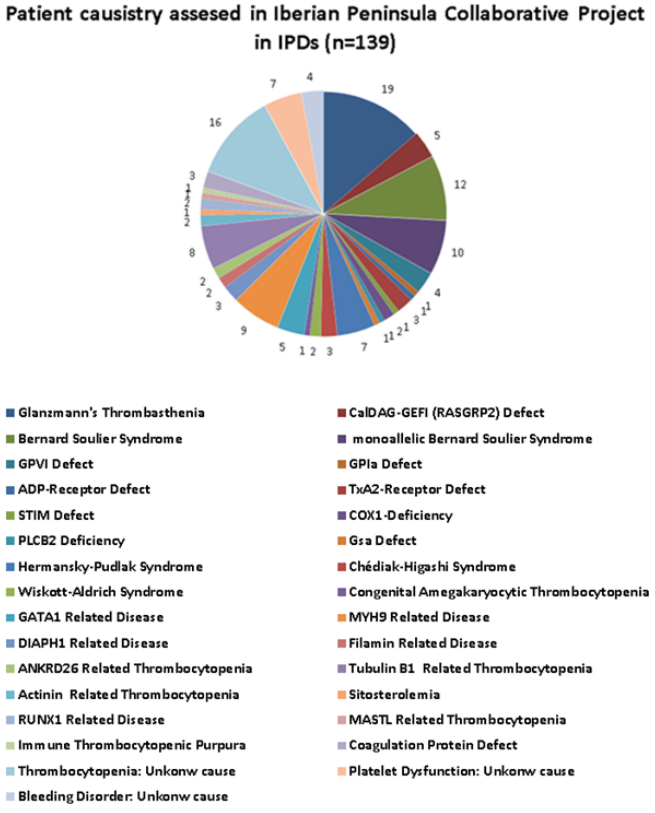

FIGURE 1 Patient causistry assessed in Iberian Peninsula Collaborative Project in IPDs (n=139)

Conclusions: A multicenter project has been consolidated that has assisted diagnose of the largest series of IPDs ever described in the Iberian Peninsula. (*ISCIII&Feder[PI14/01956,SETH&GRS 1370/A/16)*

OC 59.2 | Homozygous Frameshift in ABCC4 Causes a Novel Inherited Platelet Disorder Characterised by Increased cAMP Levels and Excessive Bleeding

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Background: *Abcc4-/-* mice present with bleeding, altered platelet cAMP homeostasis and, in one study, reduced membrane expression of glycoprotein VI (GPVI). In humans *ABCC4* is highly expressed in megakaryocytes and platelets. In genome-wide association studies (GWAS) the minor allele of single nucleotide variant rs9524862 in *ABCC4* has been associated with a higher platelet count and higher transcript levels. Reduced ABCC4 protein expression has been observed in patients with storage pool disorder, without identification of causal genetic variants.

Aims: To determine the genetic cause of unexplained bleeding and platelet disorders we performed whole genome sequencing (WGS) for 896 patients recruited from 16 referral centres to the NIHR BioResource - Rare Diseases project.

Methods: In this study the analysis of WGS results was focussed on high impact variants in loci identified by GWAS to be associated with platelet traits. Functional phenotyping included platelet aggregometry, nucleotide and cAMP assays, electron microscopy (EM), GPVI expression by flow cytometry and sequencing of platelet RNA (RNA-seq).

Results: Two affected cases from a single pedigree carry a novel homozygous frameshift variant in *ABCC4* (13:95815456 CT>C, p.Ser743fs*2) that cosegregates with bleeding (Fig. 1). The variant is absent from 8,176 in-house controls and 60,706 individuals from the ExAC database.

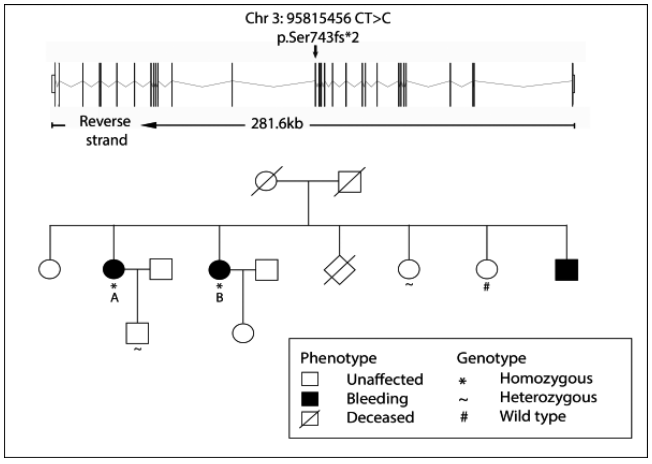

FIGURE 1 Phenotype and genotype relationship for the pedigree with a homozygous frameshift and premature stop codon at Chr 3: 95815456 CT>C, p.Ser743fs*

Presence of the premature stop codon was confirmed by RNA-seq. EM showed normal dense granules and GPVI expression was within the normal range. Platelet cAMP was significantly elevated compared to controls, including a wild-type sibling (p< 0.0001, Fig. 2).

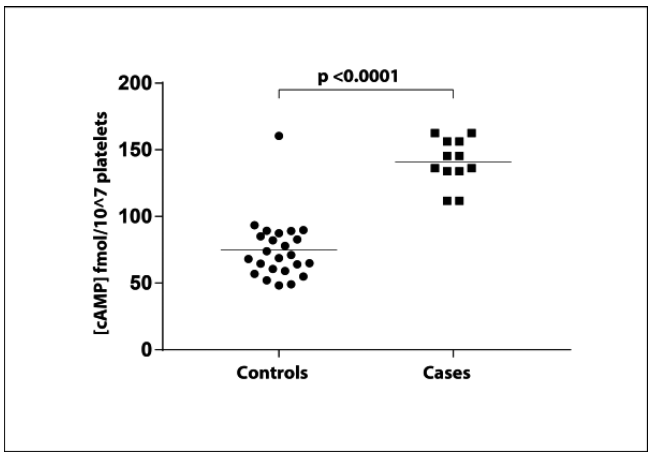

FIGURE 2 Platelet cAMP levels measured by ELISA in cases (labelled A and B in Fig. 1). Measurements were in triplicate on two occasions

Conclusions: This is the first report of a bleeding disorder associated with a variant in *ABCC4*. The variant is associated with increased intracellular cAMP levels resulting in reduced platelet responsiveness. Confirmatory studies are in progress, including platelet proteomics and phenotyping of megakaryocytes obtained by forward programming of patient-derived induced pluripotent stem cells.

OC 59.3 | A New Dominant Platelet P2RY12 Variant Affecting Receptor Function Identified in a Family with Severe Bleeding History

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Background: Inherited bleeding disorders due to platelet defects are still underdiagnosed worldwide leading to inappropriate care.

Aims: To improve diagnosis, we developed a novel platelet candidate gene array for Next Generation Sequencing (NGS) targeting platelet surface receptors and transcription factors important for platelet production.

Methods: A Caucasian family was referred to us for a history of easy bruising and serious bleeding requiring blood transfusion following surgical procedures. Full blood count, blood film examination, platelet aggregometry, flow cytometry were performed before NGS. Informed consent was obtained and the study was approved by our local ethics committee.

Results: All affected individuals had markedly impaired ADP-induced platelet aggregation with primary wave only. Responses to collagen, TRAP and epinephrine were also reduced. A single nucleotide substitution in the *P2RY12* gene was identified in all affected individuals (NM_176876 exon2 c.G794C p.R265P). Platelet P2Y12 surface and total expression was normal as was ligand binding; however, receptor function was impaired. A hemagglutinin-tagged version of the R265P P2Y12 variant like the wild type expressed well at the cell surface of transfected CHO cells but produced minimal ADP-mediated inhibition of forskolin-induced adenylyl cyclase activity. R265 is located within the extracellular loop 3 (EL3), and has been identified as one of four amino acids important for the functional integrity of the receptor as it maintains the binding pocket conformation and allows rotation following ligand binding.

Conclusions: P2Y12, a member of the G protein-coupled receptor family, is one of the 2 platelet ADP receptors. P2Y12 defects are associated with increased bleeding risk. Only a small number of mutations have been reported so far with a majority of recessive transmission. Our study describes a new dominant mutation and confirms the important role of P2Y12 EL3 domain and of the R265 for the functional integrity of the receptor.

OC 59.4 | Characterisation of Two Novel FLI1 Variants Causing Substitution of Arginine 340 in the ETS Domain of FLI1 in Patients with Dense Granule Secretion Defects

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Background: FLI1 is a transcription factor which regulates genes expressed during megakaryocytopoiesis. We previously reported *FLI1* defects affecting residues 337 and 343 in the ETS domain of FLI1 in two families with a bleeding diathesis in which the predominant defect was a reduction in platelet dense granule secretion.

Aims: To characterise two novel *FLI1* variants affecting residue 340 in FLI1 which were identified in patients with platelet function disorders.

Methods: Whole exome sequencing (WES) was undertaken for two patients; Patient 1 (P1) was investigated following enrolment in the UK GAPP study with a history of bleeding, and reduced platelet dense granule secretion. Patient 2 (P2) and her two sons had a history of bleeding and a diagnosis of storage pool deficiency. P2 also suffered from recurrent infections and all three family members had a mild history of eczema. The ability of FLI1 variants to transactivate the *GP6* promoter and undergo nuclear translocation were assessed

in HEK293T and Dami cells using a dual luciferase reporter assay, and following their expression as GFP-fusion proteins respectively.

Results: Novel heterozygous *FLI1* alterations predicting p.R340C and p.R340H substitutions in the ETS domain of FLI1 were identified by WES of DNA from P1 and P2 respectively. Compared with cells expressing wild-type (WT) FLI1, both variants showed significant reductions in transactivation capacity in HEK293T (reduction: R340H 74%; R340C 88%) and Dami (reduction: R340H 39%; R340C 32%) cells ($p < 0.001$ for all comparisons). Both variants also showed reduced nuclear accumulation when compared with WT-FLI1 in HEK293T ($p < 0.0001$) and Dami ($p < 0.05$) cells.

Conclusions: We have identified novel *FLI1* variants in two patients with storage pool disease which reduce nuclear accumulation and transcriptional activity of FLI1 *in vitro*. Given the role of FLI1 in regulating megakaryocyte-specific genes, these variants may explain the bleeding tendency observed in the patients.

OC 59.5 | A Novel Splice-specific GFI1B-p32 Mutation in a Family with Recessive Macrothrombocytopenia

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Background: Growth Factor Independent 1B (GFI1B) is a transcription factor of the erythroid and megakaryocytic lineages. A shorter p32 splice-isoform lacking the first 2 of 6 zinc fingers. So far reported germline mutations cause autosomal-dominant macrothrombocytopenia with a grey-platelet syndrome phenotype.

Aims: We report a family whose affected members present with life-threatening bleedings. The index patient and her children had recurrent hematoma and petechiae since childhood with platelets less than 45/nL. Her brother also had low platelet counts and died age 33 due to spontaneous cerebral hemorrhage. Her parents, husband and the children of the deceased brother were clinically unaffected.

Methods: Blood smears were analyzed by immunofluorescence microscopy. Platelet function was tested by aggregometry and flow cytometry. We applied targeted next generation sequencing to screen DNA for variants in 59 genes reported to play a role in platelet biogenesis or function.

Results: Aggregometry with ADP or arachidonic acid was impaired. We found a novel homozygous single nucleotide insertion in GFI1B (NM_004188.5; c.551insG), expected to cause a premature stop-codon and which segregated with the phenotype. The unaffected mother, the husband and two nephews were heterozygous, confirming an autosomal-recessive trait. Dysplastic micromegakaryocytes and peripheral platelets were CD34-positive with reduced alpha-granule markers. Platelet RNA showed residual homozygous c.551_G insertion in the p37 transcript which was markedly reduced and an unexpected expression of p32.

Conclusions: Our findings imply that the first two zinc fingers of GFI1B are dispensable for human erythropoiesis, but essential for normal megakaryopoiesis and functional platelets. While previous mutations affect both isoforms, this insertion variant results in a premature stop-codon and affects only the p37 isoform. The transcriptional regulation of erythropoiesis is uncoupled from that of megakaryopoiesis through alternative splicing of GFI1B.

OC 63.1 | Childhood-onset Congenital Thrombotic Thrombocytopenic Purpura (Upshaw-Schulman syndrome): The French Reference Center for Thrombotic MicroAngiopathies Experience

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Background: Congenital thrombotic thrombocytopenic purpura (TTP) is a rare, inherited thrombotic microangiopathy (TMA), also named Upshaw-Schulman syndrome (USS). USS patients exhibit an ADAMTS13 activity < 10% linked to bi-allelic recessive mutations of the *ADAMTS13* gene. The first TTP episode may occur during childhood including the neonatal period.

Aims: Based on the experience of the French TMAs Reference Center, our work aimed to study the correlation between the phenotype and the genotype of ADAMTS13 deficiency in childhood-onset USS.

Methods: A cross-sectional analysis of the French TMAs Registry was performed from Jan 1st 1999 to Dec 31th 2016 to identify, among childhood-onset TMA patients, those exhibiting an inherited ADAMTS13 deficiency (USS). We measured plasmatic ADAMTS13 activity using ELISA Technozym® Chr-VWF73 kit (Technoclone), ADAMTS13 antigen, and we screened ADAMTS13 autoantibodies. Genomic DNA was screened for mutations by direct sequencing of *ADAMTS13* gene. An exhaustive analysis of clinical records was assessed.

Results: 27 patients from 24 families were enrolled (sex ratio: 1.3F/1M) and all of them had a severe ADAMTS13 deficiency (activity < 10%,

undetectable antigen), no ADAMTS13 autoantibodies and bi-allelic mutations of *ADAMTS13*. Two groups of patients were identified as a function of the presence of N-terminal mutation(s) of *ADAMTS13*: 10 patients had no N-terminal *ADAMTS13* mutation (group 1) while 17 had one or two N-terminal *ADAMTS13* mutation(s) (group 2). Group 1 was associated with an ADAMTS13 activity >1% [median 1.1%, IQR: 0.6-2.2], while group 2 was associated with an ADAMTS13 activity < 1% [median 0.6%, IQR: 0.4-0.9]. 21 patients developed the disease before 1 year-old, 16 of whom in the neonatal period. 23 patients needed prophylactic plasma infusions.

Conclusions: Childhood-onset congenital TTP is a very rare disease with a prevalence in France of 0.8 cases/ million children in 2016. N-terminal mutations of *ADAMTS13* may be associated with a more severe biologic phenotype.

OC 63.2 | Hereditary Thrombotic Thrombocytopenic Purpura - Incidence of Acute Events under Plasma Prophylaxis

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Background: Hereditary TTP, Upshaw-Schulman syndrome (hTTP) is a rare, recessively inherited disorder and the result of congenital severe ADAMTS13 deficiency caused by *ADAMTS13* mutations. Acute TTP episodes can be prevented by regular plasma infusions.

Aims: Incidence rate of acute TTP events under documented plasma prophylaxis in hTTP.

Methods: Patients diagnosed with hTTP (having an ADAMTS13 < 10% in the absence of a functional ADAMTS13 inhibitor on at least two occasions > 1 month apart; a patient or family history with at least one TTP episode; two *ADAMTS13* mutations and/or a plasma infusion trial documenting full recovery and a half-life of infused ADAMTS13 of 2-3 days) enrolled in the hTTP registry (ClinicalTrials.gov NCT01257269) since 09/2012. Medical data are recorded retrospectively up to enrolment and then prospectively during annual follow-up or when an acute TTP episode occurs.

Results: As of December 31st 2016, 194 subjects had been enrolled into the study: 108 confirmed patients (of which 7 were lost to follow-up and 7 have died since enrollment) and 32 suspected patients (ADAMTS13 gene analysis pending) as well as 54 family members. Mean follow-up time was 2.8 years (IQR 1.6 - 4.9 years).

A total of 336 TTP events (including 47 prospective events) were documented in 77 hTTP patients. Twenty-four patients on regular plasma prophylaxis experienced 92 acute events during 473 person-years (incidence rate 0.19 [95% CI 0.16-0.24]). Number of subsequent TTP episodes in all patients with at least one documented prospective TTP episode was 0.4 episodes/year (IQR 0.1-1.05 episodes/year). Because of acute events 11 hTTP patients switched from a on demand treatment to plasma prophylaxis during the observation time.

Conclusions: The annual event rate is low under regular plasma prophylaxis in hTTP. The rate of subsequent events is higher once a prospective event has occurred and could help to identify patients, that will benefit from prophylactic treatment.

OC 63.3 | Clinical and Laboratory Characteristics of Patients Affected with Acquired Thrombotic Thrombocytopenic Purpura Enrolled in the Milan TTP Registry

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening thrombotic microangiopathy associated with the congenital or immune-mediated severe deficiency of ADAMTS13.

Aims: To report demographic and disease-related data of acquired TTP patients recorded in the Milan TTP registry.

TABLE 1 Demographic characteristics of patients included in the Milan TTP registry

Sex, %	Female	76.7
	Male	23.3
	White	98.2
Ethnicity, %	Black or African American	1.2
	Asian	0.3
	American Indian or Alaskan Native	0.3
Geographical origin, %	Europe/Italy	97.6/82.4
	Asia	0.7
	Africa	1
	South America	0.7

Methods: We performed a cross-sectional analysis of acquired TTP patients enrolled in the Milan TTP registry for an acute episode of TTP occurred between January 2002 and November 2015. Acquired TTP was defined as the occurrence of thrombocytopenia and microangiopathic hemolytic anemia, in absence of alternative causes.

Results: The Milan TTP registry included 416 acquired TTP patients, for a total of 837 acute events. Demographic and disease-related data are reported in Tables 1 and 2. Eighteen percent of patients had concomitant autoimmune disorders, mostly autoimmune thyroiditis (35%). Among potential triggers of acute episodes, infections were the most prevalent (26%), followed by assumption of estroprogestinics in women (4%). At presentation, systemic, bleeding and neurological signs and symptoms were the most prevalent. Clinical and laboratory parameters were less severe in relapses than first episodes. Almost all acute events were treated by plasma exchange and steroids, 12% by rituximab. ADAMTS13 activity at baseline was severely reduced (< 10%) in 88%, moderately reduced (10-45%) in 11% and normal in 1% of events. Anti-ADAMTS13 antibodies were positive in 95% of the cases. The TTP-related mortality in our population was 5%. The median time to remission was 13 days (IQR 724), shorter for relapses than first events (median difference 8 days, 95%CI 5-11). Of 348 survivors of the first TTP episode with at least 6 months of follow-up, 49% had a recurrence.
Conclusions: Acquired TTP is a rare, potentially fatal disease that may require a long hospitalization and recur in nearly half of the patients. A harmonized data collection system could be a powerful tool to improve our knowledge and management of acquired TTP.

TABLE 2 Clinical and laboratory characteristics of acute TTP events

		All events	First events	Relapses
Clinical signs and symptoms, %	None	6	0.3	13
	Systemic (fatigue, fever, abdominal pain, headache, jaundice, vomiting)	70	84	54
	Bleeding (hematuria, meno-metrorrhagia, mucosal bleeding, gastrointestinal bleeding, ecchymosis, purpura)	68	70	65
	Neurological (stroke, seizures, coma, personality change, focal neurological signs, transitory ischemic attack)	44	62	23
	Renal (acute renal failure, anuria, need for dialysis, creatinine >1.3 mg/dl)	19	24	14
	Cardiovascular (acute coronary syndrome, ECG ischemic abnormalities)	10	13	6
Laboratory data at presentation, median (IQR)	Platelet count, 10^9/l	18 (10-33)	14 (8-22)	27 (13-48)
	Hemoglobin, g/dl	9.3 (7.7-11.5)	8.0 (7.0-9.3)	11.5 (10.1-12.7)
	LDH, IU/l	1240 (656-1828)	1496 (955-2248)	784 (506-1356)

OC 63.4 | ML355, a Selective 12-LOX Inhibitor, Prevents Immune-mediated Thrombocytopenia and Thrombosis

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Background: FcgRIIa plays a central role in the pathogenesis of several immune-mediated thrombocytopenia and thrombosis (ITT) disorders, including heparin-induced thrombocytopenia and thrombosis (HITT). Devastating clinical manifestations of ITT or HITT are pulmonary thromboembolism and thrombocytopenia, which are associated with significant morbidity and mortality. Although we had

demonstrated that platelet 12-lipoxygenase (12-LOX) is important for FcgRIIa-mediated platelet activation *ex vivo*, it was not known whether 12-LOX intervention could prevent the development of thrombocytopenia or thrombosis *in vivo*.

Aims: To assess whether pharmacological inhibition or genetic ablation of 12-LOX prevents pulmonary thromboembolism and thrombocytopenia in mouse models of ITT.

Methods: Transgenic mice expressing human FcgRIIa on platelets with (hFcR/ALOX12$^{+/+}$) or without (hFcR/ALOX12$^{-/-}$) 12-LOX were retro-orbitally administered with IRDye 800CW a-GPIX antibody to induce ITT-like symptoms. HITT transgenic mice were orally gavaged with 7.5 mg/kg of ML355 or vehicle control prior to KKO (autoantibody derived from a HITT patient) and heparin administration for 7 days. Blood was collected from saphenous vein to measure platelet count at indicated time points. Lungs were harvested and imaged on Li-COR Odyssey for accumulation of a-GPIX labeled platelets.

Results: Following administration of a-GPIX, hFcR/ALOX$^{+/+}$ mice had significant reduction in platelet count compared to hFcR/ALOX$^{-/-}$ mice. In addition, pulmonary thrombosis was significantly reduced in hFcR/ALOX$^{-/-}$ compared to hFcR/ALOX$^{+/+}$. HITT transgenic mice treated with ML355 had a reduction in pulmonary thromboembolism and increase in platelet count compared to vehicle control treated HITT mice.

Conclusions: Targeting 12-LOX via genetic ablation or pharmacological inhibition with ML355 attenuated ITT and HITT *in vivo*, suggesting ML355 is a potential novel therapy to treat ITT syndromes.

OC 63.5 | Rituximab Use Is Associated with Longer Relapse Free Survival in Patients with Acquired Thrombotic Thrombocytopenic Purpura

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Background: Rituximab is an effective drug to induce remission during the acute phase of thrombotic thrombocytopenic purpura (TTP) in refractory patients.

Aims: To study the effect of rituximab treatment during the acute phase (primary or relapse) of TTP on relapse rate of the disease in a cohort of patients affected by acquired TTP.

Methods: We analyzed the clinical data of 166 patients enrolled in the Milan TTP Registry referred for an acute episode of TTP from 2011 to 2015. After evaluation of inclusion criteria, 92 patients for a total of 109 acute episodes which reached remission were included. Patients have been followed until a new acute episode or the end of the observational period (censored data June 30th, 2016).

Results: All the 109 acute episodes were treated with plasma exchange (PEX) and steroids. Rituximab was used in 24 acute episodes (R+ group), while remaining 85 were treated without (R- group). The two groups did not differ in term of sex, age, and number of acute episodes. Autoimmune disorders were more prevalent in the R+ group (33.3% *vs* 22.4%). Hb levels, platelet count and LDH levels were also similar in the two groups. As expected, the median number of PEX to reach remission was higher in the R+ group (19, IQR 11-31) than in R- group (9, IQR 6-14). Median follow up was similar in the two groups (28 months for R+ and 33 months for R- group). Nineteen relapses out of 85 acute episodes were observed in the R- group and one out of 24 episodes in the R+ group, for a hazard ratio of 5.66 (95% C.I. 0.76-42.41). The efficacy of rituximab remained also restricting the analysis at the first two years of follow up, the period with the highest probability of relapse.

Conclusions: The administration of rituximab in acute phase of TTP appeared to be beneficial in reducing the relapse rate by 5 folds.

OC 69.1 | New Insights in Pathogenesis, Diagnosis and Phenotype of FLI1-associated Thrombocytopenia

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Background: Congenital macrothrombocytopenias (CMTPs) are a family of rare diseases, of which a significant fraction remains to be characterized. Using two high-throughput sequencing strategies (308-gene panel or whole exome sequencing), we identified two families

carrying novel *FLI1* variants (p.R337Q and p.K345E) associated with CMTP.

Aims: To study the pathogenesis, phenotype and diagnosis of *FLI1*-associated CMTP.

Methods: We have studied $CD34^+$-derived megakaryocytes (MK) and used immunofluorescence and luciferase reporter system assay in transfected MSR cells. Platelet phenotype was studied using aggregation assay, flow cytometry, ATP release assay and mepacrine test. We used the innovative Focused Ion Beam / Scanning Electron Microscopy (FIB/SEM), which allowed the 3D ultrastructural analysis of platelets.

Results: The carriers had moderate thrombocytopenia (131-154 $x10^9$/l) with slightly increased mean platelet volume (10.7-13 fl; normal range 7-9 fl). The FLI1 variants nuclear accumulation and transcriptional activity properties were reduced. *In vitro* study of carriers' MK revealed a maturation defect and reduced proplatelet formation potential. The carriers' platelets exhibited dense granule abnormalities that may account for the observed bleeding diathesis. We observed defects in aggregation upon low dose ADP, collagen and TRAP, a defect in ATP secretion, a reduced mepacrine uptake and release and a reduced CD63 expression after stimulation. Remarkably, FIB/SEM revealed not only giant alpha-granules but also an almost absence of dense granules (Figure), likely due to a biogenesis defect. Furthermore, we developed the flow cytometry-based quantification of intraplatelet MYH10 (a biomarker of *FLI1* and *RUNX1* alteration), which could constitute a useful diagnostic tool.

Conclusions: Overall, this study provides new insights into the pathogenesis, phenotype and diagnosis of *FLI1*-associated CMTP, notably suggesting a role for FLI1 in dense granule biogenesis.

OC 69.2 | Identification of the DIAPH1 R1213* Variant in a Family with Macrothrombocytopenia and Hearing Loss

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Background: Recently, the R1213X variant in *DIAPH1* has been identified as a causative mutation for congenital macrothrombocytopenia (MTP) and sensorineural hearing loss in 2 unrelated pedigrees (Stritt S, Blood 2016).

Aims: Functional and molecular characterization of three patients from the same pedigree presenting with MTP and sensorineural hearing loss.

Methods: The index cases were a Spanish man, his daughter and son (43, 15 & 7 yr), all presenting with sensorineural hearing loss detected in the first 5 yrs of life. DNA was analyzed by whole exome sequencing (WES) and/or Sanger sequencing. Phenotyping included: blood cell count/film, platelet aggregation, platelet adhesion in cone and plate test, glycoproteins (GPs) expression and platelet activation by flow cytometry, immunofluorescence and immunoblotting of selected proteins.

Results: Patients developed moderate MTP (52-84$x10^9$/L) and neutropenia (1.1-1.6$x10^9$/l) in the first 5 yrs of life. WES identified the *DIAPH1* R1213* variant in both the father and the daughter, and Sanger sequencing confirmed the variant in the three relatives. Platelet studies showed moderately increased GPs expression (αIIbβ3, GPIb/IX, αIIbβ1) and no abnormalities in shear-induced adhesion, aggregation to distinct agonists or granule secretion. Western blot revealed normal platelet expression levels of DIAPH1, actin and β1-Tubulin. Platelet immunofluorescence exhibited no obvious alteration in DIAPH1 localization pattern. However, α-tubulin staining showed normal microtubular coils but aberrant microtubules distributed throughout the cytoplasm in patient platelets.

Conclusions: We report the third unrelated family with MTP and hearing loss resulting from *DIAPH1* R1213* variant. While no platelet adhesion, aggregation and secretion defects were observed, our findings support the relevant role of *DIAPH1* in the regulation of platelet cytoskeletal organization. *(ISCIII & Feder [PI14/01956], SETH & GRS 1370/A/16)*

OC 69.3 | Genotype-phenotype Correlation in Wiskott-Aldrich Syndrome: Four Novel Mutations in WAS Gene

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Background: Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency characterized by thrombocytopenia, small platelets, eczema, recurrent infections and increased risk of autoimmune diseases and cancer. It is caused by mutations in the gene coding for the Wiskott-Aldrich syndrome protein (WASP). Some mutations in *WAS* gene may be associated with milder manifestations, such as X-linked thrombocytopenia (XLT).

Aims: To determine the disease-related genotype, to quantify the level of WASP expression in hematopoietic cells, and to correlate these data with clinical manifestations in WAS patients.

Methods: We performed genomic DNA extraction from peripheral blood leukocytes, sequenced *WAS* gene and quantified the level of WASP expression in leukocytes by flow cytometry in WAS patients.

TABLE 1 Characterization of the mutations found in the WAS gene

Patient ID	Diagnosis	Clinical score	Mother as a carrier	Site of mutation	Type of mutation	Nucleotide change	Mutation	Novel mutation	Level of WAS expression
HSD and BSD (siblings)	WAS	4	Yes	exon 1	Nonsense	c.155C>T	p.Arg41X	No	Not determined
DLCS	WAS	3	Yes	exon 1	Nonsense	c.140_143 DelTT	p.Phe36X	No	Reduced
JCM MFB	XLT____ XLT	2____ 1	Yes	exon 2 exon 2	Missense Missense	c.168C>T c.207C>T	p.Thr45Met p.Pro58Leu	No___ Yes	Reduced Reduced
JPMP	WAS	3	Not determined	exon 2	Deletion	c.233delC	p.Asn78 AsnfsX48	Yes	Not determined
JCF	WAS	4	Yes	exon 4	Missense	c.431G>A	p.Glu133Lys	No	Not determined
LSMN	WAS	4	Yes	exon 6	Splice	Not determined	Not determined	Not determined	Not determined
GVSR	WAS	4	Yes	exon 7	Deletion	c.743_746 delAT	p.Asp237 AspfsX21	Yes	Not determined
BGS	WAS	4	Yes	intron 8	Splice	IVS8 as -2A>G	p.Asp259 fsX69	No	Reduced
PLSV	WAS	3	Yes	exon 10	Deletion	c.1040delA	p.Lys336 ArgfsX108	Yes	Reduced

Results: We included 16 patients from 15 distinct families with clinical suspicion of WAS or XLT. The diagnosis was confirmed in 11 patients (9 WAS and 2 XLT). WASP expression was evaluated in 5 patients, and was shown to be reduced in peripheral blood leukocytes from all tested samples (3 WAS and 2 XLT) when compared to normal control. A total of 10 mutations in the *WAS* gene were identified, including four novel mutations (p.Lys336ArgfsX108, p.Asp237AspfsX21 and p.Asn78AsnfsX48 associated with WAS, and p.Pro58Leu associated with XLT), which are shown in table 1.

Both patients with XLT presented missense mutations located in the upstream portion of the *WAS* gene (exon 2). Among the 9 patients with WAS, four of them showed nonsense mutations in the upstream region of the WAS gene, while the other five patients had missense, nonsense and splice site mutations located in the downstream portion of the WAS gene (from exon 4 and beyond).

Conclusions: Our findings support the hypothesis that milder forms of WAS are associated with missense mutations in exons 1 and 2 of *WAS* gene (upstream region), while the most severe manifestations are caused by missense and splice site mutations located in the downstream region or nonsense mutations.

OC 69.4 | Identification of Two Novel Mutations in RASGRP2 Affecting Platelet CalDAG-GEFI Expression and Function in Patients with Bleeding Diathesis

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Background: The *RASGRP2* gene encodes the Ca^{2+} and DAG-regulated guanine nucleotide exchange factor I (CalDAG-GEFI), which plays a key role in integrin activation in platelets and neutrophils. To date, few *RASGRP2* variants have associated to platelet dysfunction and bleeding in patients.

Aims: To assess the platelet phenotype in two patients with lifelong mucocutaneous bleeding and to unravel their genetic defect by high-throughput sequencing (HTS).

Methods: Blood cell counts and platelet function were assessed in two unrelated Portuguese children. Platelet aggregation, flow cytometry, clot retraction and spreading assays were done. Integrin expression and function in neutrophils were also studied. DNA was analyzed by HTS using a 71 gene panel (Lozano ML. Blood 2016). Platelet CalDAG-GEFI level was quantified by immunoblotting.

Results: The patients had normal platelet and neutrophil counts and morphology. Platelet phenotyping showed: prolonged PFA-100 closure times; normal expression of major GPs receptors; severely reduced platelet aggregation response to ADP and collagen (both patients); aggregation response to PAR1 and arachidonic acid markedly impaired in one patient; PMA-induced aggregation unaffected;

platelet secretion, clot retraction and spreading minimally affected. HTS analysis showed two new homozygous variants in *RASGRP2*: c.706C>T (p.Q236X) and c.887G>A (p.C296Y). In both patients, CalDAG-GEFI protein was hardly detected in platelet lysates, and platelet αIIbβ3 activation, as assessed by fibrinogen binding, was greatly impaired in response to all agonists except PMA. Patient neutrophils showed normal integrin expression, but impaired Mn^{2+} induced fibrinogen binding.

Conclusions: We report two unrelated patients with bleeding diathesis, new homozygous mutations in *RASGRP2*, sharply reduced CalDAG-GEFI platelet expression, and defective activation of αIIbβ3 in platelets and β2 in neutrophils. *RASGRP2* mutations are an increasing form of inherited platelet disorder. (*ISCIII&Feder-PI14/01956,SETH&GRS 1370/A/16)*

OC 69.5 | Phenotyping of Platelets of Patients with Inherited Platelet Disorders on Blood Smears by Immunofluorescence Microscopy - Experience of the Greifswald Laboratory

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Background: Diagnostic evaluation of hereditary platelets disorders is still challenging. High-throughput sequencing identified many genes with their pathogenic variants. But it is still difficult to correlate the many variants within the genome to the phenotypes of inherited platelet disorders. Specialized laboratory testing for detailed phenotyping is only available in few centers. Furthermore the need for relatively large blood volumes for functional testing precludes testing in children. Together with A. Pecci, C. Balduini, Pavia; S. Kunishima, Nagoya, P. Nurden, Bordeaux, we developed a method to identify characteristic changes in the distribution of specific platelet proteins on blood smears.

Aims: Identification of characteristic changes in platelet proteins on blood smears

Methods: We use standard blood smears, prepared by the treating physician and shipped by regular mail to the laboratory. After fixation and permeabilization, staining with specific antibodies, allows to "phenotype" platelets by microscopy using characteristic changes of the distribution of certain proteins as markers for hereditary platelet disorders.

Results: Assessing blood smears of 1250 patients referred to our laboratory with unclear platelet disorders, we achieved the diagnosis in 282 (23%) patients: *MYH9*-disorders (147), Bernard Soulier syndrome (BSS) (25), gray platelet syndrome (2), *GFI-1b* mutation (3), ß1-tubulin defects (19), Wiscott-Aldrich syndrome (WAS) (1), Glanzmann thrombasthenia (16), alpha storage pool defects (32), delta storage pool defects (37). Diagnostic sensitivity and specificity of the method was high for *MYH9*-disorders, biallelic BSS, Glanzmann thrombasthenia, and gray platelet syndrome.

Conclusions: Immunofluorescence microscopy offers a diagnostic approach requiring minimal blood volume for patients with unknown inherited platelet disorders who lack access to specialized laboratories and thereby supports interpretation of results of high-throughput methods for platelet disorders.

OC 74.1 | Low-dose Decitabine Improves Platelet Recovery in Patients with Isolated Thrombocytopenia Following SCT

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Background: Isolated thrombocytopenia is a common complication of stem-cell transplantation (SCT), which was defined as consistent low platelet counts with recovery of the other two cell lines after transplantation. Previous studies have demonstrated that decitabine, a hypomethylating agent, may increase platelet counts by promoting megakaryocyte maturation and platelet release in mouse model. Here, we conduct a clinical trial to validate this effect in post-SCT setting.

Aims: We performed a prospective open-label study to evaluate the treatment of low-dose decitabine in patients with hematological malignancies who received allogeneic SCT and suffered from isolated thrombocytopenia.

Methods: The inclusion criteria were:

(1) Platelet count ≤ 30 × 109/L persistently at day 60 post-SCT or later;

(2) Recovered neutrophil and hemoglobin;

(3) Full donor chimerism; and

(4) No response to conventional treatments for a duration of at least 4 weeks.

From July 2013 to July 2016, 38 patients were randomly assigned into either the control group to receive conventional treatment only, or the test group to receive additional decitabine (15mg/m^2, intravenously daily for 3 consecutive days).

Results: Major response was observed in 16 out of 19 patients (84.2%) in decitabine group, with a median time of 22 days to achieve platelet transfusion-independence. In contrast, 3 out of 19 patients in the control group (15.8%) showed a major response.

For bone marrw morpholocial analysis, all 38 patients showed low levels of megakaryocytes at week 0. However, the megakaryocyte counts in decitabine group were significantly increased at week 4, while no significant difference was recorded in control group. However, reactive oxygen species (ROS) and megakaryocyte counts increased in the test group.

Conclusions: Our data showed an encouraging efficacy of decitabine in patients after SCT suffering from isolated thrombocytopenia owing

to remarkably increased megakaryocyte counts via regulating ROS and megakaryocyte reconstitution.

OC 74.2 | Circulating Histone-associated Thrombocytopenia Has a Significant Adverse Impact on Outcome in Critically Ill Patients

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Background: Thrombocytopenia is frequently encountered in the intensive care unit (ICU) and associated with adverse outcomes. Circulating histones have been reported to associate with significant thrombocytopenia in a small case-control study.

Aims: To determine the clinical relevance of circulating histones on thrombocytopenia development in a large ICU cohort.

Methods: A longitudinal retrospective cohort study of 470 ICU patients admitted between February 2008 and June 2013 to the ICU of the Royal Liverpool University Hospital were followed for the first 96 hours of ICU admission. 68 patients were excluded due to identifiable causes of thrombocytopenia. Daily circulating histone levels were measured in the remaining 402 patients.

Results: Circulating histones were detectable in 150/157 (~95.5%) of thrombocytopenic patients as compared to 103/245 (~42%) of non-thrombocytopenic patients (p< 0.001). Daily histone levels were significantly higher in thrombocytopenic patients as compared to non-thrombocytopenic patients. Highest admission levels were in moderate (platelets 50-99x10^9/L) and severely (platelets< 50x10^9/L) thrombocytopenic patients. Admission histone levels robustly predicted the development of moderate-severe thrombocytopenia [AUC 0.844 (95% CI 0.810-0.877)]. Among 157 thrombocytopenic patients, 67 had DIC as defined by ISTH criteria. However, the remaining 90 thrombocytopenic patients did not have DIC. When these were stratified by admission histone levels, those with levels ≥30 μg/ml (60/90 patients) were significantly associated with more severe thrombocytopenia, longer ICU stay, longer cardiovascular and ventilation support and higher 28-day mortality than those with levels< 30 μg/ml (30/90 patients).

Conclusions: Circulating histones are significantly associated with moderate-severe thrombocytopenia in the ICU and adverse clinical outcome, even in the absence of DIC.

OC 74.3 | Lack of Effect of Inhaled Nitric Oxide on Platelet Function or Bioenergetics in Patients with Acute Submassive Pulmonary Embolism: Results of a Randomized, Placebo-controlled Trial

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Background: Prior work has shown platelet hyperactivation with acute pulmonary embolism (PE), and **nitric oxide (**NO**)** signaling attenuates platelet activation.

Aims: Test if adminis**tration of inhaled NO+**O2 to subjects with submassive PE will attenuate platelet hyper-reactivity more than O2 alone.

Methods: Phase II, (NCT01939301), 3-center, randomized, double blind, controlled trial. **Patients** had CT proven acute PE, were normotensive, and had RV dysfunction using explicit criteria. Subjects were randomized to either 50 ppm NO+O2 (treatment) or O2 only (placebo), delivered by nasal cannula and identical equipment for 24 h with blood sampling before and after treatment to assess whole-blood thromboelastography (TEG, Haemoscope 5000®, including platelet mapping); O2 consumption was measured in isolated platelets using high fidelity respirometry (Oroboros®) under basal and maximal (uncoupled) conditions. Plasma [NO3-] **was** confirmed by HPLC.

Results: We enrolled 76 patients with complete data**,** randomized to groups (N=38 each) that were well-matched for **PE severity**, comorbidities as well as TEG parameters (R **time**, angle and **maximum amplitude** without and with **heparin, arachidonateor** ADP) and platelet O2 consumption. After 24 hours of NO2+O2 or placebo, no TEG nor O2 consumption parameter was significantly different between groups. However, with NO+O2, plasma **NO3-** concentrations (μM) increased from a median of 11.7 (IQR 8.5,16.1) at enrollment to 26.9 (IQR 22.3, 38.9, P< 0.001) at 24 hours, and with placebo, plasma nitrate was unchanged from 14.9 (9.5, 19.1) at enrollment to 15.7 (11.4, 20.2).

Conclusions: Inhaled NO, delivered by nasal cannula at 50 ppm does not change TEG parameters without or with thrombin, arachindonate or ADP stimulation, nor did NO change platelet O2 consumption. The increased plasma NO3- concentrations indicate adequate drug delivery. Inhaled NO is therefore unlikely to produce clinically important changes in platelet function or bioenergetics during acute PE.

OC 74.4 | The Hypercoagulable State in Patients with Immune Thrombocytopenia (ITP) and its Association with Bleeding Symptoms

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Background: In immune thrombocytopenia (ITP), major bleeding is uncommon even when platelet counts are very low. Additionally, the severities of bleeding symptoms vary widely among patients at similar platelet counts. We proposed that the different degrees of hemostatic compensation from coagulation system are responsible for these phenomena. Thrombin generation test (TGT) is a new assay designed to measure the hyper-function of the whole coagulation system.

Aims: This cross-sectional study was aimed to explore the TGT variables in primary ITP patients and their associations with bleeding symptoms.

Methods: Primary ITP patients aged ≥18 years with platelet count of < 50 x 10^9/L were enrolled. Bleeding symptoms were evaluated using WHO bleeding scale (WHO BS) and ITP bleeding score (ITP BS). Moderate/severe bleeding was defined as WHO BS ≥2. TGT was measured in platelet-poor plasma triggered by 1 pM recombinant tissue factor and 1 µM phospholipid, with and without addition of thrombomodulin (TM).

Results: Of 27 patients enrolled, 23 (85%) were female. The mean age was 46 years (range, 17-88). Compared to healthy control (N =90), ITP patients had significantly higher mean endogenous thrombin potential (ETP, 1402 ±267 *vs.* 1213 ±257 nMxmin, p=.001), peak height (197 ±63 *vs.* 106 ±35 nM, p< .001), and velocity index (57.3 ±33.4 *vs.* 18.7 ±1.4 nM/min, p< .001). The mean ratio of ETP with TM to ETP without TM was higher in ITP patients (76 ±12 *vs.* 52 ±12, p< .001), suggesting a defect of the protein C pathway. Five ITP patients (18%) had moderate/severe bleeding. Mean ETP was significantly lower in this group (1165 ±273 *vs.*1456 ±240nM x min, p=.02). In addition, there was a negative correlation between ETP value and ITP BS in ITP patients (r=-.40, p=.04).

Conclusions: ITP patients show increased thrombin generation that is negatively correlated with bleeding severity. TGT may be used as an adjunctive measure to assess an individual's tendency to bleed.

OC 74.5 | Effects of the Btk Inhibitor Ibrutinib on Platelet Function during Inflammation and in Primary Hemostasis

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Background: Ibrutinib is an inhibitor of Bruton's tyrosine kinase (Btk) used to treat hematological malignancies, including chronic lymphocytic leukemia. Bleeding is observed in up to 50% of ibrutinib-treated patients, but the origin of bleeding remains unclear. Platelets are critical for forming hemostatic plugs at sites of mechanical vascular injury. They also play a role in vascular integrity at sites of inflammation. Immunoreceptor tyrosine-based activation motif (ITAM) signaling in platelets is necessary to protect against inflammatory hemorrhage and Btk signals downstream of ITAM receptor activation.

Aims: The objective of this study was to investigate potential mechanisms of ibrutinib-associated bleeding during inflammation and in primary hemostasis.

Methods: We used two inflammatory models - the skin reverse passive Arthus reaction (rpA) and LPS-induced lung inflammation. To selectively inhibit Btk in platelets, thrombocytopenic mice were reconstituted with ibrutinib- or vehicle-treated wild-type (wt) platelets. To study primary hemostasis, platelet plug formation and bleeding was analyzed at sites of laser-induced injury to the saphenous vein.

Results: Ibrutinib dose-dependently inhibited platelet aggregation in response to GPVI or CLEC-2 agonists. In both inflammatory models, thrombocytopenic mice showed robust hemorrhage. Interestingly, transfusion of ibrutinib-treated platelets prevented hemorrhage to the same extent as vehicle-treated platelets. In our hemostasis model, ibrutinib did not prolong bleeding in wt mice. However, ibrutinib treatment significantly impaired hemostasis in P2Y12-deficient mice compared to vehicle treatment.

Conclusions: Our findings demonstrate that ibrutinib alone does not impair platelet-driven hemostasis at sites of inflammation or mechanical injury in wt mice but increases bleeding in P2Y12-deficient mice. This suggests bleeding in ibrutinib-treated patients may be due to a combination of factors inhibiting platelet function.

VASCULAR BIOLOGY & ANGIOGENESIS

OC 67.1 | Endothelial Cells Are Important Players in the Pathogenesis of Thrombosis in Myeloproliferative Neoplasms

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Background: Thrombosis is the most frequent complication during evolution of myeloproliferative neoplasms (MPN) but the events causing these clotting abnormalities remain unclear. Recent studies have demonstrated the presence of $JAK2^{V617F}$ in endothelial cells (EC) in some patients with thrombotic complications.

Aims: To assess whether $JAK2^{V617F}$ ECs promote a prothrombotic phenotype and participate to thrombus formation.

Methods: We performed two complementary approaches.

(i) Human Umbilical Venous Endothelial Cells (HUVEC) were stably transfected with human $JAK2^{V617F}$ or $JAK2^{WT}$ as control.

(ii) For *in vivo* study, we used an inducible Cre-Lox mice model which allows expression of $JAK2^{V617F}$ in endothelial cells: *Pdgfb-iCreERT2;JAK2V617F/WT* mice.

Results: We showed that

1. expression of JAKV617F in HUVEC promotes adhesion of mononuclear cells (MNC) and neutrophils,
2. leukocyte rolling and adhesion are increased in mesenteric venules of *Pdgfb-iCreERT2;*$JAK2^{V617F/WT}$ mice
3. P-Selectin and vWF expression is increased at the surface of JAK2V617F HUVEC,

4. P-Selectin plasma concentration and endothelial cell expression are increased in *Pdgfb-iCreERT2;JAK2*$^{V617F/WT}$ mice,
5. blocking of P-Selectin decreases leukocyte adhesion on JAK2V717 HUVECs *in vitro* and leukocyte rolling and adhesion *in vivo*,
6. pulmonary thrombus formation is increased in *Pdgfb-iCreERT2;JAK2*$^{V617F/WT}$ mice,
7. blocking P-Selectin protect *Pdgfb-iCreERT2;JAK2*$^{V617F/WT}$ mice against thrombosis,
8. hydroxyurea (HU) diminishes leukocyte rolling and adhesion *in vivo* and *in vitro*, and *in vivo* thrombosis.

Conclusions: This study demonstrates that JAK2V617F ECs have a proadhesive phenotype due to P-Selectin overexpression. More, JAK2^{V617F} EC promotes thrombus formation. Finally, we show that HU decreases the pro-adhesive phenotype of JAK2V617F ECs. In summary, our results show that JAK2V617F endothelial cells contribute to thrombotic complications in MPN.

OC 67.2 | Modification of the VEGF165 C-terminus by Activated Thrombin-activatable Fibrinolysis Inhibitor (TAFIa) Inhibits Neuropilin-1 (Nrp1)-dependent Induction of Angiogenesis in vitro and in vivo

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Background: Vascular endothelial growth factor 165 (VEGF165) contains a C-terminal (CT) Arg-Arg that is required for binding to Nrp1 and enhancing angiogenesis by Nrp1-mediated activation of the VEGF receptor 2 (VEGFR2). CT Arg's are subject to cleavage by carboxypeptidase B-like enzymes such as TAFIa.

Aims: To characterize the CT Arg removal of VEGF165 by TAFIa and determine the effects of TAFIa-generated des-Arg-VEGF165 forms on Nrp1 binding and induction of angiogenesis in vitro and in vivo.

Methods: TAFIa-mediated cleavage of VEGF165 was determined by HPLC. Nrp1 binding was measured by Octet-Red, ELISA and on mCherry-Nrp1 expressing HEK293 cells. Nrp1-mediated VEGFR2 signaling was determined on endothelial cells (EC). EC migration was evaluated in a transwell migration assay, and angiogenesis in vivo by Directed In Vivo Angiogenesis Assay (DIVAA).

Results: TAFIa rapidly removed Arg165 from synthetic VEGF CT peptides, but did not remove Arg164. Binding to soluble or cellular Nrp1 of TAFIa-generated or recombinant des-Arg-VEGF165 was reduced to ~42%, but binding to VEGFR1 and VEGFR2 was normal. Des-Arg-VEGF165 failed to induce EC ERK1/2 signaling and TAFIa reduced VEGF165-mediated EC migration to baseline ($p < 0.01$). To evaluate angiogenesis in vivo (DIVAA), angioreactors containing growth-factor-reduced Matrigel pre-mixed with VEGF165 were implanted in WT or TAFI$^{-/-}$ mice. After 12 days, visual inspection of angioreactors from TAFI$^{-/-}$ mice revealed increased vessel ingrowth. Accordingly, hemoglobin and lactate dehydrogenase activity were increased in angioreactors from TAFI$^{-/-}$ compared to WT mice ($p < 0.01$), indicating that TAFI deficiency promoted VEGF165-driven vessel ingrowth.

Conclusions: TAFIa significantly diminished VEGF165 binding to Nrp1, thereby attenuating VEGF165-driven angiogenesis by removing its CT Arg165. The CT-Arg-Arg is a common motif among most VEGF-A isoforms suggesting that the anti-angiogenic properties of TAFIa may play an important role in the regulation of revascularization in injured tissue.

OC 67.3 | Deficiency of Androgen Dependent TFPI-Regulating Protein (ADTRP) Promotes Vascular Malformations via Wnt-mediated up-regulation of Metalloproteases

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Background: ADTRP is a novel protein that co-regulates with, and supports the function of tissue factor pathway inhibitor (TFPI) (Blood 2011;118:4463). We also found that ADTRP is a negative regulator of Wnt signaling and that its deficiency affects vascular development (Blood 2016;128:556).

Aims: We investigated the role of ADTRP in vascular homeostasis and the mechanisms underlying vascular defects due to ADTRP loss of function.

Methods: We used in vitro cell based assays and mouse and zebrafish models to investigate vascular defects induced by ADTRP deficiency.

Results: Genetic inhibition of ADTRP in zebrafish and mice leads to vascular malformations including abnormal dilations, tortuosity and branching, increased permeability, ruptures and micro-hemorrhages. These defects resemble phenotypes observed in mice with over-activation of Wnt, supporting our hypothesis that ADTRP is a negative regulator of Wnt signaling in vascular cells. We observed that ADTRP deficiency associates with increased expression of two Wnt-regulated matrix metalloproteases, MPM2 and MMP9. Silencing ADTRP in cultured endothelial cells increased MMP9 in a Wnt-dependent manner. ADTRP knockdown in zebrafish increased MMP9 mRNA by over 8 fold. In situ hybridization showed enhanced expression of MMP9 in cells surrounding the defective vessels. Gel zymography revealed increased gelatinolytic activity in ADTRP-morpholino knockdown vs. scrambled morpholino in zebrafish. Similarly, MMP9 protein was increased in the endothelial cells, macrophages and mast cells surrounding vascular malformations in the skin of ADTRP knockout mice. Treatment with the Wnt inhibitor IWR1 reversed MMP9 expression in

ADTRP null mouse fibroblasts and in zebrafish embryos, demonstrating that MMP9 expression induced by ADTRP deficiency is downstream Wnt signaling.

Conclusions: ADTRP deficiency leads to increased Wnt signaling and subsequent up-regulation of metalloproteases that degrade the perivascular matrix and destabilize the vessel wall.

OC 67.4 | The von Willebrand Factor-binding Protein Bridges the Bacterial Cell Wall via Clumping Factor A and Endothelial von Willebrand Factor Allowing Shear-resistant Binding of Staphylococcus aureus to Inflamed or Damaged Endothelium

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Background: When establishing endovascular infections, *Staphylococcus aureus* (*S. aureus*) overcomes shear forces of flowing blood by binding to von Willebrand factor (VWF). Staphylococcal von Willebrand factor-binding protein (vWbp) interacts with the A1-domain of VWF, enabling bacterial adhesion to endothelial cells (ECs) and subendothelial matrix. However, it is unknown how this secreted protein binds to the bacterial cell wall. Surface proteins of *S. aureus* are linked to the bacterial cell wall by sortase A (SrtA). A mutation in this gene leads to an anchoring defect of about 20 *S. aureus* surface proteins.

Aims: We hypothesized that vWbp interacts with a staphylococcal surface protein, mediating the adhesion of *S. aureus* to VWF and vascular endothelium under shear stress.

Methods: We studied the binding of *S. aureus* to vWbp, VWF and ECs in a micro-parallel plate flow chamber using various mutants deficient in SrtA and SrtA-dependent surface proteins, and *Lactococcus lactis* expressing single staphylococcal surface proteins. *In vivo* adhesion of bacteria was evaluated in the murine mesenteric circulation using real-time intravital vascular microscopy.

Results: Absence of SrtA and Clumping factor A (ClfA) reduced adhesion of *S. aureus* to vWbp, VWF and activated ECs. ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13) and an anti-VWF A1 domain antibody, when combined reduced *S. aureus* adhesion to activated ECs by 90%. Selective overexpression of ClfA in the membrane of *Lactococcus lactis* enabled these bacteria to bind to VWF and activated ECs but only in the presence of vWbp. Absence of ClfA abolished bacterial adhesion to the activated murine vessel wall.

Conclusions: vWbp interacts with the A1-domain of VWF and with the SrtA-dependent staphylococcal surface protein ClfA. The complex formed by endothelial VWF, secreted vWbp and bacterial ClfA anchors *S. aureus* to vascular endothelium under shear stress.

OC 67.5 | Cadherin 6 (cdh6) is Required for Thrombus Formation in vivo

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Background: The regulation of hemostasis and thrombus formation is a tightly controlled event that has catastrophic consequences when it is deregulated. Adhesion molecules have a critical role in hemostasis and thrombosis by mediating multiple cell-cell interactions. We now evaluate the role of the cell adhesion protein, cadherin-6 (cdh6) in vivo using cdh6$^{-/-}$ mice.

Aims: The goal of this study was to determine the contribution of cdh6 to hemostasis and thrombosis in vivo.

Methods: The role of cdh6 in hemostasis and arterial thrombosis in vivo was evaluated using cdh6$^{-/-}$ mice in both the Rose Bengal and ferric chloride ($FeCl_3$) models. The function of platelets from cdh6$^{-/-}$ mice were evaluated with aggregometry and flow cytometry.

Results: The cdh6$^{-/-}$ mice were prolonged in the Rose Bengal thrombosis model to 52±11 min from 35±5 min in wild type mice (p=0.019). Similarly, the cdh6$^{-/-}$ mice were prolonged in the $FeCl_3$ model. Five of eight mice did not occlude by the conclusion of the experiment at 30 min. In contrast, wild type mice occluded at 10±2 min (n=8). In tail bleeding assays, there was no difference between wild type and cdh6$^{-/-}$ mice (119±38 vs 159±61 s, p=0.11) We next evaluated the response of platelets from cdh6$^{-/-}$ to ADP, PAR4 agonist, or convulsion. There was no difference in aggregation, GPIIbIIIa activation, or P-selectin expression. These data suggest that cdh6 is mediating thrombosis via platelet interactions with other cells. Finally, we tested if targeting cdh6 with a blocking antibody interred with thrombosis in C57Bl6 mice. The time to occlusion was prolonged in both thrombosis assays.

Conclusions: These combined studies show that cdh6 forms a complex with the necessary proteins required to mediate adhesion in platelets. Our results demonstrate that cdh6 has a physiologically important role during thrombus formation in vivo, but does not impact hemostasis as measured by tail bleeding time. Finally, our studies show that cdh6 is a targetable receptor in thrombosis.

NURSES FORUM

NUR 04.5 | Knowledge and Awareness of Pharmacists about Venous Thromboembolism and Anti Coagulation in Pakistan

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Background: Venous thromboembolism (VTE) is considered to be the most common preventable cause of hospital-related deaths.

Hospitalized patients undergoing major surgeries and those with acute medical illness are at an increased risk of VTE. Since management of VTE is multidisciplinary, it is critical that pharmacists should have adequate understanding of the anti coagulation.

Aims: The purpose of this study was to investigate pharmacists' awareness and knowledge of VTE, thromboprophylaxis and perioperative management of anti-coagulated patients in a hospital.

Methods: Cross-sectional survey design was shared between two tertiary care hospitals, The Aga Khan university hospital Karachi and shifa international Islamabad. Pharmacists working as clinical, operational and ambulatory care were enrolled in the study. A questionnaire was made with multiple choice questions and was distributed for responses.

Results: 60 pharmacists were requested to participate in the study and 41responded with a response rate of 68.33%. The responses were as follow: All pharmacists agreed that VTE prophylaxis is clinically important, 75% pharmacists were aware of institutional policy for thromboprophylaxis, 95% agreed that their institute should have peri-operative guidelines available for safe care, 100% agreed that pharmacists can play important role in VTE guideline development in their institutes, only 33% pharmacists were aware of perioperative management of heparin, only 52% were aware of appropriate use of DVT prophylaxis in hospitalized patients, 53.66% knew that patients can develop DVT after discharge, only 34.15% aware of VTE occurrence within 90 days of hospitalization in majority of hospitalized patients.

Conclusions: Pharmacist knowledge about VTE in hospital setting requires improvement.in Pakistan . Focusing the need of pharmacists and providing them with learning opportunities through discussions and lectures may improve the current situation.

NUR 05.1 | Delays in the Presentation of Chemotherapy Induced Venous Thromboembolism: Scope of the Problem and Potential Solutions

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Background: Thrombosis is the commonest cause of chemotherapy related mortality. Currently, the data does not support routine pharmacological prophylaxis for all patients and good practice recommends patients are warned of the signs and symptoms of venous thromboembolism (VTE) which would necessitate medical review. Qualitative data suggests patients receive adequate timely information pertaining to febrile neutropenia but not for cancer associated thrombosis. This could lead to delays in the diagnosis and treatment of VTE that has been precipitated by chemotherapy.

Aims: To evaluate the behaviors and understanding of chemotherapy patients who develop VTE.

Methods: Prospective observational cohort study of cancer patients attending a regional cancer associated thrombosis clinic. Data was collected on patients with a history of chemotherapy within the previous six weeks and symptomatic VTE. Length of time from development of symptoms to presentation to healthcare professionals was noted along with their recollection of information they had been given pertaining to VTE.

Results: Data was collected on 40 patients with VTE, presenting with deep vein thrombosis (DVT) (n=23), pulmonary embolism PE (n=16), DVT and PE (n=1). Patients with DVT experienced leg swelling (n=17), leg pain (n=4) or both (n=2). Patients with PE experienced dyspnea (n=8), chest pain (n=5) or both (n=3). Time to presentation ranged from 4 hours to 40 days (mean 18 days, median 8 days, mode 4 days). 28 patients had no recollection of receiving information about the risk of VTE. All patients were knowledgeable about febrile neutropenia.

Conclusions: Patients receiving chemotherapy do not to appear recall information regarding the risks and symptoms of VTE leading to delayed presentation and treatment. Their knowledge of febrile neutropenia suggests important information can be retained by patients and that oncology services need to consider ways to prioritise the knowledge transfer pertaining to chemotherapy induced VTE.

NUR 05.2 | Significant Reduction in Hospital Acquired Thrombosis: Impact of a National Risk Assessment Target & Local Real-time Reporting

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Background: Since 2010 National Health Service (NHS) hospitals in England have been incentivised to risk assess for venous thromboembolism (VTE) all patients admitted, using a national tool, together with guidance on appropriate thromboprophylaxis (TP).

Aims: The impact of this initiative in reducing hospital-acquired thrombosis (HAT) is reviewed over 7 years within a large hospital. Similarly, those HAT events associated with inadequate TP are assessed.

Methods: This was an observational cohort study reviewing all cases of HAT diagnosed between January 2010 and December 2016 obtained in real-time from radiology records. Crude incident rates (CIR) are determined by comparison against patients admitted, expressed as events per 1,000 patients. HAT cases associated with inadequate or inappropriate TP are also assessed over the same period. This data is obtained from hospital notes and drug charts.

Results: In 2010 there were 217 HAT events from 103845 admissions (CIR 2.09 per 1000 admissions, 95% CI 1.81 - 2.37). By 2016 this has reduced to 176 HAT events from 119128 admissions (CIR 1.47 per 1000 admissions 95% CI 1.28-1.71) relative risk (RR) 0.718 (95% CI 0.589 to 0.875; p = 0.001). Over the same period, 50 of the 217 HAT events were associated with inadequate TP, falling to 7/176 in 2016 RR 0.140 (95% CI 0.0653 to 0.3002; p= 0.0001).

Conclusions: National guidance on VTE prevention and mandatory risk assessment are associated with significant reductions, both in total HAT events and those associated with inadequate TP. Locally, real-time feedback on HAT events to senior clinicians, has very likely contributed to the improvements in HAT metrics.

NUR 05.3 | Impact of a Thrombosis Pharmacist in Optimizing Anticoagulation Management in Paediatric Patients

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Background: Thromboembolic disease is a new epidemic in tertiary paediatric centres. These thrombotic complications are prevented and treated with anticoagulants. However, anticoagulants are high-alert medications associated with major patient safety incidents. Pharmacists specialized in managing anticoagulation have shown improved clinical outcomes in adult patients. To our knowledge, this thrombosis pharmacist pilot program, is the first for a paediatric hospital in Canada.

Aims: We evaluated the impact of this program on clinical outcomes, assessed family/patient satisfaction and knowledge on low molecular weight heparin (LMWH) post-discharge, obtained staff feedback, and determined net cost benefit.

Methods: A thrombosis pharmacist pilot program was implemented on February 29, 2016 and ended on June 3, 2016 to optimize LMWH and warfarin use in the inpatient setting. This project compared health outcomes against a pre-implementation period (August 1 to November 30, 2015). Surveys were conducted on telephone for patients and families, and conducted online for staff.

Results: Data from 94 and 64 patients during the pre- and post-pilot periods respectively demonstrated an increase in clot resolutions by 23.65%, reduction in bleeding events by 2.26% and length of stay by 320 patient days (during pilot period), an improvement in family/patient satisfaction and LMWH knowledge by 15.63%, and an increase in anticoagulation control (for LMWH and warfarin) during the pilot period. Staff highly recommended the thrombosis pharmacist position to continue. The net cost benefit was $348 806.

Conclusions: A paediatric thrombosis pharmacist can provide substantial improvement to anticoagulation management and may lead to significant cost avoidance to healthcare payers.

NUR 05.4 | St. Joseph's Healthcare Hamilton Perioperative Anticoagulation Clinic Quality Improvement Project

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St. Joseph's Healthcare Hamilton, Hemostasis & Thrombosis, Hamilton, Canada

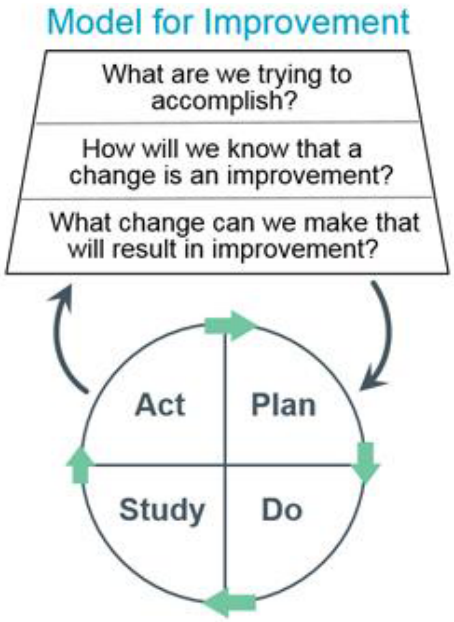

FIGURE 1 Model for Improvement by Institute for Healthcare Improvement

Background: Nurse practitioners (NPs) were introduced to the Thrombosis service at St. Joseph's Healthcare, Hamilton in December, 2014. Multiple quality improvement (QI) initiatives were undertaken to increase efficiencies of the perioperative anticoagulation clinic.

Aims: The aims of the QI project were fourfold:

1. Maximize clinic capacity (increase number of patients seen, decrease no show rate, eliminate wait list);
2. Improve patient care (Reminder phone calls, minimize unnecessary laboratory testing, minimize patient anxiety, decrease wait times, minimize delayed/cancelled procedures);
3. Achieve economic savings (maximize scope of NP practice, laboratory savings); and
4. Ensure physician satisfaction.

Methods: The Institute for Healthcare Improvement's Model for Improvement (Figure 1) was used to identify and implement changes.

Results: Multiple steps were undertaken to improve efficiency of the Perioperative Clinic. The expanded scope of NP practice resulted in increased appointment efficiency (assessment of appropriateness of anticoagulation agent, patient education); overall clinic time increased by 20 percent. Together, these steps increased clinic capacity by 160%. Implementation of reminder phone calls reduced the no show rate by 85% (17.5% to < 2%). All changes led to elimination of the wait list. Laboratory testing was reduced by 80.9%, resulting in decreased patient wait time with estimated economic savings of $1423.41/month in lab testing alone; no increase in peri/postoperative bleeding events noted. Finally, a Thrombosis physician survey revealed physician were 'very satisfied' with NP quality of care.

Conclusions: Since December 2014, multiple QI strategies were implemented by NPs in the perioperative anticoagulation clinic. These led to increased efficiencies including increased clinic capacity, elimination of wait list, economic savings and physician satisfaction.

NUR 05.5 | National Quality Improvement for Transition from Pediatric to Adult Care: Evaluation of a Pilot Program

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Background: In 2016, the National Hemophilia Program Coordinating Center (NHPCC), The Dartmouth Institute Microsystems Academy (TDIMA) and 10 Hemophilia Treatment Centers (HTCs) participated in the first US pilot National QI Program on TBD. The national transition QI framework has three interactive aspects: outcome measure (Healthy People 2020 transition measure); utilization of tools and evidence based practices ("Got Transition" and HTC developed tools) and; QI management. HTCs used standard QI assessment methodology to identify specific aims to improve TBD.

Aims:

1. To utilize focus groups to gather recommendations for improvement of future programs.
2. To determine feasibility of using the standardized QI methodology for future dispersion.

Methods: Evaluators from the Center for Program Design and Evaluation at Dartmouth College and Michigan State University conducted 3 focus groups with participants of the pilot program using a semi-structured interview guide. The quantitative data were analyzed using grounded theory methods to evaluate QI program and determine the feasibility of using this model for future dispersion.

Results: Forty-five out of 72 (63%) participated in focus groups conducted by external evaluators. 77% of respondents indicated they would definitely or probably recommend the program to colleagues an 75% indicated they were satisfied with program. Barriers to implementing QI initiative included competing priorities (49%); time management (42%); team dynamics 20%) and date collection (13%).

Conclusions: This was the first US national pilot program focused on quality improvement for transition from pediatric to adolescent care. Most of the staff indicated that the NHPCC/TDIMA program is a feasible model to implement at other HTCs. Areas of suggested program improvement include adjusting workload to align with clinical demands, clarification of program expectations and additional didactic training.

NUR 05.6 | Nurse as Change Agent for Improved Nurse Safety and Patient Satisfaction

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Background: Low molecular weight heparins (LMWH) are the anticoagulants of choice in pediatrics except in patients with mechanical heart valves. Enoxaparin, a LMWH administered by subcutaneous injection twice daily, is used off-label in pediatrics. It is supplied as prefilled safety syringes with a 27g, 1/2" fixed needle and in multidose vials. Needle/syringe choices vary when using vials. Thick needle gauges cause more injection-related trauma/pain and long needles risk accidental intramuscular injection. Using pre-filleds can lead to dosing errors and drug waste, especially in children. Needles without safety devices risk nurse injury. At our hospital, about 27,000 enoxaparin doses were dispensed for inpatients in 2016.

Aims: To improve nurse safety & patient satisfaction through nurse-initiated change.

Methods: Assessed practices for enoxaparin administration

- Patient c/o bruising/pain at injection sites
- Doses < 20 mg needed dilution to read dose
- Needle-stick risk with non-safety needles
- Safety needle/syringe search revealed desired syringe was not available in US

Planned quality improvement

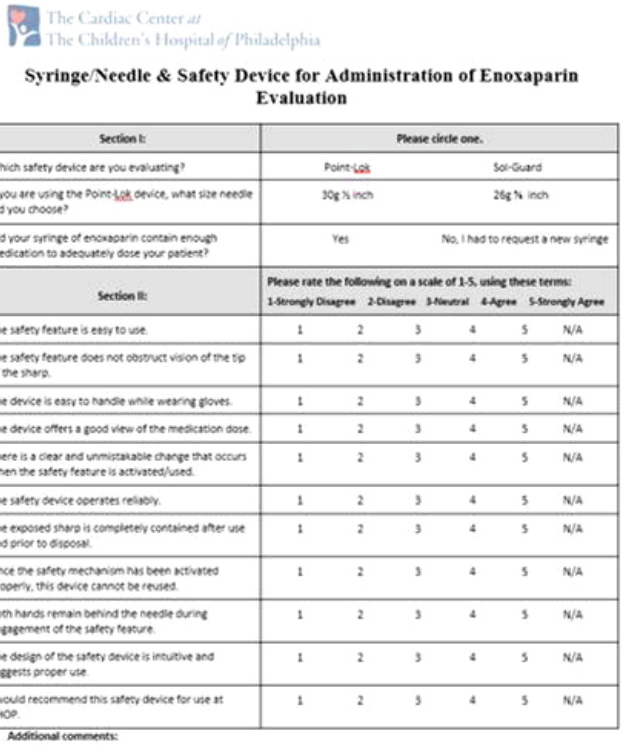
- Followed institutional processes for new product and safety approvals
- Identified a manufacturer to make a fine gauge/short, fixed-needle safety syringe
- Developed pilot program trialing newly manufactured syringes

Implement

- Developed nursing & pharmacy education
- Pilot roll-out on Cardiac units only

Evaluate

- Nurses completed pre and post implementation questionnaire



The Cardiac Center at The Children's Hospital of Philadelphia

Syringe/Needle & Safety Device for Administration of Enoxaparin Evaluation

Section I:	Please circle one.					
Which safety device are you evaluating?	Point-Lok			Sol-Guard		
If you are using the Point-Lok device, what size needle did you choose?	30g ½ inch			26g ⅝ inch		
Did your syringe of enoxaparin contain enough medication to adequately dose your patient?	Yes			No, I had to request a new syringe		
Section II:	**Please rate the following on a scale of 1-5, using these terms:** **1-Strongly Disagree 2-Disagree 3-Neutral 4-Agree 5-Strongly Agree**					
The safety feature is easy to use.	1	2	3	4	5	N/A
The safety feature does not obstruct vision of the tip of the sharp.	1	2	3	4	5	N/A
The device is easy to handle while wearing gloves.	1	2	3	4	5	N/A
The device offers a good view of the medication dose.	1	2	3	4	5	N/A
There is a clear and unmistakable change that occurs when the safety feature is activated/used.	1	2	3	4	5	N/A
The safety device operates reliably.	1	2	3	4	5	N/A
The exposed sharp is completely contained after use and prior to disposal.	1	2	3	4	5	N/A
Once the safety mechanism has been activated properly, this device cannot be reused.	1	2	3	4	5	N/A
Both hands remain behind the needle during engagement of the safety feature.	1	2	3	4	5	N/A
The design of the safety device is intuitive and suggests proper use.	1	2	3	4	5	N/A
I would recommend this safety device for use at CHOP.	1	2	3	4	5	N/A

Additional comments:

FIGURE 1 Blank pre/post evaluation questionnaire

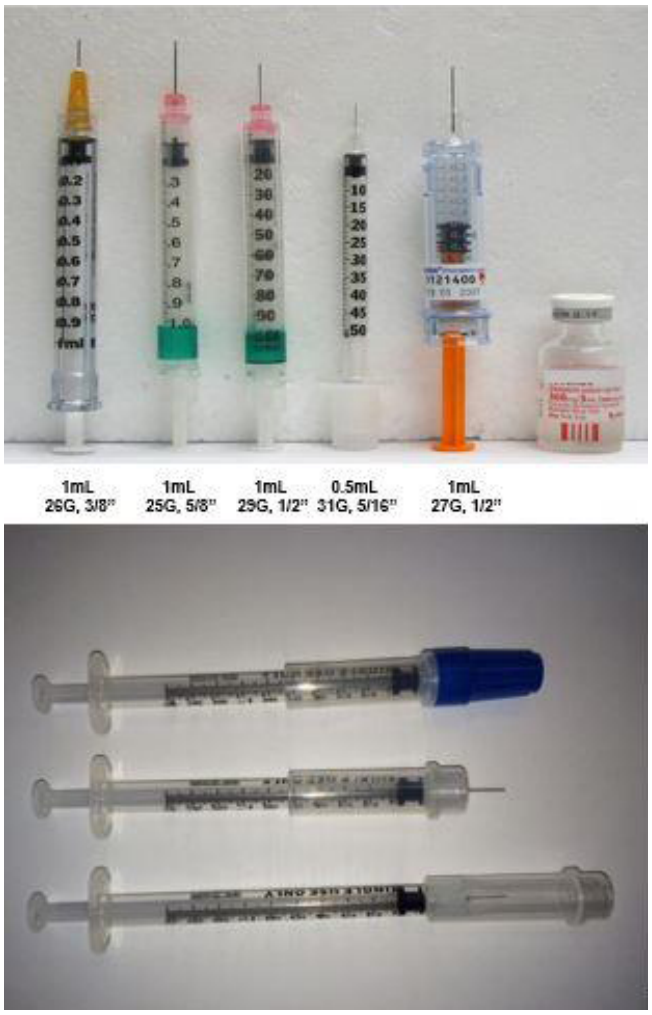


FIGURE 2 Images of syringe/needle types

- Monitored: staff needle-stick injuries,patient harm reports, pharmacy dose/fill errors
- Interviewed patients/families

Re-assess administration practice of enoxaparin

Results: Supported use of new syringe. Expanded needle use for enoxaparin hospital-wide

- Increased patient & nursing satisfaction (Fig. 1 & Fig. 2)
- New syringe caused work-flow challenges for pharmacy

Conclusions: Nurses are educated and positioned for success in:

- Identifying opportunities to improve nurse & patient safety
- Developing & executing actions yielding system-wide change
- Improving patient satisfaction.

NUR 05.7 | Lung Function, Functional Capacity and Heart Rate Recovery at Discharge in Patients with Acute Pulmonary Embolism

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Background: Acute pulmonary embolism (PE) is a cardiovascular disease with symptoms as dyspnea and hypoxemia as a consequence of haemodynamic disturbances. No previous studies exist focusing on lung function, functional capacity and heart rate recovery (HHR2) at discharge after PE.

Aims: The aims was to examine and describe lung function, functional capacity and HRR2 at discharge in patients with PE and compare to reference values for spirometry and six minute walk test (6MWT).

Methods: Thirty-one patients with first time PE admitted to the Acute Medical Unit, Sahlgrenska University Hospital were included. The patients had no earlier heart- or lung disease. Size of PE was calculated by Qanadli score (QS) percentage (mean QS 45.9 % (SD 5.2). Forced vital capacity and forced expiratory volume in one second were registered (n=30) and 6MWT performed (n=28) at the day of discharge. HRR2 was calculated after the 6MWT. Spirometry and 6MWT results were compared to reference values.

Results: This study shows that patients with PE have significantly reduced lung function ($p < 0.05$) and functional capacity ($p < 0.001$) at discharge compared to reference values. HRR2 was abnormal in 50% of all included patients. When excluding patients with beta blocker (n=7) 39% of the patients had an abnormal HRR2.

Conclusions: This study indicates that patients with PE have a reduced lung function, reduced functional capacity and abnormal HRR2 in up to 50% of the patients. Further studies is needed concerning long term follow-up of lung function, functional capacity and HRR2 after PE.

NUR 05.8 | Long-term Mental Well-being of Adolescents and Young Adults Diagnosed with Venous Thromboembolism. A Multistage Mixed Methods Study

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Background: Critical and chronic illness in youth can lead to impaired mental well-being. Although, venous thromboembolism (VTE) is a potentially traumatic and life-threatening condition, the long-term mental well-being of adolescents and young adults (AYAS) with VTE is unclear.

Aims: To investigate the long-term mental well-being of AYAS (13-33 years) with VTE.

Methods: This multistage mixed methods study included 3 sub-studies:

1) a Danish nationwide registry-based study investigating the mental well-being of AYAS with incident VTE using psychotropic drug purchase as proxy,

2) a qualitative study with 12 semi-structured interviews exploring AYAS's lived experiences following VTE, and

3) a Danish nationwide registry-based cohort study of the mental well-being of AYAS with VTE vs. AYAS with insulin dependent diabetes mellitus (IDDM) using psychotropic drug purchase as a proxy.

An integrated mixed methods interpretation of findings from the 3

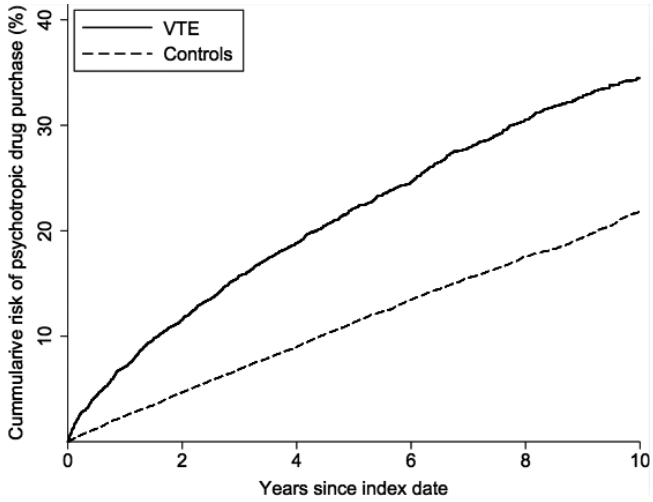

FIGURE 1 Cumulative risk of psychotropic drug purchase in 5,027 AYAS with a first VTE diagnosis versus 19,292 sex- and age matched population controls]

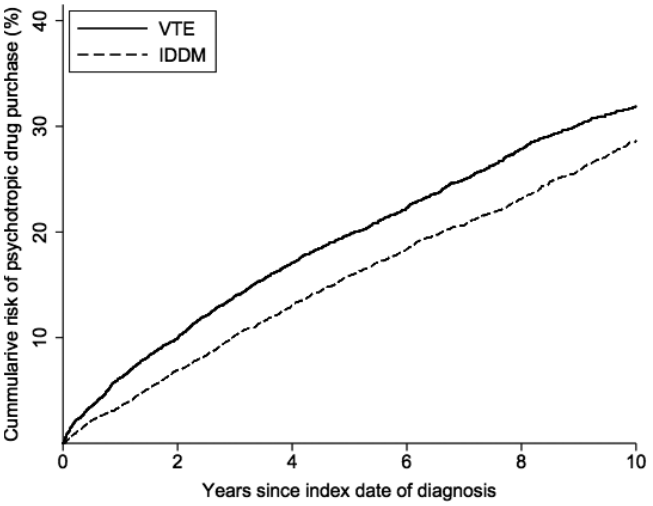

FIGURE 2 Cumulative risk of psychotropic drug purchase in 4,552 AYAS with a first VTE diagnosis compared with 5,189 AYAS with IDDM

sub-studies were conducted through narrative weaving and joint displays.

Results: The integrated mixed methods interpretation showed that the mental well-being of AYAS with VTE had a chronic perspective, with a persistently higher risk of psychotropic drug purchase among AYAS with VTE compared with sex and age matched population controls(Figure 1) and AYAS with IDDM (Figure 2). Impaired mental well-being was largely connected to fear of recurrence and concomitant uncertainty. Thus, to navigate uncertainty was important for the long-term mental well-being. Perceived health threat played a more profound role for the long-term mental well-being than illness severity, as the potential life-threat was the pivot pointing both back to the initial VTE and forward to the perception of future health-threat and the potential risk of dying of a recurrent event.

Conclusions: Our findings show that VTE has a negative impact on the long-term mental well-being of adolescents and young adults and highlight these patients' need for adequate support.

NUR 05.9 | Prevention of Hospital-acquired Venous Thromboembolism: The Project of Effective Quality Improvement

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Background: Venous thromboembolism (VTE) is the most common preventable cause of hospital death. Several pharmacological and mechanical methods are available to prevent VTE in hospitalized patients. Yet, despite the awareness of the medical staff that the majority of hospitalized medical and surgical patients have multiple risk factors for VTE and despite the existence of evidence-based guidelines, preventive methods are quite underused. An established institutional protocol, including systemic VTE risk assessment and electronic alert of high risk patients could promote VTE prophylaxis.

Aims: To improve the use of personalized VTE prophylaxis in hospitalized patients.

Methods:

1. Creating a model of quality improvement steps.
2. Adoption of the Padua risk assessment scale (RAS) and its computerization.
3. Development of educational program on VTE risk assessment and prevention methods for nurses, physicians and hospital administration.
4. Recruitment of nurses dedicated to the project in each hospital department.
5. Development of evidence-based institutional guidance to improve the management of high-risk VTE inpatients.

Results: The project has been continued for 8 years. In 2009, 2674 (17.5%) of 15315 patients were assessed for VTE risk. In 2016, 14803(99.5%) of all patients hospitalized at Rambam are evaluated for VTE risk factors using the Padua RAS. Over the 8-year follow-up, the thromboprophylaxis rate for high risk patients has increased from 957(62.2%) in 2009 to 5479(75.0%) in 2016. The total improvement in prevalence of VTE prophylaxis is 20%.

Conclusions: The project of Effective Quality Improvement has led to a rise in VTE risk assessment of hospitalized patients and to an upward trend in prescription of VTE prophylaxis.

We intend to examine the actual influence of the results obtained on VTE prevalence among inpatients.

NUR 05.10 | A Unique National Plan for Prevention of Severe Familial Hemophilia

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Background: Hemophilia (H) has an estimated incidence of 1/5000 male births. The Israeli national comprehensive care hemophilia center treats over 650 hemophilia patients.

Aims: A unique hemophilia prevention program has been running since 2009 aiming to decrease the incidence of familial hemophilia.

Methods: The National Hemophilia prevention program was led by a multidisciplinary team. Hemophilia patients and their families were interviewed and potential carriers were screened. Carriers were provided with genetic consultation and prenatal diagnosis and Preimplantation Genetic Diagnosis (PGD) was offered.

Results: Since the plan was implemented in 2009, 353 families were approached, 417 potential carries were tested and 249 carriers were diagnosed. 117 prenatal diagnostic procedures were performed. Out of 35 pregnancies diagnosed with H, 31 were terminated. PGD was offered to 108 carries. 64 women underwent a total of 158 IVF cycles that yielded 126 embroyonal returns and a total of 43 healthy babies were born. Notably, the numbers of carriers referred for IVF and PGD gradually increased with time (5-29 cases in 2009 and 2016 respectively) .Since 2009, 25 children with severe H were born despite preventive measures.

Conclusions: Our national prevention programs leads to a decreased incidence of new familial cases with severe hemophilia. The aim of this program is to reduce the burden on affected families and the health care system.

NUR 05.11 | Responding to Patient's Needs: Presenting a Framework of Support for Psychological Distress Following VTE

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Background: High levels of anxiety, trauma and uncertainty are common following Venous Thromboembolism (VTE). Findings from qualitative and quantitative study have also highlighted the emotional support needs of patients who have experienced VTE (Noble et al, 2014; Hunter et al, 2016) and point to a need for intervention to improve patient wellbeing and wider health outcomes.

Aims: The aim of this research was to develop a cost-effective intervention and/or support tools which could improve patients psychological wellbeing following VTE.

Methods: Evidence based psychological and self-help interventions were identified and a corresponding intervention pack (Coping with VTE; COVET) was developed to support VTE patients. The LENS framework was developed to assist health professionals in identifying psychological distress and providing targeted intervention.

Results: The COVET and LENS framework will be presented. The COVET intervention provides psychoeducation and is sensitive to anxieties and uncertainties commonly reported following VTE. These include:

Psychoeducation about VTE and its treatment
- Anxiety management techniques
- Support for coping with symptoms of trauma
- Information about coping with uncertainty

The COVET may be delivered as a stand-alone intervention or within the context of the proposed LENS framework.

Conclusions: The presented COVET intervention and the LENS framework aim to provide much needed tools for health professionals to support VTE patients and reduce distress following VTE. The tools presented are flexible, cost-effective and have the potential to be delivered live (i.e. nurse-led, face-to-face) or in distal settings (i.e. self-help, online). Clinical implications are discussed.

NUR 05.12 | Effect of Comprehensive Hemophilia Education Programme (CHEP) for Children and Youth on Knowledge and Quality of Life - A Pilot Project

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Background: Many children and youth are newly diagnosed to have hemophilia in developing country like India. When newly diagnosed it is common that parents are apprehensive and if properly educated many of the problems can be solved.

Aims: The aim of the study was to evaluate the effectiveness of comprehensive hemophilia education programme on knowledge and quality of life among children and youth with hemophilia.

Methods: An evaluative study was conducted at two centres (intervention and control)of hemophilia comprising 10 children in each group. Children (5-12yrs) and youth (13-24yrs) were selected by convenient sampling. Data were collected by using demographic proforma , knowledge questionnaire, Hemo QoL and A36 Hemofilia-QoL. Content validity was done based on expert opinion. Ethical committee approval was obtained before the commencement of the study and a written consent from parents was obtained.

The education programme (included five sessions on hemophilia and its management ,video on bleeds and its management,a booklet on hemophilia, a log book to record bleeds and factor replacement therapy)was given on two days when parents were attending a camp. Knowledge and quality of life was assessed before the intervention and six months after the intervention.

Results: Paired t test value for knowledge was 2.68 and the p value is 0.031, so it was found significant that education programme was effective in improving knowledge. In the age group of 8-12yrs and 13-16yrs the obtained t value for qol was 3.29 and 5.78 which was significant at 0.05 level, so the intervention was effective in improving quality of life. In the age group of 17-21 yrs the obtained t value was 1.44 and the p value is 0.38 which is not significant.

Conclusions: The study concludes that educational programme is effective in improving knowledge of mothers and it also improves the quality of life.

NUR 05.13 | Implementation of the Nurse Practitioner Role in the Thrombosis Service at St. Joseph's Healthcare, Hamilton

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Background: Nurse practitioners (NPs) were introduced to the Thrombosis service at St. Joseph's Healthcare, Hamilton in December, 2014; this represented a role change from clinical registered nurses.

Aims: The rationale for implementation of the NP role was threefold: NPs could

1. Independently assess, diagnose and treat
2. Manage atypical consults and
3. Ensure timely discharge with prescriptive authority.

Methods: The new role was evaluated using a Canadian Framework of Advanced Practice Nursing (APN) identifying four domains of practice (clinical, research, leadership, consultation and collaboration). A Thrombosis physician (MD) survey was administered to endorse NP competencies.

Results: 100% response rate for MD survey.

Clinical competencies identified include use of best practice guidelines and acting as independent practitioner and patient educator. MD survey results: 4.89/5 for NP knowledge and skill in patient care decisions; 5/5 for quality of care provided.

Research competencies include incorporating most recent research evidence into practice, NP led research and initiating regional thrombosis and knowledge translation journal clubs.

Leadership competencies include initiation of nursing staff/student thrombosis orientation, participation in national thrombosis networks and nursing practice committees. Quality improvement initiatives include 160% increase in outpatient clinic capacity with >80% reduction in lab work.

Consultation and collaboration competencies: MD survey results: 4.89/5 concur consultation with physician when appropriate, 5/5 NPs value nursing and medical aspects of care.

Conclusions: Since introduction of the NP role, achievement in each of the core competencies has been validated. Competency achievement is supported by physician colleague survey. Future goals identified include an NP led clinic for defined diagnoses and patient education initiatives.

NUR 05.14 | Nurse Practitioner-led Quality Improvement Project on Inferior Vena Cava Filter Placement and Retrieval: A Patient Focused Approach

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Background: In July 2016, Health Canada issued an alert regarding inferior vena cava (IVC) filters and their associated risk of serious complications. Recommendations included ensuring appropriate indication for filter insertion, timely follow-up and retrieval. Following this alert, our tertiary university-affiliated hospital initiated a strategy to incorporate recommendations into current practice.

Aims: To determine the indications for IVC filter insertion (compared to published guidelines), rates of IVC filter retrieval and consistency of patient follow-up prior to initiation of the IVC filter retrieval strategy. The second objective was to develop patient-centered educational material based on a patient needs assessment.

Methods: Retrospective chart review of patients with IVC filters placed at our institution from January to December 2016. REB approval was obtained. Data was extracted including date and type of filter inserted, indication for insertion and details of retrieval. A patient needs assessment was conducted via telephone to measure patient awareness and understanding of IVC filter placement, including satisfaction with education received at time of filter insertion.

Results: A total of 16 IVC filters were placed during this time; 9 were retrieved. Final results not available at this time. Complete analysis pending.

Conclusions: In addition to providing information about current practice with respect to IVC filters at a tertiary care, university-affiliated hospital, this QI project will aid in the development of a formal patient education program based on a needs assessment.

NUR 05.15 | Providing Leadership in Thrombosis - A National Network for Nurses, Midwives and AHPs

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Background: To support the National drive to implement a systematic approach to improving VTE prevention for hospitalized patients, an Exemplar Centre Network was established in 2010. Exemplar status was awarded to organisations demonstrating pre-specified quality VTE prevention care standards. This gave organisations an opportunity to showcase their work and a responsibility to provide leadership to other providers.

Aims: The VTE National Nursing and Midwifery Network (VTE NNMN) was created as a sub group of the Exemplar Centre Network

to provide support and leadership to nurses, midwives and allied health professionals working in the field. It aimed to work in alignment with the National VTE Prevention Programme in England to encourage innovation and collaborative working.

Methods: Senior nursing and midwifery support at a national level was gained. Lead nurses and midwives became members once their organisations gained exemplar status. Nurses, midwives, allied health professionals and patient representatives were invited to biannual meetings. Terms of reference were agreed and work streams set up.

Results: The network has successfully provided national leadership in VTE evidenced by the numerous clinical queries the network answers, ongoing dedication of it's members, sustained growth in new exemplar centres and it's national presence in the form of publications, social media and educational opportunities, with the aim of driving high quality care. The Network has succeeded in finding and growing talent by providing support and platforms to present and disseminate work. Resources are centralised and shared to expedite the spread of quality evidence based practice. Members report that the network has a positive impact on their professional practice.

Conclusions: A national network enables networking amongst clinicians, collaborative working and resource sharing to promote an evidence based, consistent approach to patient centred thrombosis care.

NUR 05.16 | Living with the Unpredictability of an Inhibitor as an Adolescent - Results of the SO-HEROIC Study

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Background: Haemophilia results in bleeding, predominantly into the weight bearing joints. British children with severe haemophilia are treated with factor prophylaxis. Around 1/3 will develop inhibitors making treatment complex. Tolerance is induced in many; when it fails treatment options are limited. There are no guidelines on how to treat this cohort of adolescents/young adults and clinical approaches vary widely between centres.

Aims: There is little data from a patient perspective on how living with a long-term inhibitor impacts on peoples' and families' lives. The SO-HEROIC study aims to understand the needs of these individuals, through focus groups and questionnaires.

Methods: Questionnaires were sent to participants to ask about inhibitor management, impact on lifestyles and if they would meet others for further discussions. Telephone and face-to-face interviews were conducted which were transcribed and analysed.

Results: 6 boys, with haemophilia A and a long-standing inhibitor, responded to our survey. ITT had failed in 4/6; 2/6 had not received ITT. All described limitations on physical activity and noted that they had more bleeds that took longer to resolve due to their inhibitor. 5/6 were keen to share their experiences. further 4 boys and 1 girl were identified opportunistically and agreed to in-depth interviews. All described frustrations with current treatment, the unpredictability of bleeding, and school absences. Nevertheless, some developed coping strategies and continued education to graduate levels.

Conclusions: Living with a long-standing inhibitor places extra limitations on physical and everyday activities, compounded by the unpredictability of bleeding episodes. The effect of recurrent bleeds and length of recovery for the person and family is profound. By giving greater attention to these physical and psychosocial concerns, we can promote access to and provide the necessary healthcare to ensure equal opportunity and the best possible outcomes.

NUR 07.2 | Identifying the Principles Guiding the Development and Delivery of Patient Education in Thrombosis and Hemostasis Disorders: A Literature Review

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Background: Prior work in the area of patient education has identified the importance of using learning theory and educational models to develop and deliver content that will result in improved patient outcomes. Current literature appears to focus on implementation and evaluation of teaching strategies without identifying underlying educational principles.

Aims: To identify principles of education being used to plan and deliver patient education in the field of hemostatic and thrombotic disorders.

Methods: PubMed and CINHAL were searched using the terms 'patient education' and 'hemostasis OR thrombosis'. Articles published in English from 2007-2015 were sought; 46 citations were found. Eight citations were excluded due to duplication, lack of abstract, inapplicable content; six did not meet inclusion criteria. The remaining articles were reviewed; information regarding educational principles used, disorder and learners was extracted.

Results: 26 articles evaluated the impact of educational interventions or information delivery on patient adherence, knowledge, and skills without identifying specific educational theories or models. Of these, 10 evaluated the effect of patient-directed educational interventions on patient-related outcomes; nine reported improvement. Three articles referenced educational theory/models in their development of patient education; however, they lacked the evaluation component. Only one article was specific to the population of interest.

Conclusions: While literature discussing the impact of specific educational strategies and interventions on patient outcomes is prevalent, discussion tying the use of educational theory and principles to changes

in these outcomes is lacking. Also lacking is literature connecting specific educational principles to the development and delivery of patient education in disorders of hemostasis and thrombosis. Further research is needed to identify the most effective theoretical framework for developing, delivering and evaluating patient education in this field.

POSTERS

ATHEROTHROMBOSIS & STROKE

PB 001 | ErbB Signaling Gene EGFR Centric Network Plays an Important Role in Coronary Artery Disease

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Background: Coronary Artery Disease (CAD) is a major cause of death in India and more so in young people. Combinatorial and integrative approaches to evaluate pathways and genes to gain a better understanding and potential biomarkers for risk assessment are the need of the hour.

Aims: This study aims at identifying candidate proteins associated with coronary artery disease (CAD) based on network approach.

Methods: CAD related genes were selected from CADgene database and were used for identifying common gene ontology terms across the CAD functional categories. The genes that are part of common gene ontology (GO) and disease ontology (DO) formed the seed gene for network construction. The network constructed was analyzed based on topological parameters to finally come up with backbone network and the hub molecules. Finally, pathway enrichment analysis and validation in laboratory settings on samples were carried out. The hub gene was validated on (n= 682 samples).

Results: The 608 genes from the CADgene database version 2.0 classified into 12 functional classes representing the atherosclerotic disease process were analyzed. GO homology analysis resulted into 8 GO terms. 29 CAD genes obtained after GO and DO analysis were used as a seed set for the network construction using STRING v9.1. Based on node degree, betweenness centrality and eigenvector, we identified EGFR gene as the central hub. Logistic regression analysis suggested that increased EGFR levels have 3 fold higher risk of CAD (OR 3.51(95%CI 1.96-6.28); p< 0.001), upon adjustment with hypertension, diabetes, and smoking. Furthermore, a unit increase in EGFR levels increased the risk by 2 fold for stable angina (OR 2.58 [95%CI 1.25-5.33]; p=0.01) and 3.8 fold for myocardial infarction (OR 3.82 (95%CI 1.94-7.52); p=0.001) after adjustment.

Conclusions: Our integrative analysis using gene ontologies homology across different functional classes and network analysis can help in understanding disease pathways and identify biomarkers of complex diseases.

PB 002 | Immunization with Recombinant Atherosclerosis Vaccine Candidate (AHhHmR) Does Not Promote Melanoma Lung Metastasis in B6;129S-Ldlrtm1herApobtm2sgy/J Mice

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Background: Tregs play an important role in the progress and metastasis of lung cancer and are considered as biomarkers for prediction and recurrence of lung cancer. On the other hand, Tregs have a protective role in the progression of atherosclerosis through their expansion induced by immunization with an atheroprotective antigen. Whether immunization with such antigen has a promoting effect on melanoma metastases in the lungs remains unclear.

Aims: To assess whether immunization with an atherosclerosis vaccine candidate, recombinant antigen AH^hH^mR containing epitopes derived from Apolipoprotein B (ApoB), heat shock protein 60 (HSP60) s, and complement component 5a receptor (C5aR) has an effect to promote lung metastases of melanoma cells (B16F1) in B6;129S-*Ldlr*^{tm1Her}*Apob*^{tm2Sgy}/J mice.

Methods: 5-6-week old B6;129S-*Ldlr*^{tm1Her}*Apob*^{tm2Sgy}/J mice were immunized with AH^hH^mR recombinant antigen containing epitopes derived from ApoB (AA688-707) designated as epitope A, human HSP60 (AA303-312) as H^h, mycobacterium HSP60 (AA253-268) as H^m and human C5aR (AA1-31) as R which were incorporated into a dendroaspin (den) scaffold. Melanoma cells (B16F1, $1x10^6$ in 100 µl PBS) were inoculated intravenously (tail vein) 8 weeks after the initiation of immunization. Lungs were dissected on day 12 after melanoma cell inoculation. Photos of lungs were taken, and the number of metastases was counted.

Results: The number of metastases detected in the lungs of AH^hH^mR-immunized mice was 54 ± 25 when compared to that in either AGD-den (scaffold antigen)-immunized control (63 ± 18) or in non-immunized control (93 ± 13) mice.

Conclusions: Our *in-vivo* study suggests that immunization of mice with the antigen (AH^hH^mR) did not promote the development of melanoma cell metastasis in the lung, therefore, the paradigm that Tregs are disadvantageous for the control of malignancies remains under investigation.

PB 003 | Pro-inflammatory and Pro-thrombotic Phenotype of Spontaneously Differentiated Human Monocyte-derived Macrophages in Coronary Heart Disease Patients: Implications for Plaque Morphology and Activity

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Background: The behavior of the *in vitro* differentiation of human monocyte-derived macrophages (MDMs), a representative model of macrophage infiltrating tissue, has not been explored in coronary artery disease (CAD) patients yet.

Aims: To compare the morpho-phenotype of MDMs obtained from CAD patients and healthy subjects and to assess the association between MDM profile and coronary plaque features evaluated by intravascular optical coherence tomography (OCT).

Methods: 90 CAD patients undergoing coronary angiography (stable angina or acute myocardial infarction, NSTEMI and STEMI) and 25 healthy subjects were enrolled. In 50 CAD patients OCT assessment was also performed. MDMs were obtained after differentiation (7 d, 10% autologous serum) of monocytes isolated from peripheral blood.

Results: The morphological analysis of MDMs showed a higher prevalence of round MDMs compared to spindle MDMs in CAD patients (43.94%±14.16 vs 27.22%±12.69, p< 0.001), while in healthy subjects the prevalence of these two morphotypes was similar (38.36%±6.6 vs 36.36%±7.12). MDMs of CAD patients showed higher tissue factor (TF) levels (p< 0.05), with a peak in NSTEMI and STEMI. Accordingly, MDMs of CAD patients showed a reduction in the lag time and in the time to peak, with respect to healthy subjects, with no change on endogenous thrombin potential. MDMs of CAD patients showed lower efferocytosis and lower transglutaminase-2 levels. At OCT, patients with a higher round MDMs prevalence exhibited more frequently a lipid rich plaque (p< 0.002), a thin cap fibroatheroma (p< 0.0005), a greater intra-plaque macrophage accumulation (p< 0.02), and a ruptured plaque (p< 0.0005). TF levels in round and spindle MDMs correlated with the presence of ruptured plaque, thrombus, and intra-plaque macrophages.

Conclusions: MDMs from CAD patients show a morpho-phenotypic heterogeneity with a prevalence of round MDMs displaying pro-thrombotic and pro-inflammatory properties. The MDM profile might help in predicting plaque composition and activity.

PB 004 | Regression of Atherosclerosis in ApoE Null Mice through Targeting Coagulation Factor Xa

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Background: Coagulation proteases such as thrombin and factor Xa (FXa) may regulate pro-atherosclerotic mechanisms mediated through protease-activated receptors (PARs). Whereas experimental thrombin or factor Xa inhibition reduced atherosclerosis, the effects of targeting FXa on pre-existing atherosclerotic plaques are unknown.

Aims: We investigated the effects of direct FXa inhibition by rivaroxaban on pre-existing atherosclerotic plaques in ApoE$^{-/-}$ mice

Methods: Female ApoE$^{-/-}$ mice (age: 8-9 weeks, n=10/group) received western type diet (WTD) for 14 weeks, followed by either continuation with either WTD or WTD supplemented with rivaroxaban (1.2mg/g) for 6 weeks. Quantitative and qualitative analysis of the aortic arch was performed by immunohistochemical stainings. Data were analyzed using a Mann-Whitney U test. A 2-tailed p< 0.05 was considered as statistically significant.

Results: Treatment with rivaroxaban induced a regression of pre-existing plaques in the aortic arch (-17%, p< 0.05). Moreover, plaque vulnerability upon FXa inhibition was reduced, as reflected by reduced macrophage infiltration (-39%, p< 0.05), enhanced collagen (+38%, p< 0.05) and diminished necrotic core (-31%, p< 0.05). These findings were accompanied by increased number of vascular smooth muscle cells (+22%, p=0.02) and reduced levels of MMP9 (-35%, p=0.03) and MMP13 (-50%, p=0.0009). In addition, expression of PARs and their activators, thrombin and FXa was diminished.

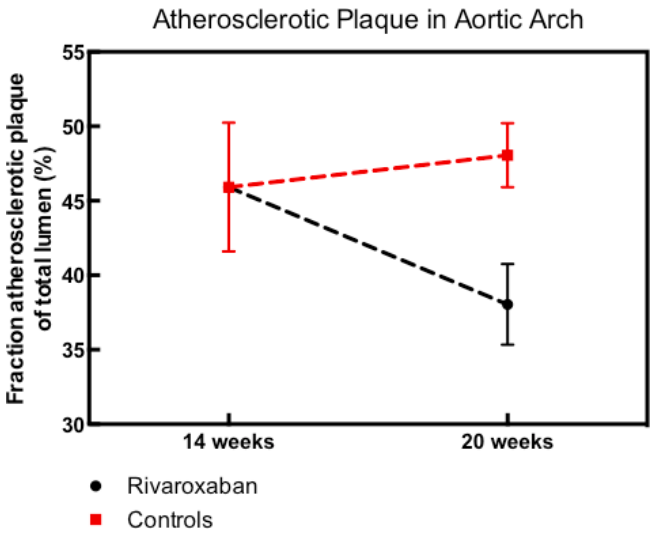

FIGURE 1 Regression of pre-existing atherosclerotic plaques in the aortic arch of ApoE null mice

Conclusions: Pharmacological inhibition of FXa induced regression of pre-existing atherosclerotic lesions and enhanced plaque stability in ApoE$^{-/-}$ mice. These findings are possibly mediated through reduced activation of PARs. Whether direct inhibition of FXa might be a promising treatment of atherosclerosis in humans needs to be elucidated in further studies.

PB 005 | The Effect of Factor XIII on the Proliferation and Migration of Vascular Smooth Muscle Cells

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Background: Plasma factor XIII (pFXIII) is a heterotetramer of FXIII-A and FXIII-B subunits. The cellular form (cFXIII), present in platelets,

monocytes, macrophages, osteoblasts and chondrocytes, is a dimer of FXIII-A. pFXIII plays an important role in blood coagulation, wound healing and maintaining pregnancy. cFXIII has been implicated in phagocytosis, cell differentiation, and extracellular matrix formation. Its role in mineralization has also been suggested. In atherosclerotic plaque vascular smooth muscle cells go through osteoblastic transformation.

Aims: 1/ To investigate if cFXIII is expressed in human aorta derived smooth muscle cells (HAoSMC) during osteoblastic transformation. 2/ To investigate the effect of extracellular FXIII on HAoSMC.

Methods: Osteoblastic transformation of HAoSMC was induced by Pi and Ca^{2+}. FXIII-A in cell lysate was measured by ELISA and Western Blot. Cell proliferation and migration were measured by EZ4U or CCK-8 cell proliferation assay kits and CytoSelect 24-Well Wound Healing Assay, respectively. Cell migration was monitored by Juli Stage Real Time Cell History Recorder. Thrombospondin-1 levels in the medium and in the cell lysate were measured by ELISA, its mRNA level was estimated by RT-qPCR.

Results: FXIII-A could not be detected in differentiated HAoSMCs. Activated recombinant cFXIII (rFXIIIa), but not the non-activated form, increased cell proliferation in concentration dependent manner. Its effect in the wound healing assay was even more considerable. The time to reach 30% and 80% confluence was decreased to less than 1/7 and 1/3 by the addition of 20 µg/mL cFXIIIa. In parallel a highly significant (67%) decreases in thrombospondin-1 concentration in the medium and a 2.5-fold increase in the cells were observed. Thrombospondin-1 mRNA did not change significantly.

Conclusions: During the course of plaque hemorrhage FXIII can be activated and exert its effect on vascular smooth muscle cells. This effect might be important in the pathogenesis of atherosclerotic plaques.

PB 006 | Insights Into the Genetic Architecture of Coronary Artery Disease in Asian Indians

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Background: Genetic approaches have identified numerous loci associated with coronary artery disease (CAD). However, most of these studies have been carried out on Caucasian population with very little information available about their association in Asian Indians. With the background of Asian Indians being high predisposed to CAD at relatively younger age refers to certain uniqueness in the genetic architecture.

Aims: The study aims at understating the genetic association of most widely studied variants in Asian Indian cohort.

Methods: The 82 potential SNPs that showed association with CAD based on literature evidence were selected for genotyping by Taqman assay in 500 CAD cases and 500 age and gender matched controls. The significant physical interaction among proteins encoded by the genes in these loci was analysed with the help of DAPPLE (Disease Association Protein-Protein Link Evaluator) algorithm. Enrichment analysis to identify the overrepresented pathways was carried out using ClueGO.

Results: The most significant associationed SNPs in Asian Indians belonged to the well-established CAD risk locus on the 9p21.3 region, KIF6, CNNM2 and the CELSR2-PSRC1-SORT1 gene cluster. The network generated by DAPPLE revealed 9 genes to be highly connected and formed two main clusters. These gene clusters mainly included lipid associated genes and cell-cycle regulating genes. The enrichment analysis using ClueGO also revealed ‚positive regulation of response to external stimuli' and ‚regulation of plasma lipids and lipoproteins' to be highly represented.

Conclusions: This analysis showed highly replicated loci, 9p21.3 to be associated with CAD in Asian Indians too. In addition SNPs belonging to KIF6 (rs20455), CNNM2 (rs12413409) and the CELSR2-PSRC1-SORT1 gene cluster (rs599839) also showed association with CAD.

PB 007 | Oral Anticoagulants as Modulators of Arterial Calcification

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Background: Vitamin K antagonists (VKA) have the undesired side effect to accelerate atherosclerotic plaque calcification. Dabigatran-etexilate (DE) is an oral anticoagulant and alternative for VKA by directly inhibiting thrombin. However, effects of DE on plaque calcification remain unknown.

Aims: The aim of this study is to compare effects of warfarin and DE on intimal calcification in an $apoE^{-/-}$ mouse model of atherosclerosis. Additionally, the prospective of vitamin K (MK7) supplementation was investigated.

Methods: Female $apoE^{-/-}$ mice received Western Type Diet (WTD; control) or WTD supplemented with either warfarin (3mg/g) or DE (7,5mg/g) for 20 weeks. Additionally, mice received the first 6 weeks WTD supplemented with warfarin followed by 14 weeks of WTD alone or supplemented with MK7 (0,1 mg/g), DE or DE with MK7. Some mice received intravenous NaF^{18} and after 30 minutes were imaged using PET and CT-scan. After 20 weeks, mice were sacrificed and aortic arches dissected, paraformaldehyde-fixed and analyzed by µCT and immunohistochemistry.

Results: Long-term warfarin treatment exacerbates atherogenesis by increased plaque size and calcification compared to DE. Moreover,

warfarin promotes micro-calcification (in 66% of mice) compared to DE (in 16% of mice). Plaque calcification was accompanied by loss of active matrix Gla protein, a pivotal vitamin K-dependent calcification inhibitor, and increased osteochondrogenic differentiation. Furthermore, supplementation of WTD or DE with MK7 reduced active calcification of the atherosclerotic plaque.

Conclusions: In contrast to DE, long-term warfarin treatment aggravates atherogenesis and intimal calcification caused by loss of calcification inhibition. Furthermore, MK7 supplementation reduced active calcification in atherosclerotic plaques. Combining DE with MK7 might be beneficial by decreasing thrombosis tendency and improving vascular vitamin K status and subsequent inhibition of vascular calcification.

PB 008 | Involvement of PAR Signaling in Thrombin Generation on Vascular Smooth Muscle Cells from Abdominal Aortic Aneurysms

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Background: In contrast to abdominal aortic aneurysm (AAA), aneurysms of the thoracic ascending aorta (TAA) are not associated with a mural thrombus. However, we have previously shown the presence of prothrombin in the media of TAA (Touat et al, ATVB 2009).

Aims: To determine (i) whether thrombin generation at the surface of VSMCs is increased in AAA, (ii) its dependence with respect to the etiology of the aneurysm, and (iii) the contribution of $a_v b_3$ integrins as a receptor for prothrombin and PAR activation to thrombin generation and thrombin-induced proliferation.

Methods: Primary cultures of SMCs seeded on fibronectin were prepared from human biopsies of TAA (n=6) and AAA (n=6). Thrombin generation was measured by thrombography in the presence of healthy plasma or plasma deficient in prothrombin, factor X or factor VII.

Results: In the presence of prothrombin deficient plasma, similar low levels of thrombin are formed on the 3 types of VSMCs indicating the presence of prothrombin within the media. There is no formation of thrombin in plasma deficient for factor VII or X. The synthesis of endogenous prothrombin has been confirmed by Western blot. There was no difference in $a_v b_3$ integrin expression between the different VSMCs. In presence of healthy plasmas, thrombin generation was significantly higher (30% increase) on VSMCs from AAA compared with VSMCs from TAA. Inhibition of thrombin generation by an antagonist of PAR-1 was more pronounced in VSMCs from AAA than from TAA without significant difference in the expression of PAR-1. This effect was associated with an increased proliferation of VSMCs from AAA in response to thrombin.

Conclusions: These results demonstrate an increase in thrombin generation on VSMCs from AAA only in the presence of plasma, which is likely mediated by PAR signaling. This new contribution of PAR in thrombin generation is also supported by an increased proliferative index.

PB 009 | Commensal Microbiota Contribute to Atherosclerotic Plaque Formation and Thrombogenicity in the Carotid Artery of Ldlr-/- Mice

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Background: Atherosclerotic plaque development depends on chronic inflammation in the artery wall, fueled by innate immune signaling pathways. The commensal microbiota is promoting low-grade inflammation and was recently linked to cardiovascular disease risk. Although implicated in atherogenesis, the role of the commensal microbiota in atherosclerosis and atherothrombosis is unexplored.

Aims: To analyze the role of commensal microbiota in atherosclerosis and plaque thrombogenicity.

Methods: We kept Ldlr-/- mice for 16 weeks on an atherogenic Western diet under germ-free isolator conditions (GF) and under SPF conditions (CONV-R). Blood cell counts, lipoprotein profile, cholesterol levels, plasma cytokines, and plasma microvesicles were determined. Plaque area of GF and CONV-R Ldlr-/- mice was histologically determined in the aortic arch, the aortic root, and the carotid artery. Thrombus formation following ultrasound-induced plaque rupture was quantified by intravital microscopy.

Results: Atherogenic Western diet fed GF Ldlr-/- mice showed significantly less atherosclerotic plaque area in the aortic arch and the common carotid artery compared with CONV-R Ldlr-/- controls. Similar to Ldlr-/- mice that were fed a normal chow diet, white blood cell counts were reduced in the GF state, while platelet numbers were unaltered. In GF Ldlr-/- mice, a reduction in the quantity of microvesicles and VLDL was observed, but total plasma cholesterol was not different. The plasma cytokine profile showed reduced levels of pro-inflammatory CCL7 and CXCL1 in GF Ldlr-/- mice, whereas T-cell related IL9 and IL27 were elevated. Following ultrasound-induced rupture of the common carotid artery plaque, the thrombus area was significantly reduced in GF Ldlr-/- relative to CONV-R Ldlr-/- mice.

Conclusions: Our results identify the commensal microbiota as an environmental factor that not only increases inflammatory markers and plaque size, but also augments thrombogenicity of ruptured plaques in atherosclerotic Ldlr-/- mice.

PB 010 | C3-Complement Activation is Found in Human Severe Atherosclerotic Plaques

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Background: Innate-immunity is a major component in the atherothrombotic process and non-structural components of the vascular extracellular matrix (ECM) are thought to regulate progression of the atherosclerotic lesions. The C3-System of the complement (C3-S) is an inflammatory pathway that has been found increased at the onset of acute myocardial infarction. The C3-S is the trigger of a cascade regulated by components as the Factor-H / CFHR family.
Aims: To investigate whether C3-S changes are found in the ECM of human atherosclerotic plaques of different severity.
Methods: Segments of human aorta with advanced atherosclerotic lesions (AAT) and without atherosclerosis (nAT) were sequentially extracted to obtain the ECM protein-fraction. The proteomic profile was analyzed by 2D-electrophoresis and mass-spectrometry. Protein and mRNA levels were analyzed by western-blot and RT-PCR. Human vascular smooth muscle cells (hVSMC) in culture were also investigated.
Results: The ECM of human aortas is enriched in active components of the C3-S with a significantly different proteomic-signature in ATT. C3 signal was more abundant in ECM of ATT-arteries and western blot analysis demonstrated a 3-fold increase in its active cleaved product C3b ($p< 0.05$) compared with nAT-ECM. C3 is expressed in hVSMC as demonstrated by RT-PCR and significantly upregulated (1.7-fold) in cells exposed to aggregated-LDL (100 µg/ml). In addition, CFHR1 and CFHR5 increased 2.5- and 3.8-fold, respectively in ATT-arteries. In contrast, Factor-H consistently detected in ECM, did not differ between ATT and nAT.
Conclusions: Our results demonstrated for the first time the presence and differential abundance of active products of C3-System in the ECM of advanced plaques, suggesting the C3-complement pathway as a novel player in vascular remodeling and in the progression of advanced human atherosclerotic lesions.

PB 011 | Relationship of Elevated Levels of Visfatin with Insulin Resistance and Prothrombotic State in Coronary Artery Disease

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Background: Recent data suggest that various adipocytokines are dysregulated in coronary artery disease and might be of pathophysiological and prognostic significance in cardiovascular complications. Visfatin has been linked to obesity, type 2 diabetes mellitus, inflammation and atherosclerosis.
Aims: The purpose of this study was to examine the relationship between visfatin and prothrombotic factors in patients with coronary artery disease.
Methods: We consecutively evaluated 223 patients, who presented with coronary artery disease. We measured anthropometric and biochemical variables including renal function, plasma lipid profile, fasting glucose, insulin and C-peptide, PAI-1, prothrombin fragment 1+2 (F1+2), and D-dimer as markers of a prothrombotic state, and C-reactive protein (CRP) as a marker of proinflammatory state. The Homeostatic Model Assessment (HOMA) index was calculated as an index of sensitivity to insulin.
Results: Coronary artery disease patients had significantly higher levels of visfatin, D-dimer, PAI-1, F1+2 compared with healthy men. On univariate regression analysis, visfatin was directly related with PAI-1 ($r = 0.173$, $P< 0.05$) and duration of diabetes ($r = 0.141$, $P< 0.05$), BMI ($r = 0.214$, $P < 0.01$), HOMA index ($r = 0.347$, $P < 0.001$), CRP ($r = 0.375$, $P < 0.001$), triglycerides ($r = 0.193$, $P< 0.01$) and LDL-cholesterol ($r = 0.190$, $P < 0.01$), and inversely related with HDL-cholesterol ($r = -0.179$, $P< 0.01$). A multivariate analysis indicated that visfatin levels are independently related with D-dimer
(β=0.203, $P< 0.01$), PAI-1 and duration of disease (respectively: β=0.195 and β=0.157, both $P< 0.05$), LDL cholesterol ($\beta = 0.164$, $P< 0.05$), HOMA index ($\beta = 0.236$, $P< 0.001$), and CRP (β=0.278, $P< 0.001$).
Conclusions: These results indicate that a prothrombotic state is associated with elevated visfatin level and duration of disease, insulin resistance and a proinflammatory status in coronary artery disease. Intriguingly, high plasma level of adipokine seem to modulate platelet activation.

PB 012 | Parameters of Complete Blood Count Do Not Predict On-treatment Platelet Reactivity in Acute Coronary Syndrome Patients

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Background: A large body of evidence clearly demonstrated that persistent high on-treatment platelet reactivity (HTPR) is associated with a higher risk for adverse cardiovascular events in acute coronary syndrome (ACS) patients. Several parameters of complete blood count (CBC) including the mean platelet volume (MPV), the platelet-lymphocyte ratio (PLR) and the neutrophil-lymphocyte ratio (NLR) were also demonstrated to correlate with clinical outcomes in ACS patients.
Aims: To investigate whether CBC parameters correlate with HTPR and could independently predict HTPR as compared with the well established vasodilator stimulated phosphoprotein platelet reactivity index (VASP-PRI) in ACS patients treated with P2Y12 receptor antagonists.
Methods: We retrospectively reviewed patients who underwent percutaneous coronary intervention (PCI) for the treatment of an ACS and who were treated with dual antiplatelet therapy between January 2014 and August 2016.
Results: Among patients that underwent PCI during this period, 565 patients had a pre procedural CBC and were further included in the analysis. Among these 565 patients, 426 (75.4 %) were men and 139 (26.6%) were women. The median age was 65 years. One hundred twenty four out of 565 patients (21.9%) presented a HTPR. Neither MPV, nor PLR, nor NLR were correlated with the VASP-PRI (r=0.029, p=ns; r=0.025, p=ns; r=-0.35, p=ns respectively). ROC curve of MPV, PLR and NLR for

predicting HTPR found areas under the curve close to 0.5 (0.528; 95%CI, 0.486-0.570 for MPV; 0.542; 95%CI, 0.500-0.584 for PLR; 0.516; 95%CI, 0.474 -0.558 for NLR) representing a worthless test for predicting HTPR. The patients were further divided into quartiles based on MPV, PLR and NLR. Rates of patients with HTPR were determined in all the quartiles, and we found no difference in rates of patients with HTPR between quartiles of MPV, PLR or NLR for any of these CBC parameters.

Conclusions: In our study, MPV, PLR and NLR do not predict HTPR in ACS patients undergoing PCI.

PB 013 | Impairment of Weight Gain and Lipoprotein Lipase Mediated-triglyceride Clearance in Integrin β3 (CD61) Deficient Mice

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Background: CD61-involved platelet function is implicated in atherosclerosis. CD61 deficiency causes platelet dysfunction and promotes atherosclerosis for which hyperlipidemia is an independent risk factor. Previous studies showed that the CD61 polymorphisms were associated with the plasma triglyceride (TG) level, and the CD51/61 blockade inhibited adipogenesis. However, the regulatory mechanisms for CD61 in lipid metabolism have yet to be established.

Aims: To better understand the mechanisms underlying the pro-atherogenic potential of CD61 gene expression focusing on the lipid metabolism in the CD61-deficient (CD61KO) mice.

Methods: The mouse body weight, tissues weight, body composition and physiological parameters were measured on chow diet. Plasma and hepatic TG levels, and the tissue and plasma lipoprotein lipase (LPL) expression and activity were assayed with established protocols. Statistical significance was determined using an unpaired two-tailed Student *t* test. The animal protocols were reviewed and approved by the local authorities to comply with the Declaration of Helsinki.

Results: The CD61KO mice exibited a weight loss (Fig. 1A) and less fat mass (Fig. 1B-C) together with an increased energy expenditure (Fig. 1D-E) and unaltered food consumption. Notably, plasma TG was significantly elevated (Fig. 2A), probably ascribable to an impaired TG clearance (Fig. 2B-D). An abnormal distribution of LPL (Fig. 2E-F, 2H) further suggested that plasma TG trafficking into peripheral tissues might be interrupted. On the other hand, the mRNA expression (Fig. 2G) and activity of LPL were found in the normal range (Fig. 2I-J).

Conclusions: The impaired TG clearance in CD61KO mice might be responsible for weight loss, reduction of the adipose tissues and elevated plasma TG level. The enhanced LPL expression in adipose tissues might provide a plausible explanation for this phenotype. Data indicate that CD61 contributes to the development of atherosclerosis most likely through regulating the key pathways of lipid metabolism.

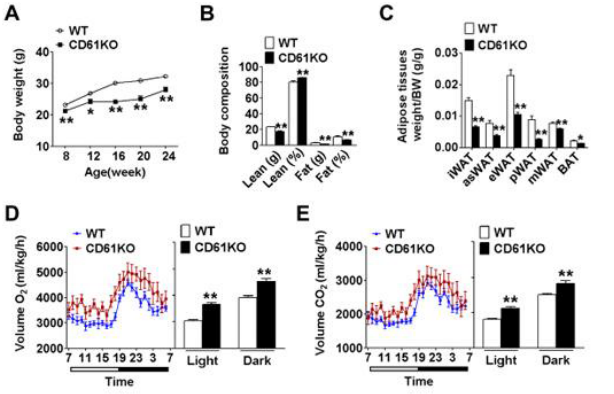

Figure 1. CD61KO results in body weight and fat mass loss, and increase of energy expenditure on normal chow diet (NCD).

(A) Body weight curves of age-matched male WT and CD61KO mice.

(B) Absorptiometry for fat and lean masses of male WT and CD61KO.

(C) Weight of different adipose tissues (iWAT (inguinal adipose tissue), asWAT (anterior subcutaneous adipose tissue), eWAT (Epididymis adipose tissue), pWAT (perirenal adipose tissue), BAT (brown adipose tissue)) after 24 weeks of NCD.

(D-E) Oxygen (O_2) consumption (D) and carbon dioxide (CO_2) release (E) during light (left) and dark phases (right) of mice with NCD for 16 weeks.

Data are presented as means ± SEM (n=8～10). $^*P < 0.05$, $^{**}P < 0.01$ *vs* WT.

FIGURE 1 CD61KO results in body weight and fat mass loss, and increase of energy expenditure on normal chow diet (NCD)

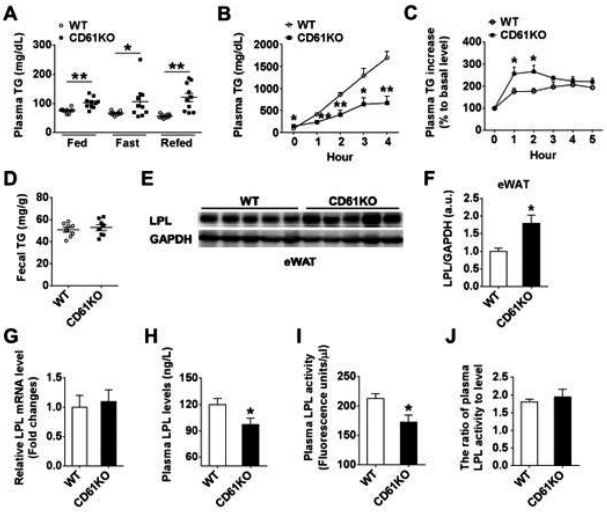

Figure 2. CD61 deficiency impairs plasma TG clearance owing to the abnormal distribution of LPL.

(A) Plasma TG level in NCD fed mice starting at the age of 10 weeks. (Fed, NCD; Fast, fasted overnight; Refed, refed NCD for 3 hour after an overnight fast).

(B) Hepatic VLDL-TG production. Plasma TG was measured after administration of poloxamer407 in mice fasted overnight.

(C) Intragastric lipid loading test. Plasma TG level at indicated time after a 15ml/kg olive oil gavage in overnight-fasted mice.

(D) Fecal TG contents were measured in NCD feeding mice.

(E-F) Representative immunobloting (E) and quantitative (F) data of LPL in eWAT of mice fed with NCD for 24 weeks.

(G) Relative LPL mRNA levels by qPCR normalized to GAPDH in eWAT of mice fed with NCD for 24 weeks.

(H-J) Plasma LPL levels (H), activity (I) and the activity over protein levels (J) were measured in mice after fasted overnight starting at 20 weeks age.

Data are represented as means ± SEM (n=5～10) . $^*P < 0.05$, $^{**}P < 0.01$, *vs* WT.

FIGURE 2 CD61 deficiency impairs plasma TG clearance owing to the abnormal distribution of LPL

PB 014 | Platelet Acetyl-CoA Carboxylase Phosphorylation: A Potential Marker for Atherothrombotic Coronary Artery Disease

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Background: In human platelets, Acetyl-CoA Carboxylase (ACC), the downstream specific substrate of the AMP-activated protein kinase, is mainly phosphorylated in response to thrombin compared to other platelet agonists. In clinical situation, such as coronary artery disease (CAD), associated with thrombin generation, ACC phosphorylation (P-ACC) can be an interesting marker of thrombin action on platelets.

Aims: In the current prospective clinical trial (ACCTHEROMA), we investigate platelet P-ACC as a marker of atherothrombotic CAD.

Methods: A total of 188 consecutive patients admitted for coronary angiogram were included from March 2015 to February 2016. Blood samples were drawn immediately after sheath insertion at the cath lab. Platelets were isolated and protein extracts were analysed by immunoblotting and electrochemiluminescence (ECLIA). CAD was assessed in all patients by coronary angiogram. Global atherosclerotic burden was evaluated by coronary (CAC Agatston score) and extra-coronary (Aortic- AoC score) calcification score on thoraco-abdominal scanner with prospective ECG-gating in a randomly selected subgroup of patients(n=68).

Results: Patients with demonstrated CAD (CAC Agatston score >100 and/or at least 1 vessel disease on the angiogram) have higher platelet P-ACC compared to non-CAD (p< 0,001). Quartile analysis of platelet P-ACC revealed a significantly higher proportion of patients (45%) with unstable CAD (acute coronary syndrome) in the 4th quartile (p=0, 01). More importantly, after adjusting for established cardiovascular risks factors, platelet P-ACC was an independent predictor of unstable CAD

(OR: 7.03, p=0,001). Likewise, ECLIA test shows significant correlation with immunobloting for platelet P-ACC analysis (p< 0,001).

Conclusions: Platelet P-ACC is a potential marker for screening patients with CAD at high ischemic risk. Optimizing ECLIA test for platelet P-ACC analysis seems to be a promising tool for future clinical studies. (ACCTHEROMA, NCT03034148).

PB 015 | Recombinant Atherosclerosis Vaccine Candidate Antigen (AHhHmR)-induced Tregs in B6;129S-Ldlrtm1herApobtm2sgy/J Mice Do Not Promote Melanoma Cell (MC) Proliferation

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Background: Atherosclerosis is an inflammatory disease in which plaque builds up inside the arteries. Natural regulatory T (nTreg) cells have a protective role in the progression of atherosclerosis. Atheroprotective antigen-induced specific regulatory T (iTreg) cells seem to provide more protection than nTreg. On the other hand, Treg can interfere with the function of anti-tumour immune effectors. However, whether the iTregs have a promoting effect on cancer cell growth remains unclear.

Aims: To assess whether atheroprotective antigen-induced Treg cells have an effect to promote MC proliferation when immunization with this antigen reduces atherosclerotic lesion through Treg expansion in B6;129S-*Ldlr*^{tm1her}*Apob*^{tm2sgy}/J mice.

Methods: Mice were immunized with atheroprotective antigen (AH^{h}H^{m}R) containing epitopes derived from Apolipoprotein B (A), human heat shock protein (HSP)60 (H^{h}), mycobacterium HSP60 (H^{m}) and complement component 5a receptor (R) which were incorporated into a dendroaspin (den) scaffold. Treg cells were isolated from the spleen of the immunized mice at the end of week 12 after being fed a high-fat diet and co-cultured with MCs for different time periods. The cell proliferation experiment was performed using Quick Cell Proliferation Assay Kit II (Abcam, Cambridge, UK), according to manufacturer's protocol.

Results: When the MCs were co-cultured with Treg cells, no effect on MC proliferation was detected in the presence of Treg cells either from control (non-immunized or AGD-den-immunized mice) or antigen-immunized mice. In addition, no significant difference expressed as OD values was observed when MCs were co-cultured with AH^{h}H^{m}R or AGD-den or with PBS as stimulators in a serum-free medium.

Conclusions: The results showed that Treg cells either from non-immunized or antigen-immunized mice have no direct proliferation promoting effect on the MCs in co-culture experiment, indicating that the notification of Tregs as disadvantageous for the control of malignancies should remain under investigation.

PB 016 | Peripheral Immune Changes and Persistence of Tolerance to Atherosclerosis by Immune Modulation with Multi-antigenic Construct in Apobtm2Sgy Ldlrtm1Her/J Mice

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Background: Inflammatory pathogenic T-cell response to self-antigens like LDL and heat shock proteins (HSP) as well as exogenous antigens from pathogens has been implicated in the initiation of an autoimmune response during atherogenesis. We have shown that oral administration of a multiantigen molecule expressing peptides derived from ApoB100, human HSP60 and outer membrane protein of Chlamydia pneumonia induces tolerance to peptides and controls the disease development.

Aims: To study the longevity of the tolerance by AHC molecule and changes in spleen cell population in response to immune therapy and its association with disease progression.

Methods: Apobtm2Sgy Ldlrtm1Her/J knockout mice (5-6 weeks) were given five oral doses of AHC molecule on alternate days to induce immune tolerance and fed on a high-fat diet (HFD) for 0,6,12,18,24 and 30 weeks with HFD (21.5%) alone fed mice as a control.

Results: Histological analysis revealed reduction in plaque at 6 (62%, p=0.002), 12 weeks (46%, p=0.001), 18 (23%, p=0.001) but later at 24 and 30 weeks confounds to 20% (p=0.001). We observed a significant increase in regulatory T cells in the spleen at 0 (60%, p=0.02), 6 (50%, p=0.02) and 12 weeks (43%, p=0.02) in corroboration with adaptive immunity to native LDL and Ox-LDL. We also observed lower levels of IFN-γ at 6 (42%, p=0.009), 12 (31%, p=0.015), 18 (33%, p=0.007) and 24 weeks (24%, p=0.015) and inflammatory Ly6C monocytes 21% at 6 week (p=0.02) and 70% at 12 week (p=0.005). The CD11b+CD11c+ were higher at 0 (46%, p=0.02), 6 (63%, p=0.028) and 12 weeks (64%, p=0.04) while $CD11c^{+}CD103^{+}$ cells were 26.5% higher at 0 week (p=0.02) and 55% at 6 week (p=0.02).

Conclusions: Our results suggest that oral treatment with multi-antigenic molecule can control the progression of atherosclerosis efficiently in early stages by inducing regulatory T cells and tolerogenic dendrites. The effect of tolerance reduced at later stages of the disease. Optimizing immune tolerance could induce long-term protection against the disease.

PB 017 | Association of vWF Antigen/ADAMTS13 Activity Ratio with Gensini Score in Type 2 Diabetes Mellitus

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Background: High levels of von Willebrand factor (vWF) and low levels of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) were associated with risk of coronary artery disease (CAD) and development of atherosclerosis. Moreover, elevated vWF was also associated with diabetes mellitus (DM).

Aims: This study aimed to assess the association of ADAMTS13 activity and vWF levels with severity of coronary stenosis in DM patients.

Methods: Type 2 DM with CAD patients (n = 62) and non-DM without CAD subjects (control group, n = 84) were recruited. Severity of coronary stenosis was considered by using Gensini score and the DM with CAD subjects were divided into 2 groups; high (Gensini score ≥ 20, n = 46) and low-medium score (Gensini score < 20, n = 16). vWF antigen and activity were measured by in-house ELISA and collagen binding assay (CBA), while ADAMTS13 activity was determined by residual-CBA.

Results: vWF antigen/ADAMTS13 activity (vWF/ADAMTS13) ratio was likely to increase in DM with CAD patients compared to control group (p for trend = 0.046). However, no significant differences of other parameters were observed between both groups. DM patients with high Gensini score showed elevated ADAMTS13 activity and tendency of decreased vWF/ADAMTS13 ratio compared to those with low-medium score [(73.4±17.2% vs 59.5±22.5%, p = 0.037) and (1.1±0.6 vs 1.5±1.3, p for trend = 0.024), respectively]. Furthermore, multivariate regression analysis showed that after adjustment for hypertension and HDL-C, DM with high vWF/ADAMTS13 ratio were significantly associated with low-medium Gensini score [OR (95% CI) = 5.4 (1.1, 26.9)].

Conclusions: This study suggested that high vWF/ADAMTS13 ratio was associated with low-medium Gensini score, which may indicate the association of ADAMTS13 and vWF levels with early atherosclerosis in DM patients. However, further study with larger sample size is needed to confirm the association.

PB 018 | Activated Protein C Protects against Accelerated Atherosclerosis in Diabetes by Epigenetically Restricting p66Shc Expression in Macrophages

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Background: Atherosclerosis is two- to four-fold increased in diabetic patients. The mechanisms of accelerated atherosclerosis in diabetes remain poorly defined.

Aims: As plasma levels of activated protein C (aPC) a protease known for its cytoprotective effects, are reduced in diabetes we hypothesized that aPC protects against diabetes induced atherosclerosis.

Methods: ApoE-/- mice were made diabetic or fed HFD for 20 weeks. Subgroup of mice were either fed chow diet or were given SGLT2 inhibitor to reduce blood lipid and blood glucose level. Atherosclerotic plaques macrophages and SMC were isolated by laser capture dissection microscopy.

Results: Diabetic ApoE-/- mice displayed smaller but less stable plaques with more macrophages and less smooth muscle cells (immunohistochemical analyses) compared to HFD ApoE-/- mice. Expression of $p66^{Shc}$ and CD36 was increased in macrophages, but not in SMCs isolated by laser dissection from plaques of DM ApoE-/- mice as compared to HFD ApoE-/- mice. Likewise, $p66^{Shc}$ expression was increased in human atherosclerotic lesion from diabetic patient compared to non-diabetic patient. Bone marrow transplantation from

p66^{Shc}-/- into ApoE-/- revealed complete protection from glucose induced atherosclerosis. HFD induced atherosclerotic plaques regressed after normalizing blood lipid levels, while hyperglycaemia induced atherosclerotic plaques did not regress despite restoring normoglycemia, suggesting epigenetic regulation of atherosclerotic plaque in diabetic mice. *In vitro* glucose induces p66^{Shc} and CD36 expression in macrophages. Glucose induced p66^{Shc} expression remained high even when normoglycemia is restored, indicating epigenetic control of p66^{Shc}. Treatment with aPC reduced p66^{Shc} expression, strongly induces expression of DNMT-1 and decreases plaque size.

Conclusions: These data shows that aPC reverses hyperglycemia induced and epigenetically sustained expression of p66^{Shc} and CD36 in atherosclerotic plagues macrophages, providing new insight into aPC dependent vascular protection.

PB 019 | Restoration of Blood Flow Contributes to the Regression of Atherosclerosis Plaque in Mice

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Background: Disturbed flow (d-flow) is being unveiled as an equal essential player as lipid in the initiation and progression of atherosclerotic plaques. Both mouse models and clinical statin treatment were in certain extent proved to induce plaque regression by lowering of LDL level. However, the role of blood flow in atherosclerosis regression has not been illustrated.

Aims: We aimed to establish a practical method to study the effects of blood flow on atherosclerosis regression based on hemodynamic regulation.

Methods: A single slipknot was applied to three branches of the left carotid artery (LCA) in ApoE$^{-/-}$ mice. After 2 weeks on HFD, the blood flow in LCA was reconstructed by knot release. Ultrasonography was used to verify successful induction of d-flow and the restoration of the blood flow. Atherosclerotic plaques in carotid arteries were stained with Sudan IV.

Results: After the release of the knots, an abrupt increase in flow velocity indicated a successful restoration of blood flow from the LCA to its branches. At this point, the restored flow in ligated LCA was still lower compared to the untied right carotid artery (RCA). After the procedure, mice were given chew diet for additional 4 weeks and blood flow was re-accessed using carotid ultrasonography. Results showed no significant difference of blood flow between LCA and RCA, indicating nearly complete regression of carotid atherosclerosis. The blood flow of LCA in the control group maintained low and d-flow even after 4 weeks with chew diet. The *en face* staining with Sudan IV clearly showed regression of carotid atherosclerosis. Meanwhile, the vascular lumen of the LCA became larger and the infiltration of monocytes decreased in the atherosclerotic plaque.

Conclusions: The results give us the first proof of the influence of blood flow on the regression of atherosclerotic plaque. Moreover, the results revealed a practical tool for a study model of the atherosclerosis regression and the evaluation of anti-atherosclerotic drugs.

PB 020 | Nanoliposomes for Transfection Of CD39 mRNA: A Step towards Nanotheranostics for Delivery of Anti-inflammatory Therapeutics against Thrombosis

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Background: Genetic therapy using modified mRNA for specific therapeutic protein expression for disease treatment and vaccination displays a new field of therapeutic and diagnostic medicine in cardiovascular research. Non-viral vectors transfection using biocompatible nanoliposomes enables safe and efficient delivery of the CD39 mRNA. CD39 is an ecto-nucleoside triphosphate diphosphohydrolase which degrades adenosine 5'-diphosphate (ADP), a major player of the platelet activation cascade, therefore provides a promising anti-thrombotic strategy.

Aims: Generation of cell-favorable cationic nanoliposomes as nanotheranostic agents to successfully deliver therapeutic CD39 mRNA for anti-thrombotic therapy.

Methods: Cationic nanoliposomes (DC-Cholesterol/DOPE-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) were generated as transfection vehicles for either eGFP mRNA, or the therapeutic anti-inflammatory, CD39 mRNA.

Results: We observed no toxicity using these nanoplexes and noted good cell viability after transfection. Nanoplexes for the transfection of eGFP mRNA showed an increase in fluorescence signals on microscopy as compared to mRNA control after 24 hours in chinese hamster ovary (CHO) cells. Nanoplexes for the transfection of CD39 mRNA showed increase CD39 expression signals on flow cytometry as compared to mRNA control after 24 hours using CHO cells. We have also demonstrated efficient transfection across several cell lines (CHO, HEK293 and A549), as well as long-term protein expression (120 h and 168 h) using these nanoplexes.

Conclusions: A non-toxic, safe and efficient nanoliposome for the delivery of therapeutic mRNA for gene therapy is useful for diseases such as inflammation and thrombosis. This approach supplies the primary basis to use CD39 mRNA as an anti-inflammatory therapeutics for future nanotheranostics approach.

PB 021 | Association of Plasma Soluble Thrombomodulin and HDL-C with Coronary Artery Stenosis

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Background: Arterial wall damage is the early stage of atherosclerosis which is a major underlying cause of coronary artery stenosis. Thus, biomarkers of endothelial injury are considerable interest in predicting the severity of coronary artery disease (CAD). Thrombomodulin can be released from damage endothelial cells and it has been shown that the soluble form of this protein (soluble thrombomodulin; sTM) is a specific marker of endothelial injury.
Aims: This study aimed to determine the association between sTM and CAD risk factors with coronary artery stenosis in Thai women.
Methods: A total of 135 Thai females at the age of 61.8+8.2 years were recruited in the study. Based on coronary angiography, the subjects were classified into 3 groups; 75 females without a significant stenosis vessel, 18 females with a single stenosis vessel and 42 females with multiple stenosis vessels. Levels of sTM were measured by using an enzyme-linked immunosorbent assay (ELISA) technique.
Results: Plasma sTM levels among 3 groups of subjects were significantly different (3.1±1.3, 3.3±1.6 and 3.7±1.6 ng/ml, respectively, p=0.041). In addition, the significant differences of plasma HDL-C concentrations were found among those subject groups (48.3+14.5, 42.6+10.2 and 38.4+10.1 mg/dl, respectively, p< 0.001). Inverse correlation between the levels of plasma sTM and HDL-C was demonstrated in this population (r=-0.254, p=0.003). Binary logistic regression analysis revealed that high sTM levels and low HDL-C concentration were independently associated with vascular stenosis [adjusted OR (95% CI) = 3.2 (1.0, 10.0) and 2.5 (1.2, 5.2) respectively].
Conclusions: These findings suggested that raised plasma sTM concentration released from endothelial damage during the plaque progression as well as low levels of HDL-C which involved in disposal of blood cholesterol were likely to be used for predicting coronary stenosis.

PB 022 | Protective Auto Antibodies and their Inverse Correlation with Antigen Levels in Indian Patients with Acute Coronary Syndrome

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Background: Auto antigens and their antibodies in peripheral circulation have been shown to be associated with atherosclerosis. Several studies support the dual activity of these auto antibodies either playing a causative or protective role in atherosclerosis. Sudden cardiac death is highly prevalent in Indian subcontinent.
Aims: To assess level of circulating athergenic autoantigens and auto antibodies in acute coronary syndrome patients from India.
Methods: Peripheral blood was collected and plasma was isolated from patients (N= 195) admitted after myocardial infarction and equal number of age and gender matched controls without any prior history of cardiac disease. IgG and IgM antibodies for APO B 100, HSP 60, Ox-LDL and LDL were assessed using ELISA. All the plate carried a common control for normalizing the titre. The level antigens in circulation for HSP 60 and Ox-LDL were measured following manufacture guidelines.
Results: The auto antibodies were found to be less in circulation in MI patients for APO B 100 ((IgG (1.87±0.02 vs 1.62±0.02, p< 0.001) and IgM (0.68±0.02 vs 0.57±0.03, p< 0.01)), HSP 60 ((IgG (2.12±0.01 vs 1.91±0.02, p< 0.01) and IgM (0.79±0.02 vs 0.68±0.02, p< 0.01)), Ox-LDL ((IgG (0.96±0.02 vs 0.88±0.02, p< 0.05) and IgM (0.41±0.02 vs 0.34±0.02, p< 0.001)) and LDL ((IgG (1.63±0.01 vs 1.46±0.02, p< 0.001) and IgM (0.63±0.02 vs 0.55±0.02, p< 0.01)) compared to the controls. The level of HSP 60 antigen (34.63±3.66 vs 48.54±3.52, p=0.01) and their ratio to their respective antibody (16.8±2.04 vs 26.67±2.53, p< 0.01) was high in patients compared to controls. There was a negative correlation between IgM auto antibodies to Ox-LDL (r=-0.11, p=0.04), LDL (r=-0.15, p< 0.01) and number of diseased vessels.
Conclusions: The study suggests that auto antibodies may play a protective role in our population by antigen removal by auto antibodies in circulation there by reducing the antigen load.

PB 024 | Role of RhoA/Rho Kinase Pathway Activation in Cocaine-induced Leukocyte Transendothelial Migration

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Background: Cocaine use increases the risk for serious cardiac and cerebrovascular events. The pathogenic mechanisms are not fully understood although accelerated atherosclerosis and thrombus formation are prominent findings. Leukocyte transendothelial migration (TEM) is one of the crucial steps in the development of atherosclerosis (AE) and, RhoA/Rho kinase (ROCK) pathway plays a key role in pathogenesis of AE. We hypothesized that cocaine enhances leukocyte TEM associated to activation of endothelial ROCK.

Aims: To assess the effect of cocaine on leukocyte TEM and the contribution of ROCK activation to this phenomenon.

Methods: In cultured Human Aortic Endothelial Cells (HAEC) exposed to cocaine (10μM) or vehicle, ROCK activity was determined by western blot (phosphorylated versus total myosin light chain phosphatase 1 levels) and, E and P-selectin exposure by immunofluorescence. For TEM assays, HAEC were grown onto transwell filters and co-cultured with leukocytes for 90 minutes. Migrated leukocytes were quantified in the lower chamber and the cells on the grids were stained for VE-cadherin and CD45. All the experiments were conducted in the presence or absence of Y-27632 (10 μM), which targets the catalytic domain of ROCK.

Results: HAEC exposed to cocaine expressed significantly higher amount of E- and P-selectin and showed increased activation of ROCK (p: 0.038). These phenomena were inhibited by Y-27632 (p: < 0.05). Intercellular gaps and VE-cadherin junction dissociation were observed in HAEC treated with cocaine which was associated with a 2.6-fold increase in leukocyte TEM. Treatment with Y-27632 preserved the monolayer architecture and intercellular VE-cadherin localization and reduced dramatically the leukocyte TEM (p: 0.004).

Conclusions: We showed that activation of RhoA/ROCK pathway plays a key role in cocaine-induced leukocyte TEM. Its inhibition (e.g. with statins) may provide a novel target in the management of cocaine-associated vascular complications.

PB 025 | High Circulatory Level of Inflammatory Cells and their Relation to Recurrent Cardiac Events tn Indian Population

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Background: Inflammatory and regulatory response play key role in immune regulation. We reported imbalance between peripheral inflammatory and regulatory subsets in myocardial infarction (MI) in our previous study.

Aims: To study the imbalance between the inflammatory and regulatory subsets in patients with MI, clinically recovered MI, patients with recurrent cardiac event and patients with history of CAD.

Methods: Peripheral blood was collected from the patients admitted after first MI, clinically recovered patients 6-18 months after the first event, patients with history of CAD, patients with recurrent event, age and gender matched controls without any prior history to CAD. All the patients were followed up for 2 years. The inflammatory (Th17 and Tc17) and regulatory Tcells ($CD4^+CD25^{High}$ and Tregs) were assessed by flowcytometry. Statistical analysis was performed in SPSS version 17.0.

Results: Immune imbalance observed in MI was recovered in clinically recovered MI with reduced peripheral inflammatory cells Th17 (4.45±0.36 vs 3.09±0.37, p< 0.01), increased regulatory subsets $CD4^+CD25^{High}$ (3.69±0.45 vs 5.30±0.33, p< 0.01), Tregs (3.07±0.47 vs 4.23±0.43, p< 0.05) and decrease in inflammatory to regulatory Tcell ratio (Th17/Tregs (1.71±0.16 vs 0.784±0.06, p< 0.01 and Th17/$CD4^+CD25^{High}$ (1.43±0.13 vs 0.604±0.04, p< 0.01)) compared to their first sample. The Th17 cells was high in the patients with the history of CAD (4.45±0.36 vs 5.22±0.22, p< 0.01) and patients who had incident of recurrent cardiac event later (4.45±0.36 vs 4.76±0.49, p=0.67) compared to MI patients without any previous history of CAD or future events during follow up.

Conclusions: Our results suggest that immune imbalance could be one of the causative for MI and high circulatory level of inflammatory cells could be a cause of recurrent cardiac events.

PB 026 | Lipoprotein a [Lp(a)] and Hemostatic Variables in Young and Post Menopausal Women: Are their Levels Affected by Hormones Use?

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Background: Lipoprotein (a) [Lp(a)] is a risk factor for atherogenesis and due to its structural homology with plasminogen may contribute to thrombogenesis by reducing fibrinolysis. Use of oral contraceptives (OC) and hormone replacement therapy (HRT) may favor hypercoagulability and, consequently, add clinical significance to elevated levels of Lp (a).

Aims: To evaluate the lipid profile focused on Lp (a) and some hemostatic variables and investigate whether the use of hormones can affect their plasma levels in young (OC) and postmenopausal women (HRT).

Methods: A total of 82 women were distributed into four groups: young people in use of OC (YP n = 20), or not (YN n = 20) and menopausal people in use of HRT (MP n = 17) or not (MN n = 25). Levels of Lp(a) were determined by turbidimetry while the lipidic profile for conventional methods. Plasma levels of plasminogen activator inhibitor type 1 (PAI-1), 1+2 prothrombin fragment (F1+2) and plasminogen were determined by ELISA and D-Dimer (D-Di) by ELFA. Mann-Whitney test was used to compare the medians of the pairs of groups: YNxYP, MNxMP and YNxMN. Informed consent was obtained from all partipants.

Results: Lp(a) levels were not significantly different among all groups. Increased levels of TC, F+2 and DDi were observed in OC users while no change in lipidic and hemostatic profiles was found, except reduction in TC and LDL levels in HRT users. Comparative analysis between YN x MN showed increased levels of TC, LDL, PAI-1, F1+2 and DDi, and reduced levels of HDL (Tables 1 and 2).

TABLE 1 Hemostatic variables (median and 25th and 75th percentile). PAI-1 in ng/mL; F1+2 in pmol/L; D-Di in ng/mL; Plasminogen in µg/mL

Group (n)	PAI-1	P -value	F1+2	P -value	D-Di	P -value
YN (20)	33.55 (17.05-42.33)	0.3438	218.30 (176.40-278.00)	0.0009	156.10 (128.80-282.60)	0.0679
YP (20)	21.90 (15.95-37.88)		385.50 (283.80-628.40)		234.60 (188.70-311.80)	
MN (25)	50.00 (32.70-75.5)	0.5053	378.70 (285.60-517.00)	0.6631	328.80 (225.40-510.50)	0.442
MP (17)	61.20 (38.65-68.60)		374.80 (308.10-458.80)		278.60 (208.20-339.50)	
YN (20)	33.55 (17.05-42.33)	0.0136	218.30 (176.40-278.00)	0.0001	156.10 (128.80-282.60)	0.0004
MN (25)	50.00 (32.70-75.65)		378.70 (285.60-517.00)		328.80 (225.40-510.50)	

TABLE 2 Lipid variables (median and the 25th and 75th percentile). Total cholesterol (TC), High density lipoprotein (HDL), and Lipoprotein low density

Group (n)	TC	P -value	HDL	P -value	LDL	P -value	Lp(a)	P -value
YN (20)	156.00 (134.25-172.75)	0.0144	67.00 (49.03-71.00)	0.1556	84.00 (52.70-97.05)	0.457	21.95 (9.40-51.97)	0.9892
YP (20)	173.00 (162.00-192.75)		72.50 (60.10-79.75)		84.80 (75.05-100.85)		19.05 (8.85-42.58)	
MN (25)	202.00 (175.50-245.50)	0.0416	47.20 (38.25-56.30)	0.3496	131.50 (108.70-148.0)	0.0083	22.10 (10.95-66.05)	0.1956
MP (17)	177.00 (161.50-237.50)		51.60 (37.75-66.00)		95.80 (87.80-128.70)		12.70 (3.60-52.05)	
YN (20)	156.00 (134.25-172.75)	0.0001	67.00 (49.03-71.00)	0.0309	84.00 (52.70-97.05)	0.0001	21.95 (9.40-51.97)	0.4932
MN (25)	202.00 (175.50-245.50)		47.20 (38.25-56.30)		131.50 (108.70-148.40)		22.10 (10.95-66.05)	

Conclusions: Use of hormones or aging did not affect the levels of Lp(a). However, use of oral contraceptive (OC) seems to be atherogenic and thrombogenic, while the use of HRT seems to protect against atherogenesis but did not significantly influence thrombogenesis. Aging by itself contributed significantly to the atherogenic and thrombogenic potentials, as expected. Supported by CAPES, CNPq and FAPEMIG - BRAZIL.

PB 027 | Selected Factors of Haemostasis and Some Clinical Parameters in Patients with Peripheral Arterial Disease with Regard to Atherosclerosis

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Background: A hypercoagulable state is found in the blood of patients with atherosclerosis leading to thrombotic events such as cardiac infarction, cerebral stroke or peripheral arterial disease.

Aims: The objective of this study was the evaluation of selected haemostasis factors depending on the clinical parameters in the patients with symptomatic PAD.

Methods: The study group consisted of 80 patients with symptomatic PAD (M/F 53/27) at an average age of 63.5 ± 9 years, and the control group members were healthy volunteers (M/F 20/10) at an average age of 56 ± 6 years. The testing involved determining the concentrations of tissue factor (TF), tissue plasminogen activator antigen (t-PA Ag), type-1 tissue plasminogen activator inhibitor (PAI-1 Ag), fibrinogen, D-dimers and platelet count (PLT) in citrated venous blood. The obtained results were subjected to analysis depending on the age of the patients, the levels of ABI, IC distance, BMI levels, the concentration of low-density lipoproteins (LDL) and triglycerides (TG), in smokers with regard to the period of cigarette smoking (the number of pack-years).

Results: Increased concentrations of all the haemostatic parameters were fund in the blood of the patients involved in the study. There

was observed a positive correlation between D-dimers levels and the age, a negative correlation was revealed between D-dimers levels and ABI value, a negative correlation was between the PLT count and ABI value, a negative correlation was between fibrinogen concentration and IC distance, a negative correlation was observed between PLT and IC and a positive correlation was between t-PA Ag and BMI as well as between PAI-1 Ag and BMI.

Conclusions: A shortened intermittent claudication distance and decreased ankle-brachial index value (PAD progression indices) correlated positively with increased fibrinogen concentration level and platelet count and D-dimers concentration which suggested the coexistence of hypercoagulability and secondary fibrinolysis and inflammatory process.

PB 028 | MicroRNA Target Prioritization to Identify Potential Therapeutic Candidate in Cardiovascular Disease

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Background: Micro RNA profiling can often reveal several hundreds of genes getting regulated for a particular biological system of interest. Multiple algorithm based target prediction tools are now available that ranks the potential targets on the basis of the complimentarity and the degree of conservation between mi RNA and 3'UTR target sequence across species. However, in cases where the number of target genes of interest is in the order of hundreds and thousands, a gene by-gene approach becomes impractical.

Aims: To prioritize potential micro RNAs at each stage of cardiovascular disease development and to explore the therapeutic potential of these miRNAs in treatment of cardiovascular disease.

Methods: Atherosclerosis was induced in groups of Apob^{tm2Sgy} Ldlr^{tm1Her}/J mice by feeding them with diet rich in cholesterol. Micro RNA profiling was performed with RNA extracted from ascending aorta at 6, 18 and 30-week time points. The disease specific correlation of our data and parent ontology ranking helped us to identify potential mirnas at each stage of cardiovascular disease development.

Results: We demonstrated a novel algorithm based approach (miRrank) which ranks the targets on the basis of functional ontology. Totally, 25 miRNAs were enriched across the disease development and the expression levels were experimentally verified using mice aorta samples. According to the AHA drug classification and cytoscape analysis we observed that the targets of mmu-mir-497-5p and mmu-mir-185-5p belong to calcium channel blocker category and these mirnas potentially be used as a therapeutic candidate in treatment of cardiovascular disease. Thus, calcium channel blockers play a very essential role to control the entry of calcium ions into the cells.

Conclusions: We believe that our method will significantly add biological value to the existing tools and identify bio markers with potential use in therapy as shown above.

PB 029 | Plasminogen Deficiency Attenuates Atherosclerosis in a Murine Model of Type II a Familial Hypercholesterolemia

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Background: Due to atherosclerosis is asymptomatic nature, it is difficult to investigate the progression of this disease in humans. Animals, especially mice with select genetic alterations, are very useful to investigate this disease. Mice with a double deficiency of LDLr and Apobec1 (*Ldlr$^{-/-}$/Apobec1$^{-/-}$*) show high levels of plasma total cholesterol on a normal chow diet, most of which is distributed in the LDL fraction. Consequently, spontaneous atherosclerotic plaques form in the aorta, a situation that is similar to human familial hypercholesterolemia.

Aims: Many studies have defined a relationship between atherosclerosis and the fibrinolytic system. So, we examined the role of plasminogen in atherosclerosis with *Ldlr$^{-/-}$/Apobec1$^{-/-}$* mice.

Methods: We established *Ldlr$^{-/-}$/Apobec1$^{-/-}$/Plasminogen$^{-/-}$*(*Ldlr$^{-/-}$/Apobec1$^{-/-}$/Plg$^{-/-}$*) triple deficient mice from *Ldlr$^{-/-}$/Apobec1$^{-/-}$* mice and *Plg$^{-/-}$* mice. In triple-deficient mice, we investigated the levels of total cholesterol (total-C), LDL-C and HDL-C and the size of plaques in the aortic sinus.

Results: We have found that total cholesterol levels were significantly higher in *Ldlr$^{-/-}$/Apobec1$^{-/-}$/Plg$^{-/-}$* mice than in *Ldlr$^{-/-}$/Apobec1$^{-/-}$* mice. Furthermore, almost all of the cholesterol accumulated in the LDL fraction, and the HDL cholesterol level was not different between both groups. Although the cholesterol levels are very high in this murine model, these mice showed much smaller plaques in the aortic sinus. These results suggest that a Plg deficiency in a *Ldlr$^{-/-}$/Apobec1$^{-/-}$* background might restrict uptake of oxidized (Ox)-LDL in monocyte/macrophages as well as other cell types.

Conclusions: In the *Ldlr$^{-/-}$* background, not only Ox-LDL but native LDL should also be cleared by scavenger receptors. Hence, we examined continuously whether Plg affects the expression of various scavenger receptors in macrophage of *Ldlr$^{-/-}$/Apobec1$^{-/-}$* mice in order to reveal important roles of Plg in atherosclerosis in a model of familial hypercholesterolemia.

PB 030 | Integrated microRNA and mRNA Expression Profiling to Identify mRNA Targets of Dysregulated miRNAs in Coronary Artery Disease Condition

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Background: The interplay between miRNA and their mRNA has been proposed to play an important role in biological and pathological process of many diseases. We have constructed and explored the interactive molecular networks impacted by predicted mRNA targets of differentially expressed miRNAs in patients with coronary artery disease.

Aims: To identify mRNA targets of dysregulated miRNAs through the integrated analysis of miRNA and mRNA expression profiling in patients with and without Coronary artery disease.

Methods: The miRNA and mRNA expression profiling was carried out on 20 subjects, Including 10 CAD patients and 10 healthy controls on the Agilent platform. Data was analysed with Gene SpringGx12.5 and integrated network analysis using Ingenuity Pathway Analysis (IPA. The expression pairing was applied to identify the anti-correlated miRNA-mRNA changes. The connections with in the IPA interaction network were visualised and analysed based on their topological parameters in Cytoscape.

Results: The integrated analysis of miRNA and mRNA expression data identified 6 miRNAs that were up regulated and one miRNA that was down regulated. We identified RUNX1as key target based on anti-correlated miRNA-mRNA interaction. The core analysis results revealed dis-regulation of key pathways, such as Cytokine signaling, Cell cycle regulation and Circadian rhythm. The interaction network analysis based on the topological parameters confirmed the top hub miRNA node in the network to play a key role in regulation RUNX1.

Conclusions: Using this integrated approach, we identified RUNX1 a novel target gene of dysregulated miRNAs, that could have potential role in cardiovascular disease development.

PB 032 | Predictive Value of Neutrophil to Lymphocyte Ratio in Long-term Outcomes of Left Main and/or Three-Vessel Disease in Patients with Acute Myocardial Infarction

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Background: Patients with acute coronary syndrome due to left main and/or three-vessel disease (LM/3VD) are at the highest risk of adverse cardiovascular events. Neutrophil to lymphocyte ratio (NLR) has been proposed as a marker of cardiovascular risk.

Aims: We sought to evaluate the independent predictive value of NLR for LM/3VD in AMI patients.

Methods: Patients (n=806) admitted with LM/3VD in AMI and who underwent PCI between January 2013 and December 2013 were followed up for 2 years. NLR was calculated as the ratio of neutrophil to lymphocyte based on the laboratory data on admission. We used Cox regression models to examine the relation between NLR and clinical outcomes.

Results: In this cohort, median NLR in the entire study population was 2.60 (interquartile range, 1.86-4.04). By receiver operating characteristics curve analysis, the optimal cut-off value of admission NLR to predict 2-year all-cause mortality was 3.39 (area under the curve 0.753, sensitivity 77%, specificity 70%). NLR was divided into two sub-groups based on an optimal cut off value of 3.39. Of the 806 patients, 255 patients (31.6%) had NLR≥3.39. The high NLR group had higher prevalence of prior myocardial infarction, prior PCI and Intra-aortic balloon pump (IABP). During the follow-up period, the high NLR group was associated with a significantly higher rate of long-term all-cause death (6.7% vs 0.9%, p< 0.001), cardiac death (5.5% vs 0.9%, p< 0.001) and MACCE (24.7% vs 15.8%, p=0.002) compared to the low NLR group. In multivariate analysis, after adjusting for risk factors, NLR≥3.39 was determined as an independent predictor of 2-year all-cause mortality (hazard ratio[HR] 3.08, 95% confidence interval [CI] 1.06 to 8.97, p=0.039) and MACCE (hazard ratio 1.44, 95% CI 1.01 to 2.05, p=0.046) for LM/3VD. The NLR was significantly and positively correlated with hsCRP levels (r=0.231, p< 0.001).

Conclusions: Our study demonstrates that admission NLR≥3.39 is an independent predictor of LM/3VD in patients with AMI after PCI.

PB 034 | Myocardial Infarction is a Risk Factor for Venous Thromboembolism (VTE) Recurrence in Women with a First VTE

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Background: Previous reports suggest that myocardial infarction (MI) is a transient risk factor for incident venous thromboembolism (VTE). Whether patients with a history of MI and VTE have an increased risk of VTE recurrence (reVTE) remains unsettled.

Aims: To assess whether a history of MI is associated with risk of reVTE in a cohort of patients with incident VTE.

Methods: A total of 5494 VTE patients were recruited from the Tromsø Study and the MEGA study and followed from the date of their first VTE until the date of recurrence, death, or the last date they were known to be recurrence free. Cox regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) of reVTE according to MI status adjusted for age and body mass index, and stratified by sex since men have a higher recurrence risk than women.

Results: During a median follow-up of 11.3 years, 792 patients experienced a reVTE. Women with a history of MI before the first VTE

had a 2.4-fold higher risk of reVTE (HR 2.44; 95%CI 1.27-4.69) than those without a history of MI. No association was observed in men (HR 1.20, 95%CI 0.71-1.94). When competing risk by death was taken into account, the corresponding subdistribution hazard ratios were 2.14 (95%CI 1.08-4.24) in women, and 0.99 (95%CI 0.60-1.66) in men. Similar risk estimates were observed when MIs that occurred during follow-up were modeled as intermediate exposures using a time-varying Cox-model.

Conclusions: Women with a history of MI had an increased risk of recurrence after a first VTE. Although the risk of a first VTE is highest during the first months following an MI, our findings suggests that MI is a persistent rather than transient risk factor for venous thrombosis, especially in women.

PB 035 | Lack of Tissue Factor Cytoplasmic Tail Attenuates Cardiac Injury after Myocardial Infarction

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Background: The interplay of immune cells has an important role in driving both acute inflammatory as well as regenerative responses post myocardial infarction (MI). The phosphorylation of tissue factor (TF) at its cytoplasmic tail (CT) influences inflammatory response, angiogenesis and tumor development. However, the role of TF CT in infarct healing and cardiac remodeling is still unknown.

Aims: The main aim of this study was to elucidate whether, phosphorylation of CT of TF affects infarct healing and remodelling.

Methods: LAD ligation is an established method to study post-MI tri-phasic immune cell response. MI was induced in 9 to 12 weeks old male C57BL/6J and mice specifically lacking CT of TF ($TF^{\Delta ct}$) by permanent ligation of the left anterior descending (LAD) coronary artery. We assessed left ventricular function at specific time points (1,7,14,21,28 days) after MI by high frequency ultrasound. Infiltration of immune cells was investigated by performing flow cytometry analysis after enzymatic digestion of the myocardium.

Results: Determination of the ejection fraction by ultrasound showed that $TF^{\Delta ct}$ had preserved left ventricular function compared to C57BL/6 controls at d1, d7, and d21 post MI, respectively. Flow cytometric analysis revealed a significant influx of $CD45.2^+$ leukocytes as well as $CD45.2^+$ /$CD3^-$/$CD11b^+$/$Ly\text{-}6G^+$ neutrophils into infarcted areas at d1 post MI in C57BL/6, which was augmented in $TF^{\Delta ct}$ (n=7-8, $p< 0.05$). Interestingly, initially increased influx was followed by dampened inflammatory response at d7 post MI in $TF^{\Delta ct}$ compared to C57BL/6 controls (n=8-9, $p< 0.01$). Importantly, post infarct survival after 28d post MI was significantly higher in $TF^{\Delta ct}$ compared to B6J mice (n=17, $p< 0.05$).

Conclusions: We show here that deletion of the cytoplasmic tail of TF is protective in acute as well as chronic MI by preserving LV function and increasing survival. In subsequent studies we will investigate whether phosphorylation of TF-CT in inflammatory cells is one of the key mechanisms in cardiac injury.

PB 036 | Free Ubiquitin Promotes Cardiomyocytes Proliferation and Survival In vitro and In vivo via CXCR4 Pathway

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Background: Ubiquitin (UB) is one of the most highly conserved low molecular weight (8.5 kDa) protein. Supplementation of free ubiquitin (extracellular ubiquitin) has been shown to limit inflammation and attenuate tissue injury in several diseases. However, the biological effect of ubiquitin on cardiomyocyte (CM) has not been defined.

Aims: To examine the ability of free ubiquitin to promote CM proliferation and enhance cardiac function after MI.

Methods: In the present study, we investigate the effect of free UB on the proliferation and apoptosis of cardiomyocytes under hypoxia and the potential relationship of free UB/CXCR4 to Akt, YAP pathways. Mouse models of acute myocardial infaction (AMI) were established. Free UB was injected into the myocardium. Cardiomyocyte apoptosis, infarct size and cardiac function were analyzed.

Results: Improved CM survival was found in ubiquitin treatment compared with controls after hypoxia. Knock-down of ubiquitin by mixed shRNAs targeting its coding genes ubiquitin B (*UBB*) and ubiquitin C (*UBC*) reduced the growth of CM by inducing apoptosis and modulating the cell cycle progression. Moreover, injection of free ubiquitin after MI significantly improved survival, cardiac function, and reduced infarct area 4 weeks after MI. Expression of proliferation activator (cyclin D1, E1, p-AKT) were increased and negative cell-cycle and apoptotic regulators (p21, caspase-3) were decreased under hypoxia and MI conditions. Ubiquitin activates various proliferation pathways, including Akt and YAP through ubiquitin/CXCR4 axis.

Conclusions: Our results demonstrate the critical role of ubiquitin in CM proliferation and survival after MI. Targeting ubiquitin may serve as a potentially novel approach for MI prevention and therapy.

PB 037 | Development of a Novel Flow Cytometric Immunobead Array to Quantify von Willebrand Factor Antigen and Ristocetin Cofactor Activity and its Application in Acute Myocardial Infarction

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Background: Both von Willebrand disease (VWD) and acute myocardial infarction (AMI) generate quantitative and qualitative changes of von Willebrand factor (VWF).

Aims: To develop a rapid, and precise flow cytometric immunobead array (FCIA) to quantify VWF antigen (VWF:Ag) and risrocetin cofactor activity (VWF:Rco), and to apply it in AMI.

Methods: Microbeads coated with anti-VWF mAb SZ29 were incubated with diluted plasma for VWF:Ag assay, and microbeads coated with anti-GP Ibα mAb SZ151 were bonded with recombinant fragment GPIbα, then incubated with diluted plasma and ristocetin simultaneously for VWF:Rco assay. Finally, both binding VWF microbeads were detected with FITC conjugated sheep-anti-human VWF IgG antibody by flow cytometry, respectively. 21 VWD patients, 105 controls (CTL) and 146 AMI patients were analyzed. VWF:Ag and VWF:Rco were measured by FCIA l. VWF:CB was tested by ELISA. ADAMTS13 activity was measured by FRETS-VWF73. VWF multimer assay was implemented by SDS-PAGE.

Results: Both VWF:Ag and VWF:Rco by FCIA had a good linear regression (R^2 = 0.9941 and R^2 = 0.9876). The intra-assay and inter-assay coefficient variations were 9.2% and 12.6% for VWF:Ag-FCIA, 7.7% and 13.5% for VWF:Rco-FCIA. The specificity and accuracy for VWF:Rco-FCIA (98.1% and 97.62%) were higher compared with VWF:Rco-ELISA (92.38% and 92.86%, P < 0.05). Plasma VWF:Ag, VWF:Rco and VWF:CB in AMI (mean ± SD, 299.4% ± 16.8, 242.0% ± 17.3 and 309.2% ± 19.9) were significantly higher than those in controls (73.5% ± 4.1, 65.5% ± 3.4 and 75.6% ± 6.0, all P < 0.0001), while ADAMTS13 and ADAMTS13/VWF:Ag in AMI (44.9% ± 0.8 and 0.25% ± 0.02) was significantly lower than those in controls (48.8% ± 1.2, P < 0.01 and 1.04% ± 0.10, P < 0.0001). The plasma ultra-large VWF level was dramatically increased in AMI.

Conclusions: We have developed a novel FCIA quantifying VWF:Ag and VWF:Rco quickly with specific and accurate results. The FCIA can not only be utilized for screening VWD, but also has clinical value in monitoring AMI patients.

PB 039 | Post-infarct Survival and Cardiac Remodeling Depends on the Interplay of Neutrophils, Monocytes and Interferon Gamma in a Mouse Model of Myocardial Infarction

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Background: Myelomonocytic cells are involved in the initial injury as well as in the later healing mechanisms of myocardial infarction (MI). The exact interaction of inflammatory myeloid cells and prominent cytokines remains only partly understood.

Aims: The cardio protective or in part adverse role of lysozyme M positive ($LysM^+$) and granulocyte-receptor 1 positive ($Gr\text{-}1^+$) immune cells on cardiac injury and healing in a murine model of MI should be investigated.

Methods: MI was induced in 8 to 12 week-old male mice (C57BL/6 background) by permanent ligation of the left anterior descending coronary artery (LAD).

Results: Compared to LysMCre controls, $LysM^+$ cell depleted $LysM^{iDTR}$ transgenic mice (depletion 3d prior MI by diphtheria toxin application, 25 ng/g body weight) displayed a reduced influx of $Gr\text{-}1^{high}$ neutrophils into infarcted myocardium 1d post MI (measured by FACS). Additionally, cardiac mRNA expression levels of the cytokines interferon gamma and tumor necrosis factor alpha were decreased 7d post MI. Mortality after MI was significantly increased in LysM-depleted mice within 28d post MI. To estimate the role of neutrophils, we depleted C57BL/6 mice with a monoclonal anti-Gr-1 antibody and found increased mortality early after MI as well as a decrease in INFg mRNA expression. MCP-1 and CCR2 mRNA were decreased 3d after MI according to reduced amount of $Ly6C^{high}$ inflammatory monocytes in the infarcted myocardium of anti-Gr-1 treated mice. LAD ligated $INFg^{-/-}$ mice and $TNF\alpha^{-/-}$ mice displayed a significantly decreased post-infarct survival, worsening of left ventricular function and an impaired inflammatory cell infiltration.

Conclusions: We provide evidence that neutrophils, monocytes and INFg play an essential role in survival and cardiac remodeling following MI. Our data indicate that neutrophils are required for monocyte chemotaxis. We argue that therapeutic strategies to oppose the inflammatory injury in MI must consider a potentially beneficial effect of early neutrophil influx into infarcted myocardium.

PB 040 | Control of Thrombosis and Inflammation by Platelet p38α in Acute Myocardial Infarction

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Background: Mitogen-activated protein kinases (MAPKs), especially P38 play detrimental roles in cardiac hypertrophy, atherosclerosis, vascular restenosis and cardiac remodeling post-myocardial infarction (MI), which have established the foundation of recent P38 inhibitors cardioprotection clinical trial activities.

Aims: To study the activation and function of MAPKs in coronary thrombosis process in vivo and its relationship with clinical outcomes.

Methods: The relative phosphorylation levels of MAPKs in human platelets between STEMI patients and healthy subjects were assayed and analyzed by GraphPad prism. A LAD coronary artery ligation MI model was carried out to elucidate the roles of P38 in distal microvascular embolization, inflammatory response, ventricular remodeling and cardiac function post MI.

Results: The results indicated that MAPKs, especially P38 were highly and frequently phosphorylated in the STEMI patients platelets, and the phosphorylation levels of P38 in platelet was probably correlated

to no-reflow. Further results showed that P38α was the major isoform expressing in human and mouse platelets. Platelet specific P38α deficient mice presented impaired ability of thrombosis and homeostasis, but had improved cardiac function, smaller infarct size, decreased inflammatory response and microthrombus in a left anterior descending artery ligation mouse model. Signaling analysis revealed that P38 activation was one of the earliest events in platelet under the treatment of receptor agonists or hydrogen peroxide. P38α/MK2/HSP27 and P38α/cPLA2 were major pathways in regulation of platelet activation receptors-mediatedor reactive oxygen species (ROS)-induced in ischemic environment. Moreover, the distinct roles of ERK1/2 in receptors- or ROS-induced P38 activation were elucidated.

Conclusions: P38α acts as a critical regulator of platelet activation in response to injured endothelium and ischemic environment. Inhibition of platelet P38α may improve clinical outcomes in subjects with acute STEMI.

PB 041 | Identification of a Plasma microRNAs Profile Related to Arterial Thrombosis

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Background: MicroRNAs (miRNAs) regulate protein expression and are present in biological fluids. Circulating microRNAs (miRNAs) have been described as promising and non-invasive clinical biomarkers for cardiovascular diseases.

Aims: To validate a miRNAs plasma profile characteristic of patients with chronic ischemic heart disease (MI).

Methods: Optimized method for plasma miRNA isolation was used. We measured miRNA expression with the GeneChip miRNA 3.0 Array (Affymetrix) in plasma from 6 stable patients with a history of MI and 6 healthy subjects, age and sex matched. The results were analyzed with the PARTEK Genomic Suite software. The array showed a different miRNA expression profile between MI and controls. We selected 15 miRNAs differently expressed (P< 0.05; fold-change>1.5) for validation by RT-qPCR in plasma from 30 stable MI and 37 controls, age and sex matched. The results were analyzed using an elastic net penalized logistic regression model (R version 3.3.1).

Results: Statistical analysis of the RT-qPCR validation results showed that there is a list of 6 miRNAs able to distinguishing between patients and control [fold-changes ranging from -1.75 to 3.68]. We obtained a good prediction of the risk of MI applying a predictive model using these 6 miRNAs, achieving an area under the ROC curve (AUC) = 0.81 [95% CI=0.70-0.92; *P*< 0.001]. In addition, computational prediction of miRNA targets (miRWalk) on the 3´UTR region showed that they may interact with proteins involved in coagulation and complement, endothelium, and lipid transport or metabolism pathways (*P*< 0.05).

Conclusions: This study identifies a plasma miRNA profile characteristic of MI with good prediction of the risk of MI in our patients. Although these results need to be replicated in larger series, this miRNA profile may potentially be useful for MI discrimination. ISCIII-FEDER (PI14/00512; PI12/00027; RD12/0042/0029; PI14/00079; FI14/00269; CPII15/00002), GVA (PROMETEOII/2015/017), IIS La Fe.

PB 042 | Platelet Serotonin Aggravates Myocardial Reperfusion Injury via Degranulation

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Background: Inflammation during reperfusion injury after acute myocardial infarction (MI) comes with a burst of neutrophils migration. The peripheral hormone serotonin is synthesized by tryptophan hydroxylase 1 (TPH1) and mediates neutrophil recruitment during acute phase inflammation.

Aims: To assess the contribution of platelet derived serotonin on inflammation during myocardial reperfusion injury.

Methods: MI was induced for 30 minutes, followed by 24 hours of reperfusion. Heart function and infarct size was evaluated. Heart tissue was analyzed for cytokine expression and migrated inflammatory cells. Integrins were analyzed on circulating cells using flow cytometry. *Ex vivo* heart function was analyzed using the isolated working heart assay.

Results: Serotonin peaked 24 hours after MI in WT mice (150 ng/mL) and reached normal levels after 2 days (90 ng/mL). Heart function in SSRI treated and *Tph1*$^{-/-}$ mice compared to WT was improved and infarct size was reduced (40 in SSRI; 35 in *Tph1*$^{-/-}$; 53 in WT; % area at risk (AAR)). This effect was absent *ex vivo*. WT mice revealed increased MPO levels in the heart and neutrophil content in the AAR was reduced in *Tph1*$^{-/-}$ mice (14 vs. 28 in WT per mm^2 tissue). Depletion of neutrophils reduced infarct size to 37 %AAR in WT mice. Neutrophils had decreased expression of CD11b in SSRI (70%) treated and *Tph1*$^{-/-}$ mice (30%) compared to WT. *In vitro* stimulation with serotonin induced degranulation of neutrophils and increased CD11b expression, which was reversable by addition of a protein transport inhibitor.
Human neutrophils revealed the same phenotype and ACS patients showed a correlation of plasma 5-HT and neutrophil CD11b (R^2=0,72).

Conclusions: Serotonin directly mediates neutrophil migration during myocardial reperfusion injury by inducing degranulation and subsequent upregulation of CD11b. Intervening in serotonin-neutrophil crosstalk might provide novel anti-thromboinflammatory treatment strategies.

PB 043 | Smoking and Fibrinogen Levels Modify the Risk of MI due to FXIII V34L: Results from the MAMI Study

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Background: Coagulation factor XIII (FXIII), after activation by thrombin cleavage in the final step of the coagulation cascade, participates in cross-linking fibrin to form a stable clot. The Leu34 form of the common FXIII V34L polymorphism (rs5985) accelerates the rate by which thrombin activates FXIII during coagulation.

Aims: To determine the risk of Myocardial Infarction (MI) due to FXIII V34L in combination with smoking status and fibrinogen levels.

Methods: Data on 394 cases and 465 controls was obtained through an interviewer-led questionnaire as part of the Maltese Acute Myocardial Infarction (MAMI) Study. FXIII activity (%) was measured using a microtitre assay with fibrinogen and 5-(biotinamido)pentylamine as substrates. Fibrinogen was quantified using the Clauss assay. FXIII V34L was genotyped by PCR-RFLP with Hhal. Differences between median FXIII activity were evaluated using the Mann Whitney and Kruskal-Wallis tests. Age and gender adjusted odds ratios (OR) with 95% confidence interval (CI) were determined.

Results: In controls, median FXIII activity was markedly higher in individuals with the FXIII Leu34 allele (V/V 76.1%, V/L 125.8% and L/L 180.3%; $P < 0.001$). FXIII activity was almost identical in cases (84.5%) and controls (88.5%; P=0.42) and FXIII V34L does not appear to change the risk of MI ($OR_{V/L}$ 1.3; 95% CI 0.6-2.5, $OR_{L/L}$1.3; 95% CI 0.9-1.7). However, in smokers, risk increases with increasing number of Leu34 alleles ($OR_{V/L}$ 4.2; 95% CI 2.4-7.6, $OR_{L/L}$ 5.0; 95% CI 1.2-21.0) when compared to wild-type non-smokers. Also, men in the highest fibrinogen tertile carrying one or two Leu34 alleles had an increased risk of MI (OR 2.2; 95% CI 1.1-4.4) when compared to wild-type men in the lowest fibrinogen tertile.

Conclusions: On its own, FXIII V34L does not appear to alter the risk of MI; but in the presence of the Leu34 allele, risk is increased >4-fold in smokers and doubles in men with fibrinogen levels in the highest tertile.

PB 044 | Levels of Beta-thromboglobulin and Platelet Factor 4 Are Different between NSTEMI and STEMI: Results from the MAMI Study

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Background: Platelet factor 4 (PF4) and Beta-Thromboglobulin (β-TG; NAP2) are both released from platelet α-granules during platelet activation. The simultaneous measurement of both proteins in plasma may be a useful marker of platelet activation.

Aims: To determine the association, if any, of these two platelet-specific proteins with ST elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI).

Methods: The Maltese Acute Myocardial Infarction (MAMI) collection is composed of 394 cases with a first myocardial infarction (MI) and 465 gender and frequency matched controls. Type of MI was determined from electrocardiogram (ECG). All participants completed an interviewer-led questionnaire. Platelet poor plasma obtained from citrated blood samples was stored at -80°C. PF4 and β-TG were quantified by ELISA. The Kruskall-Wallis test was used to determine differences in median levels whilst correlation between both proteins was assessed using Spearman's rho.

Results: Levels of PF4 and β-TG are correlated (r_s 0.92; $P< 0.01$). Both PF4 and β-TG levels were lower in men when compared to women (PF4: 117.17pg/ml *vs* 134.08pg/ml; $P< =0.05$; β-TG: 119.02pg/ml *vs* 145.87pg/ml; $P<0.01$). Both decreased slightly with age whilst smoking did not have an effect. Levels of PF4 and β-TG were lower in cases when compared to controls (PF4: 97.23pg/ml *vs* 121.41pg/ml;

$P< 0.01$; β-TG: 99.51pg/ml *vs* 127.81pg/ml; $P< 0.01$). Levels were higher in patients with NSTEMI compared to those with STEMI (PF4: 104.42pg/ml *vs* 89.76pg/ml; $P< 0.01$; β-TG: 104.09pg/ml *vs* 90.13pg/ml; $P< 0.05$).

Conclusions: Our findings were different when compared to other studies which concluded that PF4 and β-TG are higher in cases than controls. Higher levels in NSTEMI may suggest ongoing platelet activation which may result in increased risk of a second event.

PB 045 | CD41 Distribution within Platelet-Monocyte Complexes in Patients with Acute Myocardial Infarction

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Background: The amount of circulating platelet-monocyte complexes (PMCs) is an early marker of immunoactivation and atherothrombosis. The elevated level of PMC is detected in patients with acute coronary syndrome (ACS), although the composition of such complexes has never been revealed. Participation of platelet-derived extracellular vesicles (PEVs) in formation of PMCs may be of particular interest in ACS.

Aims: To compare the amount and composition of PMCs in the whole blood of healthy donors and patients with ACS and stable angina.

Methods: The study included 28 patients with acute myocardial infarction (MI), 21 patients with unstable angina (UA), 27 patients with stable coronary artery disease (SA) and 20 healthy volunteers. Patients with ACS received standard therapy, including aspirin and P2Y12 inhibitors and subjected to PCI. Whole venous blood was sampled at admission and immediately fixed with 0,25% formaldehyde. Then 30 µL of fixed blood was immunolabeled with CD45-eFluor450, CD14-PerCP Cy5.5 and CD41a-APC and subjected to flow cytometry. PMCs were defined by simultaneous expression of all three markers. Data are presented as mean±SEM or ±SD when applicable.

Results: The percent of monocytes complexed with platelets is elevated in MI patients, compared to the group of healthy volunteers 8,98±7,33% vs. 3,55±0,81% (p< 0,00001) (Fig1). Flow cytometry revealed three populations of PMCs based on CD41 expression (Fig2A). Percent of PMCs with low CD41 MFI (type 1) increases significantly between MI and Control groups (32,8±9,3% vs. 40,7±14,4%, p*=0,03), while PMC with medium CD41 expression (type 2) tend to decrease (67,2±9,9 vs. 58,9±13,3, p=0,05) (Fig 2B).

Conclusions: PMC level is elevated in MI patients. Based on CD41 expression PMCs form three populations with dominant type 2. In MI patients compared to Control group there is a significant increase of CD41 low (type 1) PMC population, which is presumably formed with platelet-derived vesicles, but the nature of these complexes needs to be further clarified.

PB 046 | Cardiomyocyte Extracellular Vesicles Increase after Experimental Myocardial Infarction and Promote Endothelial Dysfunction in vitro

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Background: In acute myocardial infarction (AMI), damaged cardiac cells produce extracellular vesicles (EVs) capable of transferring biological information from the cell or organ of origin. Likewise, EVs have been shown to predict future risk of cardiovascular events in some patients, although the dynamics of their release and clearance are still unclear.

Aims: to determine changes in EV numbers and cellular origin at different time points after myocardial infarction (MI) in WT mice, and to assess in vitro whether cardiomyocyte derived EVs modify endothelial function.

Methods: Mice were subjected to MI by the ligation of left-descending coronary artery. Platelet poor plasma (PPP) was obtained by double centrifugation at baseline, day 3, 15 and 30 post-MI. EV numbers and cell origin were measured in PPP by FACs using antibodies against CD41 (platelets), CD62E (endothelial cells), CD11b (leukocytes), TER119 (erythrocytes) and connexin-43 (cardiomyocytes). In vitro, endothelial function (proliferation, migration, tube formation) was determined after stimulation with cardiomyocyte (HL-1 line) derived EVs.

Results: EV numbers were similar at baseline (806±12 EVs/mL) and after MI (EVs/mL: 741±108 Day 3, 670±84 Day 15, 704±84 Day 30). No changes were observed in those derived from leukocytes, endothelial cells, platelets or erythrocytes. Cardiac EVs increased by 35 % early after infarction, remain high 15 days post-ischemia (216±16 EVs/mL, p=0.037 vs baseline) and start to decrease at day 30 (200±13 EVs/mL). In vitro, cardiomyocyte derived EVs were internalized by endothelial cells, induced their proliferation and migration, and impaired tube formation capacity.

Conclusions: Changes in circulating cardiac EVs after MI indicate a dynamic release of vesicles according to organ damage. Those heart-derived EVs could further promote endothelial dysfunction after myocardial infarction.

PB 047 | T Lymphocyte Microparticles Are the Major Determinant of Acute Coronary Syndrome

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Background: Microparticles (MPs) are vesicles released from various cell origins following activation or apoptosis. Cell activation and apoptosis occur during atherosclerosis development and progression. MPs have been considered as markers and contributors to the inflammatory process of atherosclerosis. Each type of MPs plays different role in the pathogenesis.

Aims: This study aimed to assess the distribution of MPs from various cell origins in patients with the occurrence of acute coronary syndrome (ACS)

Methods: Thirty two ACS patients and 30 age-matched control subjects were recruited in the study. Platelet free plasma was separated from citrated blood and MPs were measured by flow cytometry. Endothelial MPs (EMPs), platelet MPs (PMPs), monocyte MPs (MMPs), neutrophil MPs (NMPs) and T lymphocyte MPs (TMPs) were determined using specific monoclonal antibodies. Phosphatidylserine positive (PS+) and phosphatidylserine negative (PS-) MPs were also classified.

Results: Proportion of male gender, dyslipidemia and smoking as well as level of fasting blood sugar were significantly increased in ACS compared to control group. Patients with ACS revealed significantly

higher levels of EMPs, PMPs, TMPs, PS+EMPs, PS+PMPs, PS+MMPs, PS+NMPs, PS+TMPs, PS-PMPs and PS-TMPs than those in control subjects. Binary logistic regression analysis revealed the association of TMPs, PS+PMPs, PS+MMPs, PS+NMPs, PS+TMPs and PS-TMPs with ACS. However, after adjustment for male gender, dyslipidemia, smoking and fasting blood sugar, only the association of TMPs, PS+TMPs and PS-TMPs with ACS still existed [OR (95% CI) = 6.53 (1.39, 30.67)].
Conclusions: This study suggested that among MPs from various cell origins, TMPs were the major determinant of ACS.

PB 049 | Specificity of Various Haemostatic Markers in Young and Elderly Patients with Myocardial Infarction

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Background: Numerous haemostatic factors may induce formation of various pathogenetic substrates of atherothrombosis, further contributing to the occurrence of different forms of myocardial infarction in both young and elderly patients.
Aims: To determine the difference in the values of haemostatic markers (antithrombin, protein C, fibrinogen, FVII , FVIII , vWF, FX, FXII, homocysteine, PAI-1, CRP) in patients with myocardial infarction younger than 45 years and the group of patients with myocardial infarction older than 65 years.
Methods: In a prospective study, 143 patients were examined, i.e. 80 patients with myocardial infarction younger than 45 years and 63 patients with myocardial infarction older than 65 years.
Results: Patients with myocardial infarction of older age group compared with younger patients had statistically lower values of antithrombin (p = 0.000) (87.2±12.6 vs 105.5 ±12.4 %) and protein C (p = 0.001) (104.8±17.2 vs 117.9±18%) as well as statistically higher values of fibrinogen (p = 0.000) (5.0 ± 1.9 vs 3.8 ± 1.6 g / L) and statistically significant (p = 0.005) higher CRP concentration (32.8 ± 52.2 vs 15.0 ± 25.2 mg / L). Coagulation factor VII activity was statistically insignificantly higher (p = 0.061) in younger patients with myocardial infarction than in older patients (109.3 ± 21.8 vs 101 ± 22.8%).Compared with younger patients with myocardial infarction older patients had statistically significantly higher (p = 0.004) levels of FVIII activity (198.2 ± 92.4 vs 129.1 ± 89.8%) and statistically significantly higher (p = 0.000) values of vWF activity (183.7 ± 86.5 vs 111.9 ± 47.1%).
Conclusions: The obtained significant differences in haemostatic markers indicate their possible impact on myocardial infarction in young and elderly patients, though further research is still required to fully clarify them.

PB 050 | Soluble P-selectin Levels Are higher in Patients with NSTEMI vs STEMI

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Background: P-selectin is stored in the α-granules of platelets and the Weibel-Palade bodies of endothelial cells. Upon activation, it is redistributed to the plasma membrane where it participates in binding to its receptor, P-selectin glycoprotein ligand-1, present on the surface of activated leukocytes. Soluble P-selectin (sP-sel) is a cleaved form detected in plasma and is a marker of platelet activation and endothelial dysfunction.
Aims: To investigate factors that influence levels of sP-sel and to determine whether sP-sel levels are different between controls and cases with myocardial infarction (MI).
Methods: Data on 394 cases and 465 controls was obtained through an interviewer-led questionnaire as part of the Maltese Acute Myocardial Infarction (MAMI) Study. Levels of sP-sel were determined in citrated plasma using specific ELISA. Blood samples were collected from cases at least 6 months after MI. Hypercholesterolaemia and diabetic status were determined using self-reporting and biochemical measurements (lipid profile and HbA1c respectively). Differences between medians were evaluated using the Mann Whitney test.
Results: Amongst controls, median sP-sel levels were higher in men (28.4ng/ml) than in women (26.5ng/ml, $P< 0.01$). In women, sP-sel levels increased with age (< 40y=22.2ng/ml, ≥40y=27.2ng/ml, $P< 0.001$). Smoking also increased sP-sel levels (29.3ng/ml *vs* 27.4ng/ml, $P< 0.05$). Individuals with elevated HbA1c (above 6.5%) had increased median sP-sel levels when compared to non-diabetics (30.5ng/ml *vs* 27.4ng/ml, $P=0.06$), whilst cholesterol levels did not have an effect. sP-sel levels were higher in cases with non-ST elevation MI (NSTEMI) relative to cases with STEMI (29.4ng/ml *vs* 27.6ng/ml, $P< 0.01$).
Conclusions: Levels of sP-sel are affected by gender, smoking and age. sP-sel levels are higher in cases with NSTEMI where thrombi tend to be smaller, are fibrin-poor but contain numerous platelet aggregates.

PB 051 | On-treatment Platelet Reactivity in Peripheral and Coronary Blood in Patients Undergoing Primary PCI for ST-segment Elevation Myocardial Infarction (STEMI)

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Background: Dual oral antiplatelet therapy is widely used in the setting of primary percutaneous coronary intervention (p-PCI) for ST-segment

elevation myocardial infarction (STEMI). Platelet function analysis in these patients revealed a variable level of efficacy of these treatments.

Aims: Our goal was to evaluate the on-treatment platelet reactivity in peripheral and coronary blood in patients with STEMI.

Methods: One hundred and nine patients who consecutively underwent p-PCI at Cardiology Unit of Padua University Hospital from June 2014 to June 2015 were enrolled. Before the procedure, all patients received: unfractionated heparin; intravenous aspirin 250 mg; oral clopidogrel 600 mg or prasugrel 60 mg or ticagrelor 180 mg. Samples were collected from a peripheral vein and from the culprit coronary artery. Classic coagulation parameters and impedance aggregometry were measured.

Results: ASPI-test and ADP-test in peripheral blood (23±26 and 43±29 U, respectively) were higher than in coronary blood (17±23 and 39±29 U, respectively) but the differences weren't statistically significant (p >0.05). In peripheral blood, fourteen (13%) patients had ASPI-test above a predetermined (41 U) cut-off level and thirty-one (29%) patients had ADP-test above the cut-off value of 44 U. Considering clopidogrel, prasugrel and ticagrelor, the prevalence of patients above the ADP-test cut-off level was 23%, 31%, 28% respectively. Similar results were observed in coronary blood.

Conclusions: Patients undergoing p-PCI for STEMI showed an overall platelet function slightly lower in coronary than in peripheral blood. The clinical significance of peripheral and coronary on-aspirin/thienopyridines platelet reactivity remains to be clarified.

PB 052 | Ultrasound Elastography: A New Technique to Distinguish between Acute and Chronic Deep Vein Thrombosis

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Background: Ultrasound Elastography (UE) imaging is a novel ultrasound technique for relative quantification of tissue elasticity. Its applicability to venous thromboembolic events has not been established. In particular, it is not clear if this technique may be useful to determine the age of deep venous thrombosis (DVT).

Aims: The aim of this study was to assess the role of UE in distinguishing acute from chronic DVT.

Methods: Consecutive patients with unprovoked acute and chronic (≥ three month) DVT were analyzed with a 6-15 MHz matrix array linear probe during continuous freehand manual compression of the anatomic site. UE shows the spatial distribution of tissue elasticity properties in a Region of Interest (ROI) by estimating the strain before and after the distorsion caused by external and internal forces. The mean strain values of acute and chronic popliteal and femoral vein thrombosis were compared. Furthermore, we assessed the accuracy of strain in distinguishing between acute and chronic DVT.

Results: 80 patients (mean age 65.6 years, SD 14.2; 41 males) with acute and chronic DVT were included; 116 femoral and popliteal DVT were analyzed. Mean strain value of acute femoral DVT was significantly higher than chronic femoral DVT (4.92 vs 2.62 $p < 0.001$) and mean strain value of acute popliteal DVT was significantly higher than chronic popliteal DVT (4.82 vs 2.60 $p < 0.001$). Age, sex and thrombus location did not significantly affect the strain value (data not shown). A strain value > 4 had a sensitivity of 97.6% (95% CI 85.9, 99.8), a specificity of 94.6% (95% CI 86.0, 98.2), a positive predictive value of 91.1% (95% CI 77.9, 97.1), a negative predictive value of 98.6% (95% CI 91.3, 99.9), and a positive likelihood ratio of 18.06 (95% CI 6.95, 46.9), and a negative likelihood ratio of 0.025 (95% CI 0.003, 0.175).

Conclusions: UE appeared a promising technique to distinguish between acute and chronic DVT.

ClinicalTrials.gov Identifier: NCT02809638

PB 053 | General Practitioner-performed Compression Ultrasound for Deep Vein Thrombosis (Practicus Study): A Prospective, Multicenter Cohort Study

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Background: Currently, compression ultrasonography (CUS) is universally recognized as the procedure of choice for the diagnosis of suspected deep vein thrombosis (DVT) of the lower limb.

Aims: The aim of this study was to assess the diagnostic accuracy of CUS performed by General Practitioners (GP) in primary care setting for symptomatic proximal DVT of the lower limb.

Methods: We prospectively evaluated, in a multicenter cohort study, all the consecutive outpatients referred for suspected DVT from May 2014 to May 2016; all patients underwent bilateral proximal lower limb CUS first by GP and then by physicians expert in vascular ultrasonography, every group blinded with respect to each other. This test was repeat after 5-7 days in all negative or unclear exams. Inter-observer agreement and accuracy were calculated.

Results: We enrolled 1107 patients; DVT was diagnosed by expert ultrasound physicians in 200 patients with an overall prevalence of 18.1% (95% CI 15.8, 20.3). GP agreement with the physician in DVT diagnosis was excellent (Cohen's k 0.86, 95% CI 0.84, 0.88). GP-performed CUS had a sensitivity of 90.0% (95% CI 88.2, 91.8) and a specificity of 97.1% (95% CI 96.2, 98.1) with a diagnostic accuracy of 95.8% (95% CI 94.7, 97).

Conclusions: Our results suggest that GP-performed CUS may be a potential useful alternative in the diagnosis of DVT, with a good accuracy. However, sensibility of GP-performed CUS appeared sub-optimal and future studies should incorporate in the evaluation of this technique other pre-test tools that may increase its accuracy.

ClinicalTrials.gov Identifier: NCT02114983

PB 054 | Targeted MiR126 Therapy of Abdominal Aortic Aneurysm

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Background: Abdominal aortic aneurysm (AAA) is a major health concern, carrying an astonishingly high acute mortality rate. Current condition monitoring for high-risk patients is ultrasound imaging, without secondary prevention therapies available. Limited high-risk surgical interventions are available for severe cases.

Aims: Targeted microbubbles for the delivery of microRNA-126 (miR_{126}) mimics downregulate vascular cell adhesion molecule-1 (VCAM-1) expression, rectify endothelial inflammation and ameliorate the development of abdominal aortic aneurysm.

Methods: Single-chain antibodies against VCAM-1 were conjugated onto ultrasound enhancing microbubbles, and coated with miR_{126} for targeted delivery.

Results: Using the angiotensin II induced model of murine AAA, we observed that targeted delivery of mimic-miR_{126} hinder the dilation of the abdominal aorta both before (1.12 ± 0.11 vs. 1.36 ± 0.12; mm mean ± SD; p>0.05; N=5-9) and after (0.88 ± 0.06 vs. 1.05 ± 0.05; p>0.001; N=5-9) the renal arteries. Further confirmation using 3D ultrasound vessel reconstructions, immunohistological examination, and gene expression analyses (p>0.01; N=5-9) confer an overall decrease in VCAM-1 expression and aneurysm severity.

Conclusions: We describe a novel theranostic approach towards effective reestablishment of intravascular endothelial homeostasis under pro-inflammatory conditions. This technology holds immense potential for a safe, non-invasive, targeted treatment towards conditions driven by inflammation of the endothelial, including AAA.

PB 056 | Risk Prediction Model for Peripheral Arterial Disease Patients Undergoing Revascularization within Two Years of Diagnosis

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Background: Despite standard conservative management of patients with peripheral arterial disease (PAD), about 40% will undergo revascularization procedures within 2 years. Predicting the risk for progression more accurately at time of diagnosis would provide physicians with a means to stratify patients in low and high risk subjects and to tailor treatment accordingly. Stricter monitoring of high-risk patients, leading to a more timely decision to switch from conservative treatment to invasive therapy, can prevent further progression with associated quality of life decline.

TABLE 1 Multivariate model for prediction of revascularization within two years following diagnosis

	Regression coefficient (crude)	Regression coefficient (adjusted[a])	Odds ratio (OR)(80% confidence-interval)		Regression coefficient (crude)	Regression coefficient (adjusted[a])	Odds ratio (OR)(80% confidence-interval)
Hypertension	-0.045	-0.031	0.956 (0.669-1.366)	Fontaine stage IIb-IV	0.623	0.462	1.865 (1.292-2.694)
History of myocardial infarction	-0.189	-0.139	0.828 (0.478-1.436)	Age	-0.043	-0.032	0.958 (0.937-0.979)
Cholesterol lowering drugs	-0.057	-0.043	0.944 (0.607-1.469)	Male gender	0.010	0.010	1.010 (0.699-1.460)
Diabetes	-0.197	-0.142	0.821 (0.519-1.298)	Impaired kidney function (GFR<60ml/min)	-0.607	-0.448	0.545 (0.340-0.874)
Physical function	-0.017	-0.013	0.983 (0.975-0.991)	Elevated age-adjusted D-Dimer	0.415	0.296	1.514 (1.033-2.218)
Ankle-brachial index	-1.246	-0.930	0.288 (0.108-0.763)	Constant	3.985	2.902	

[a]Regression coefficients adjusted for over-fitting with shrinkage factor = 0.74, intercept is re-estimated. To calculate the absolute probability of revascularization within 2 years of PAD diagnosis: P(revascularization) = $1/(1+e^{-Regression})*100\%$. Regression = (-0.032 x age) + (0.010 x male gender) - (0.930 x ABI) - (0.139 x MI) - (0.142 x Diabetes) - (0.031 x Hypertension) - (0.043 x Cholesterol-lowering drugs) - (0.013 x Physical Function) + (0.462 x Fontaine severe) - (0.448 x Impaired kidney function) + (0.296 x Elevated D-Dimer) + 2.902.

Aims: To develop and internally validate a prediction model for revascularization within two years following diagnosis, to assist in personalizing treatment of PAD-patients.

Methods: In a cohort of 280 newly diagnosed PAD-patients data regarding medical history, cardiovascular risk profile, D-dimer, medication use and patient-reported quality of life were collected at baseline. Primary endpoint was defined as a revascularization procedure within two years after diagnosis. A set of predictors was selected from a literature review and expert consultation, subsequently a prediction rule was developed using multivariable logistic regression and internally validated using bootstrapping techniques.

Results: In total, 108 patients (38.6%) underwent revascularization within two years of diagnosis. Predictors included into the model were hypertension, history of myocardial infarction, cholesterol-lowering drug use, diabetes, physical function, ankle-brachial index, Fontaine stage, age, gender, renal function and increased age-adjusted D-Dimer. The area under the receiver operating characteristic curve of the internally validated model was 65% (95%CI 63.7-76.3%) with good calibration.

Conclusions: We successfully developed a practical prediction model to stratify newly diagnosed PAD-patients in low and high risk for revascularization to assist physicians in optimizing individual patient management.

PB 057 | Repetitive Intravenous Thrombin Injections Result in Pulmonary Arterial Endothelial Dysfunction in a Mouse Model of Sublethal Acute Pulmonary Embolism

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Background: Acute pulmonary embolisms (APEs), resulting from deep vein thromboses (DVT), undergo a total or near-total resolution. Caused by yet unidentified mechanisms, a process of remodeling ultimately resulting chronic thromboembolic pulmonary hypertension (CTEPH) is initiated. The mechanisms that underlie the chronification of emboli and ultimately the development of CTEPH remain uncertain.

Aims: The main aim is to investigate the effect of single or repetitive sublethal experimental pulmonary embolism on the pulmonary vasculature, further comparing it to the IVC (inferior caval vein)-stenosis model.

Methods: C57BL/6J mice were anaesthetized and APEs were induced by i.v. injection of thrombin 2.5-5 Units (U) by retro-orbital injection. Repetitive thrombin doses were administered after recovery. For IVC-ligation the IVC was exposed and permanently ligated with space holder. PA vasoconstriction as well as endothelial dependent and independent relaxation was analyzed by exposure to increasing concentrations to phenylephrine, acetylcholine and glyceryl trinitrate under physiologic conditions. ROS-burden was evaluated and dihydroethidium (DHE) staining of PA cryosections.

Results: Pulmonary embolisms after injection of thrombin were confirmed by a sudden bradycardia and an increase in PA-pressure as observed by echocardiography. 12 h after repetitive (triple) pulmonary embolisms, isometric tension studies revealed a significant endothelial dysfunction in PAs explanted from embolized animals as compared untreated animals. Importantly, no APE and no PA endothelial dysfunction were detected after IVC-ligation. Further, the levels of superoxide were significantly increased in PAs from triple-embolized mice as compared to PAs from control mice or after IVC-ligation.

Conclusions: Subtotal IVC-ligation is not a subsequent model for APEs, the most important complication of DVT. Repetitive APEs by triple injection of thrombin results in PA endothelial dysfunction, which could initiate remodeling cascade resulting in CTEPH.

PB 058 | Lack of Association between Candidate Gene Polymorphisms and Ischemic Events in Patients with Peripheral Arterial Disease

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Background: Genetic basis of peripheral arterial disease (PAD) is incompletely understood.

Aims: We tested whether selected single nucleotide polymorphisms (SNPs) were associated with PAD and whether they affected cardiovascular adverse events in comparison to diabetes and smoking.

Methods: 710 patients with PAD and 650 age- and sex-matched control subjects, both groups aged 65±9 years at inclusion, were subjected to yearly physical and laboratory investigations and were managed for 5 years according to the European guidelines on cardiovascular disease prevention (ClinicalTrials.gov number NCT00761969). The occurrence of death, non-fatal myocardial infarction or ischemic stroke (major events) and revascularization procedures (minor events) was recorded. SNPs of Nurr1 gene, associated with regulation of inflammation (rs1466408, rs13428968 and rs12803), IL6 gene, encoding the inflammatory cytokine and being associated to susceptibility to diabetes mellitus (rs10499563), PECAM 1 gene, involved in leukocyte migration, angiogenesis and integrin activation (rs668 and rs12953), and Chr12 gene, associated with coronary events (rs10861032) were determined.

Results: The distribution of selected SNPs did not differ between patients with PAD and control subjects, and neither between subjects with ischemic events (n = 275) and those without events. In contrast with the negative findings of genetic testing, diabetes and smoking strongly affected survival. The hazard ratio for cardiovascular death in PAD patients with diabetes was 2.14 (CI 1.21-3.76) in comparison to patients without diabetes (p = 0.008). The 5-year survival of active

smokers with PAD was 0.80 (CI 0.75-0.62), of former smokers 0.83 (CI 0.79-0.88), and of never-smokers 0.89 (CI 0.86-0.93), (p = 0.024).
Conclusions: SNPs of Nurr1, IL6, PECAM1 and Chr12 were not associated with PAD or with ischemic events. However, diabetes and smoking were associated with worse survival.

PB 059 | Restenosis is a Predictor of Poor Outcome in Patients Undergoing Endovascular Intervention for Peripheral Arterial Disease

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Background: Few data are available on clinical outcome in patients who underwent percutaneous transluminal angioplasty (PTA) for peripheral arterial disease (PAD). We speculated that restenosis after PTA is a predictor of poor outcome, being restenosis a marker of a more aggressive atherothrombosis.
Aims: To ascertain if restenosis after PTA was associated with higher risk of cardiovascular events in patients with PAD.
Methods: A longitudinal study of 310 patients who underwent PTA for PAD (Fontaine's stages: II through IV; aged 70 ±11 years, male/female 182/126). Major adverse cardiovascular events (MACE) were the composite end-point. The study started after the PTA. Each patient was seen after one month, six months, one year and every year thereafter. At each visit, clinical examination, ABI measurement and duplex sonography (DUS) were performed. Primary patency was maintained until restenosis defined by a peak systolic velocity (PSV) ratio >2.4 and >70% diameter reduction was documented by DUS. Patients were followed-up for an average time of 4050±885 days.
Results: 117 (37.7%) patients developed restenosis. Age, sex, and ABI before PTA were similar among patients with restenosis vs. those without. Diabetes was more frequent in patients with restenosis vs. those without (33.3 vs. 20.9%, p=0.017). During the follow-up, MACEs (n=146) were more frequent in the patients with restenosis versus those without (79.5 vs. 30.1% log-rank p< 0.001). According to Cox regression analysis, age, diabetes, critical limb ischemia before PTA, and restenosis (RR 3.2 95%CI 2.3-4.5, p< 0.001) were predictors of MACE.
Conclusions: The presence of restenosis at DUS in patients who underwent PTA for PAD is associated with increased risk of arterial thrombotic events. Intervention trials are required to show the benefit of different therapeutic approaches in such patients at high risk of clinical deterioration.

PB 061 | Venous Thromboembolism Following Hantaviral Infection

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Background: The hallmark characteristics for viral hemorrhagic fevers are thrombocytopenia, vascular dysfunction and disseminated intravascular coagulation. Previously, we have shown that Puumala hantavirus, an etiologic agent for hemorrhagic fever with renal syndrome (HFRS) constitutes a significant risk factor for acute myocardial infarction and stroke (Connolly-Andersen et al., 2014, Circulation). However, there is no information available regarding whether the risk for venous thromboembolism (VTE) increases during HFRS.
Aims: Determine whether HFRS is a risk factor for VTE.
Methods: HFRS in Sweden is a notifiable disease. The personal identification numbers from all HFRS patients since 1997 were cross-linked with the Swedish National Patient Register. We used the self-controlled case series method to calculate the incidence rate ratio (IRR) for VTE during HFRS.
Results: 7244 HFRS patients were included in the study and of these 146 had a first deep vein thrombosis (DVT) or pulmonary embolism (PE). The age-adjusted IRR for a first VTE in the first two weeks following HFRS was 64.3 (95% CI 36.3-113.9). When divided into a first DVT or PE, the age-adjusted IRR was 45.9 (18-116.9) and 76.8 (37.1-158.9), respectively. The risk for a VTE remained significantly higher up to 3 months following HFRS.
Conclusions: HFRS is a risk factor for venous thromboembolic events. Although HFRS is considered a mild viral hemorrhagic fever, our studies highlight the increased risk for thromboembolic complications. Our findings support the notion that preventive measures, especially for patients with additional risk factors, might be beneficial. However, this warrants further studies.

PB 062 | Abdominal Aortic Calcification in Patients with Peripheral Arterial Disease: Hemostasis Changes in Relation to Calcinosis Grade

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Background: Peripheral arterial disease (PAD) has often assosieted with abdominal aortic calcification (AAC) and hypercoagulable states.
Aims: The objective of our research work was to determine the specific haemostatic features in patients with PAD in relation to different grade of an AAC.
Methods: A total of a 94 symptomatic PAD patients with objective signs of AAC were enrolled into the study cohort. PAD was defined as an ABI < 0.9. The exclusion criteria of our study were diabetes mellitus, abdominal aortic aneurism, previous vascular intervention and chronic renal insufficiency. AAC was verified by CT-imaging. Adopted Agatston score (As) was used for evaluating of an AAC severity. The study cohort was divided into three groups according to As: group I (mild calcification) - 30 patients, group II (moderate calcification) - 32 patients, group III (severe calcification) - 32 patients. Activity of VIII and von Willebrand factors (vWF), fibrinogen concentration, antithrombin activity and Hagemann-dependent lysis were evaluated.

Results: Three groups of patients were similar according to age, gender and other risk factors for PAD. We have not found any statistically significant differences in activity of VII factors and the Hageman-dependent lysis between groups (p>0.05 for all compared variants). The concentration of antithrombin activity was inversely correlated with the grade of aortic calcification (p< 0.01). The mean concentration of fibrinogen (4.1±1.2 g/l vs. 8.6±0.8 g/l vs. 6.7±0.6 g/l, respectively (p< 0.01 for all compared variants)) and the mean activity of vWF (136.2±12.7% vs. 188.1±15.2% vs. 161.6±11.8%, respectively (p< 0.01 for all compared variants)) were highest in patients with moderate aortic calcification.

Conclusions: Our findings have shown that an AAC may influence to haemostatic system in patients with PAD. Interestingly, that haemostasis was correlated with the grade of abdominal aortic wall calcification and, severe coagulation disorders were related to a moderate grade of calcification.

PB 063 | Thrombophilic and Cardiovascular Risk Factors for Retinal Vein Occlusion

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Background: The role of thrombophilic and cardiovascular risk factors in different manifestations of retinal vein occlusion (RVO), i.e., central or branch RVO, and at different ages is still debated.

Aims: To evaluate the association between thrombophilic and cardiovascular risk factors and the risk of RVO (overall, separately for central and branch RVO, and at different ages).

Methods: Case-control study on 313 patients with a first objectively-confirmed RVO (52% females, median age 54 yrs, 216 central and 97 branch RVO) consecutively referred at our Thrombosis Center for a thrombophilia work-up, and 415 healthy individuals (71% females, median age 41 yrs). Cardiovascular risk factors (hypertension, hyperlipidemia, diabetes and cigarette smoking) were recorded. In a multivariable logistic regression model, the risk of RVO in carriers relative to non-carriers of a particular risk factor was estimated as odds ratio (OR) and 95% confidence intervals (CI), adjusting for age, sex and the other risk factors.

Results: Antithrombin, protein C or protein S deficiency (adjusted odds ratio [95%CI]: 15.60 [2.01-121]), hyperhomocysteinemia (HHCy: 3.22 [1.38-7.49]), high factor VIII (FVIII) levels (3.08 [1.20-7.89]), factor V Leiden (2.93 [0.97-8.86]) and the presence of at least one cardiovascular risk factor (1.79 [1.00-3.23]) were associated with an increased risk of branch RVO. The association was weaker for central RVO, and limited to HHCy (2.15 [1.09-4.24]) and high FVIII (1.99 [0.90-4.42]). For HHCy, high FVIII and cardiovascular risk factors the association with the risk of RVO was stronger at an age >50 yrs (3.41 [1.29-8.99], 2.57 [1.00-6.68] and 2.03 [1.16-3.56], respectively) than ≤50 yrs (1.93 [0.85-4.36], 1.67 [0.54-5.12] and 1.22 [0.73-2.03], respectively).

Conclusions: Thrombophilic and cardiovascular risk factors are associated with RVO, particularly branch RVO. The risk of RVO associated with HHCy, high FVIII and cardiovascular risk factors is higher at an older age.

PB 064 | Patients with Cryptogenic Acute Limb Ischemia Have High Recurrence Rates Off Anticoagulation: Results of a Single Institution Retrospective Cohort Study

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Background: Acute limb ischemia (ALI) is often secondary to emboli, or progression of peripheral vascular disease (PVD). Rarely, ALI occurs in the absence of known PVD, or obvious cause. Rarer still, occasionally the cause of ALI remains truly cryptogenic. (cALI) While precipitated ALI (pALI) is well-described, cALI is a population that has not been studied, and whose management is not established.

Aims: To describe the population that develops ALI without PVD, and cALI, and recurrence rates based on therapeutic choices.

Methods: Retrospective review of patients treated from Jan, 2002 - present at our facility. We included adults seen for ALI with at least 3 months of follow up. Patients with known PVD, trauma, critical illness, or recent vascular access were excluded. We examined documentation to determine a root cause in each case. Patients with no identifiable cause for their ALI were labeled as cALI. We compared patients with pALI and cALI, evaluating rates and mean time to recurrence, and circumstances surrounding recurrence.

Results: Out of 608 patients analyzed, 39 had ALI without PVD. 30 were found to have a precipitating cause and were labeled as pALI. 9 (23%) had no identifiable cause, and were labeled as cALI. The demographics of the two groups were similar. In total, 8 patients recurred (20%) 5 with pALI (15%) and 3 with cALI (33%). Median time to recurrence was 16.5 months in the pALI group, and 23.3 months in the cALI group. Of those who recurred, a similar proportion did so off anticoagulation in both groups (60 and 66% of patients off anticoagulation, respectively).

Conclusions: Over 20% of patients presenting with ALI without PVD did not have an identifiable cause and remained cryptogenic. Recurrence rates in patients with pALI and cALI are similar, occuring in nearly 60% of patients who are not anticoagulated. This suggests that the etiology of ALI may be less important in patients without PVD, and that indefinite anticoagulation may be warranted in both precipitated and cryptogenic ALI.

PB 065 | Gene-sodium Interaction on the Developing of Hypertension: A Cohort-based Case-control Study

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Background: There are few studies concerning gene-sodium interaction between G-protein beta3 subunit (GNB3) C825T (rs5443) and dietary sodium intake on the risk of hypertension, i.e. BP salt sensitivity.
Aims: The study aims to evaluate the joint influence of GNB3 polymorphisms and sodium consumption on the occurrence of hypertension.
Methods: A cohort-based case-control study was conducted in 2014. There are 233 participants with new hypertension in the case group and 233 participants in the sex-matched the control group. The primary outcome is the occurrence of hypertension over a 10-year period. The determinants of hypertension were 3 genotypes of SNP in GNB3 (TT; CT and CC) and two dietary salt categories on the basis of the level of sodium consumption representing high (> 4800 mg/day), and low-sodium (< 2400 mg/day) diets.
Results: The occurrence of hypertension was increased with participants carrying TT genotype and high-sodium diets comparing with those carrying TC or CC genotype with low-sodium diets (adjusted OR 4.13, 95% CI 1.53-10.95) (Rothman synergy index=1.49).
Conclusions: The study suggests that GNB3 C825T polymorphism may influence the response of the renin-angiotensin system to high-sodium diet. It imply that participants carrying TT variant allele and dietary high-sodium are more likely to occurrence of hypertension.

PB 066 | Drug Induced Venous Thrombosis

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Background: Deep vein thrombosis and pulmonary embolism are major cause of morbidity. It has been recognized that drugs may play an important role in development of thrombosis whether it is chemotherapy or a vastly prescribed therapy such as oral contraceptive.
Aims: To investigate the epidemiological and clinical characteristics of patient presenting drug induced thrombosis.
Methods: Retrospective study including 1055 patients admitted in internal medicine department of La Rabta hospital in Tunisia, researching patients with drug usage prior to thrombosis.
Results: Among the 1055 patients hospitalized for venous thrombosis, 421 were already on drugs. Of these patients, intake of thrombosis inducing drug was noted in 46 cases (10.9%). Sex-ratio was 1.19. Mean age of our patients was 51 years. Elderly patients represented 34.8%. Smoking was observed in 28% of patients. The other factors, contributing to thrombosis formation are presented in table 1.
Venous thrombosis was at the lower limbs in 95.7%. It was a proximal venous thrombosis in 76% of cases. Inferior vena cava thrombosis was noted in 3 cases.
Among the incriminated drugs, we noted chemotherapy (4 cases), Thalidomide (2 cases), estroprogestative (13 cases), Androcur® (2 cases) and Carbamazepine (12 cases). Complications observed consisted in pulmonary embolism (11.2%) and post-phlebitis syndrome (15%). On treatment no thrombosis extension was observed. Treatment was based on LMWH relayed by oral anticoagulant with eviction of suspected drug whenever possible. The average duration of VKA treatment in our patients was 35 months. Recurrence of thrombosis was noted in 13% of cases.
Conclusions: Drug-induced thrombotic event are usually venous thrombosis, however arterial thrombotic event have also been reported. Better understanding of the mechanisms by which drugs exert thrombosis may facilitate their safe use in patients, especially those with predisposing risk factors.

PB 067 | Retinal Vascular Occlusions: A Retrospective Study

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Background: Retinal vascular thrombosis (RVT) are an important cause of vision loss. Some of them may result of loco-regional ocular causes. They more often occur in patients with cardiovascular pathologies or risk factors, or sometimes other systemic diseases that need to be recognized for a proper treatment.
Aims: to study clinical, etiologic and evolutive characteristics of RVT.
Methods: This retrospective study included patients hospitalized in our department between 2005 and 2016 for RVT.
Results: Ten cases of RVT were observed. It was venous thrombosis (4 cases), arterial thrombosis (4 cases) and mixed thrombosis (arterial and venous) (2 cases). The sex ratio was 1. 4 men had retinal venous thrombosis (RVeT), 3 women had retinal arterial thrombosis (RAT) and 2 women had mixed thrombosis (MT). The average age was 50 years. Patients in the RVeT group had unilateral involvement in 50% of cases affecting the central retinal vein in all cases. These RVeT were ischemic (2 cases), oedematous (1 cases) and mixed (1 case). The symptoms during RVeT were marked by progressive reduced visual acuity in 75% of the cases with a visual acuity less than 1/10 in the both patients with ischemic involvement. The etiologies found in this group are presented in table 1.RAT affected the central artery of the retina in all cases and was symptomatic of amputation of the visual field. Theetiologies of this group are presented in table 2.
MT was observed in 2 patients. It affected the retinal ciliary artery and was responsible for a reduction in visual acuity.
All patients received platelet anti-aggregation with specific treatment of background pathology.
Conclusions: A diagnosis of RVT represents a challenge for both ophthalmologists and internists. Collaboration is required to properly investigate and manage these patients. In spite of the current knowledge, there is a clear need for future studies evaluating medical interventions for this condition.

PB 068 | Thrombosis Events in Antiphospholipid Syndrome

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Background: Anti-phospholipid syndrome (APS) is an autoimmune disease characterized by the occurrence of thrombo-embolic manifestations or obstetric complications.

Aims: to report the epidemiological, clinical and evolutionary characteristics of venous thrombosis during the APS.

Methods: A retrospective study including records of patients hospitalized between 2010 and 2015 for venous thrombosis which was related to antiphospholipid syndrome.

Results: Between 1055 cases of venous thrombosis, 80 patients had an antiphospholipid syndrome. The latter was associated with systemic lupus erythematosus in 4 patients and Sjogren syndrome in one patient. . Female predominance was observed in 77.8% of patients. The risk factors for MVTE observed were: age over 50 years (27.8%), obesity (33%), venous insufficiency (5.6%), varicose veins (11.1%), bed rest Prolonged (16.7), trauma (5.6%) and surgery (16.7%). Thrombosis of the lower limbs was found in 66.7% of the cases. An unusual localization was reported in 27.8% of the patients. It was proximal in 38.9% of the cases and distal in 27.8% of the cases. A post-phlebitic syndrome was objectified in 16.7%. Six cases of recurrence were found.

Conclusions: Antiphospholipid syndorme is an important aetiology of venous thrombosis which must be searched when thrombosis occured in young patient, thrombosis have unusual localization or is reccurent.

PB 069 | Mean Platelet Volume (MPV) as a Predictor of Venous Thromboembolism (VTE) in Colorectal Cancer

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Background: Platelet activity is a major devilish in atherothrombotic events and cancer. Mean platelet volume (MPV), which is widely available as a routine parameter of the complete blood count, is a potentially useful biomarker of platelet activity in the setting of venous thrombosis. Recent studies showed that high-MPV levels associated with a increase VTE risk in cancer patients.

Aims: To investigate the role of MPV in VTE and colorectal-cancer.

Methods: A retrospective study was performed to analyze differences of MPV between patients with VTE, VTE and colorectal-cancer, and control. Two reviewers independently extracted data for meta-analysis. Differences in MPV were expressed as unstandardized mean difference.

Results: Among 170 patients, 58-control, 54-VTE, and 58-VTE with colorectal-cancer, MPV was significantly higher in VTE groups. From 403 articles, 10 studies(5 cohorts and 5 case-controls) comprising 2,265 patients. MPV was significantly higher in those with VTE (mean difference 0.61 fL,95%CI 0.34-0.88,P< 0.001). Elevated MPV increased the relative risk of VTE (RR 1.319,1.061-1.641,I2=82.5%).

Conclusions: Our evidence suggests that elevated MPV is associated with VTE and VTE with colorectal-cancer. A mechanistic study and RCT are required in order to use antiplatelet therapy.

PB 772 | Regular Physical Activity and Risk of Incident Venous Thromboembolism in the General Population

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Background: Despite extensive health benefits of regular physical activity (PA), most cohort studies investigating the association between PA and risk of incident venous thromboembolism (VTE) have failed to show a favorable effect. The lack of effect may reflect an underestimation of the true association due to fluctuations in PA during a long follow-up (regression dilution bias).

Aims: To investigate whether regular PA of moderate and high intensity during leisure time was associated with incident VTE in a population-based cohort with repeated assessments of PA.

Methods: A total of 30 046 subjects with data on PA participating in one or more surveys of the Tromsø Study (1994-95, 2001-02, 2007-08) were included, and incident VTE was registered up to 31.12.12. Self-reported weekly duration of moderate and high intensity PA was categorized (no PA, < 1h, 1-3h and >3h), and hazard ratios (HRs) adjusted for sex and BMI were calculated using time-varying Cox-regression models with age as timescale. The lowest PA category was set as reference.

Results: There were 457 VTE events during follow-up (incidence rate 1.5 per 1000). PA was not associated with risk of incident VTE in the general population (HR for upper PA category: 0.92; 95% CI 0.63-1.32). However, in elderly (≥65 years), a moderate amount of PA (1-3h/week) was associated with 38% lower risk of VTE (HR 0.62; 95% CI 0.41-0.95), 63% lower risk of provoked VTE (HR 0.37; 95% CI 0.19-0.71) and 47% lower risk of deep vein thrombosis (DVT) (HR 0.53; 95% CI 0.30-0.95). No significant effect of PA was observed for pulmonary embolism (PE) (HR 0.76; 95% CI 0.41-1.42). The risk estimates were attenuated when subjects with previous cancer or who developed cancer during follow-up were censored.

Conclusions: By applying time-varying analyses, we found that moderate amounts of PA lowered the risk of provoked VTE, and DVT in particular, in the elderly. The beneficial effect may be partially mediated by a lower incidence of cancer-related VTE among elderly who exercise regularly.

PB 773 | Impact of Regular Physical Activity on the Risk of Recurrent Venous Thromboembolism and All-cause Mortality

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Background: Venous thromboembolism (VTE) recurs frequently and is associated with an increased risk of mortality. Current evidence indicates that physical activity (PA) may reduce the risk of incident VTE, but the effect on recurrence and all-cause mortality is unknown.

Aims: To investigate the impact of moderate and high intensity PA, assessed prior to the incident VTE, on the risk of recurrence and all-cause mortality in a cohort of VTE-patients recruited from the general population.

Methods: A total of 733 patients with incident VTE and data on PA derived from the Tromsø Study surveys 4-6 were included, and recurrent VTE and all-cause mortality were registered up to December 31, 2012. The most recently reported weekly level of moderate and high intensity PA before the incident VTE was dichotomized (none vs. any), or categorized according to weekly duration (no PA, < 1h, 1-3h and >3h per week). Hazard ratios (HRs), adjusted for sex and BMI, were calculated using Cox regression models with age as timescale.

Results: There were 115 recurrences and 342 deaths during a median follow-up of 2.5 years. PA was associated with a non-significant lower risk of VTE recurrence (HR none vs. any PA 0.73; 95% CI 0.48-1.10). The impact of PA was driven by the effect in patients with incident deep vein thrombosis (DVT) (HR 0.63; 95% CI 0.38-1.03), with no effect in pulmonary embolism (PE) (HR 1.06; 95% CI 0.50-2.25). PA was associated with 39% lower risk of all-cause mortality (HR 0.61; 95% CI 0.47-0.79), which tended to be more pronounced in men than in women (HR 0.56; 95% CI 0.39-0.79 vs. HR 0.74; 95% CI 0.49-1.09). The beneficial effect of PA on all-cause mortality was restricted to patients with incident DVT and showed a dose-response effect with the weekly duration of PA (p for trend 0.01).

Conclusions: Our results suggest that a sedentary lifestyle is associated with higher risk of recurrence and mortality after incident VTE, and that PA displays a stronger protective effect on mortality with increasing weekly duration.

PB 775 | Associations between Serum Levels of Calcium, Parathyroid Hormone and Future Risk of Venous Thromboembolism - The Tromsø Study

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Background: Population-based cohorts have demonstrated that serum calcium and parathyroid hormone (PTH) are risk factors for arterial cardiovascular disease and mortality. The relationship between serum levels of calcium, PTH and risk of venous thromboembolism (VTE), however, has not been addressed in population-based cohorts.

Aims: To investigate the associations between serum levels of calcium and PTH and future risk of VTE in a general adult population.

Methods: A total of 27,712 subjects (25-87 years) who participated in the Tromsø 4 (1994-95) and Tromsø 5 (2001-02) surveys were included. Total calcium and PTH were measured in 27,685 and 8,547 subjects, respectively. Incident VTE was recorded through December 31, 2012. Cox-regression models with quartiles of calcium and PTH as time-varying exposures were used to calculate hazard ratios (HR) of VTE. Quartiles of calcium and PTH were also combined in order to assess the effect of discordants of both PTH and calcium (e.g. highest and lowest quartiles of both calcium and PTH) on VTE risk using the middle two quartiles as reference.

Results: There were 712 VTEs during a median of 15.0 years of follow-up. Serum levels of calcium and PTH were not associated with risk of VTE. However, subjects with discordant high serum levels of both calcium and PTH (calcium ≥2.45mmol/L and PTH ≥4.0pmol/L) had increased risk of VTE compared to subjects with normal calcium and PTH (multivariable HR 1.78, 95% CI 1.12-2.84). This association was not mediated by cancer or arterial CVD.

Conclusions: Serum levels of calcium and PTH separately were not associated with future risk of VTE, but subjects with high levels of both calcium and PTH had increased risk of VTE compared to subjects with normal levels.

PB 776 | Repeated Measures of Fish Consumption, n-3 PUFA Intake and Future Risk of Incident VTE

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Background: Strong evidence supports that polyunsaturated fatty acids (n-3 PUFAs) have beneficial effects on key pathways in the pathogenesis of thrombosis. However, studies on the association between fish intake and venous thromboembolism (VTE) show diverging results, and few studies have investigated the relationship between n-3 PUFA intake and VTE.

Aims: To investigate the association between weekly fish consumption and n-3 PUFA intake on the risk of incident VTE in a population-based cohort with repeated measurements of fish- and fish-oil consumption.

Methods: 29,547 subjects, aged 25-97 years, were recruited from the fourth (1994-95) and sixth (2007-08) surveys of the Tromsø study.

Information on fish consumption and fish-oil supplements was assessed by self-reported questionnaires at baseline, and updated when a subject re-entered the next survey. Incident VTEs were validated and recorded up to Dec 31, 2012. Total n-3 PUFA concentration was estimated based on the reported intake of fat and lean fish, fish as bread spread and fish-oil supplements, using standardized schemes. Quartiles of fish consumption (Q1:≤400, Q2:400-599, Q3:600-849, Q4≥850 g/week) and n-3 PUFA intake (Q1:< 7.79, Q2:7.79-20.74, Q3:20.75-33.34, Q4:≥33.35 g/week) were created. Cox-regression models with age as timescale, and fish- and n-3 PUFA consumption as time-varying covariates, were used to calculate hazard ratios (HR) of VTE adjusted for sex and BMI. The first quartile (Q1) was used as reference.

Results: There were 660 VTE events during a median of 17.8 years of follow-up. There was no association between fish intake and risk of VTE (HR Q4 vs. Q1: 1.03, 95% CI: 0.82-1.31, p for trend across quartiles: 0.8). Moreover, there was no association between n-3 PUFA intake and VTE risk (HR Q4 vs. Q1: 0.88, 95% CI: 0.70-1.109 p for trend: 0.15). Similar results were observed in subgroup analyses of unprovoked and provoked VTE.

Conclusions: Neither weekly fish consumption nor intake of n-3 PUFAs was associated with risk of VTE.

PB 777 | Residual Vein Thrombosis in Subjects with Proximal DVT: An Association with Subclinical Atherosclerosis. The VERITAS Study

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Background: Following the demonstration that there is a link between venous and arterial thrombotic disorders, the ultrasound detection of residual vein thrombosis (RVT) has recently been shown to be a powerful and independent predictor of subsequent symptomatic atherosclerotic events.

Aims: We sought to assess whether in patients with proximal deep vein thrombosis (DVT) the ultrasound persistence of RVT is associated with the presence of subclinical atherosclerosis.

Methods: An Italian multicentre cross-sectional study was conducted between 2014 and 2016. Consecutive subjects older than 40 with an episode of proximal DVT were eligible, provided that they had not experienced atherosclerotic disorders or ipsilateral DVT. Subjects were investigated at three months after the qualifying DVT by two "blind to each other" assessors. The former investigated the presence of RVT (persistence of a thrombotic burden of at least 4 mm in the transverse section under maximum compressibility); the latter evaluated the presence of intima-media tickness (IMT) (> 0.9 mm) or carotid plaques (according to NASCET classification). For each patient detailed information on risk factors for vein and arterial thrombosis was collected.

Results: We recruited 252 patients (see table 1 for population characteristics).

TABLE 1 Population characteristics

	Study population N 252
Age	67.2±12.8
Male Sex (%)	133 (52.8)
Type of event (%) DVT DVT+PE	130 (51.6) 122 (48.4)
Unprovoked DVT (%) Provoked DVT	194 (77.0) 58 (23)
Inherited thrombophilia (%)	16 (10.5)
BMI > 30 (%)	58 (23)
Hypertension (%)	117 (46.4)
Diabetes mellitus (%)	38 (15.1)
Dyslipidemia (%)	78 (31)

Subjects with RVT (139, 55.2%) had more frequently an unprovoked DVT , a DVT associated with PE and a lower prevalence of inherited thrombophilia. Persistence of RVT was significantly related to presence of IMT and of carotid plaques as shown in table 2.

TABLE 2 RVT and subclinical atherosclerosis

	3rd month RVT Absent 113	3rd month RVT Present 139	p
Age	66.3±13.1	67.9±12.5	0.524
Thrombophilia (%)	10/54 (18.5)	5/99 (5.1)	0.011
Idiopathic VTE (%)	83 (73.5)	119 (86.2)	0.011
Event type (%) TVP TVP+EP	82 (72.6) 31 (27.4)	56 (40.3) 83 (59.7)	<0.001
Hypertension (%)	52 (46.0)	65 (46.8)	0.906
Dyslipidemia (%)	30 (26.8)	48 (34.5)	0.187
Diabetes Mellitus (%)	13 (11.5)	25 (18.0)	0.153
IMT (%)	35 (31)	76 (54.7)	<0.001
Carotid plaques (%)	36 (31.9)	80 (57.6)	<0.001

Overall, the persistence of RVT was associated with an increased risk of asymptomatic atherosclerosis with an odds ratio of 2.71 (95% CI 1.61-4.55).

Conclusions: The persistence of RVT three months after an episode of proximal DVT has the potential to identify a subgroup of patients with a higher prevalence of subclinical atherosclerosis and, as such, exposed to a higher risk of subsequent arterial cardiovascular disorders.

PB 778 | Elevated Circulating CD40-L in Atrial Fibrillation and its Modulation by Ablation

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Background: Atrial fibrillation (AF) is currently the most common cardiac arrhythmia and is a major cause of morbidity and mortality. Inflammation plays a crucial role in the progression of atrial fibrillation. CD40-Ligand (CD40-L) is a cellular mediator that is elevated in inflammatory processes. CD40-L can be expressed on platelets, leading to the release of cytokines during inflammation.

Aims: The purpose of this study is to characterize and quantify the levels of CD40-L to evaluate its relevance to AF both pre- and post-ablation.

Methods: In a prospective study, plasma samples were collected from 55 AF patients at baseline prior to ablation, and at 1 and 3-month follow-up post-ablation. Normal human plasma samples (n=50) were supplied by George King Biomedical Inc. (Overland Park, KS). ELISA kits were obtained to profile CD40-Ligand (R&D Systems, Minnesota USA). Statistical analysis was performed using non-parametric Mann-Whitney t-tests.

Results: There was a significant increase in CD40-L at pre-ablation baseline in patients with AF compared to normal (401.0 pg/mL vs. 53.62 pg/mL, p=0.0034). CD40-L levels post-ablation significantly decreased. At 1 month follow up, CD40-L levels dropped (401.01 pg/mL vs. 40.59 pg/mL, p=< 0.0001). At 3 months, the levels of CD40-L remained decreased (43.28 pg/mL, p=< 0.0001). There was no significant change between levels at 1 month and 3 months (p=0.8573). There was also no significant increase in post-ablation levels of CD40-L compared to normal human plasma levels (p=0.2095).

Conclusions: Inflammation plays an important role in the pathogenesis of atrial fibrillation. In this study, patients with AF have highly elevated levels of CD40-L compared with normals. Contrary to previous reports, our study suggests that elevated levels of CD40L may play an important role in the pathogenesis of AF. It is also noted that the levels of this inflammatory mediator are modulated by atrial ablation as a medical treatment for AF.

PB 779 | Elevated Cellular Fibronectin is a Modifier of Clot Properties in Type 2 Diabetes: Association with Cardiovascular Disease

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Background: Type 2 diabetes is associated with faster formation of poorly lysable, denser fibrin clots and elevated cellular fibronectin (cFn), a marker of vascular injury.

Aims: We investigated whether cFn affects clot properties in type 2 diabetes.

Methods: In 200 consecutive patients with type 2 diabetes and 100 control subjects matched for age and sex, we determined plasma cFn along with clot formation and degradation using turbidimetric and permeability assays. The study was conducted in compliance with Declaration of Helsinki. The local ethics committee approved the study. All enrolled subjects provided written, informed consent.

Results: cFn positively correlated with age, body mass index, glucose, fibrinogen, and triglicerydes (all p< 0.05). Diabetic patients had elevated cFn (median, 3.99 [interquartile range, 2.87-4.81] µg/ml]), increased clot density ($MaxAbs_C$) and prolonged lysis time (Lys_T) compared with those without type 2 diabetes (all p< 0.01). Diabetic patients with documented cardiovascular disease (CVD, n=127, 63.5%) had increased cFn (4.53 [3.68-4.95] µg/ml), decreased clot permeability (K_s) and increased $MaxAbs_C$ compared with those without CVD (all p< 0.001). Diabetic patients with cFn in the top quartile (>4.81 µg/ml) were 2 times more likely to have CVD compared with those in the lowest quartile (odds ratio 1.80, 95% confidence interval 1.41-2.46, p< 0.001). No differences in cFn were observed in relation to microvascular complications. After adjustment for potential confounders, cFn accounted for 10.2% of variance in K_s, 18.2% of variance in clot density and 10.2 % of variance in AUC in diabetic patients.

Conclusions: This study shows that elevated cFn unfavorably modifies clot properties in type 2 diabetes, especially with CVD, which indicates novel links between vascular injury and prothrombotic alterations in diabetes.

PB 780 | Thrombin Generation Potential is Increased in Anabolic Androgenic Steroid Abuse Years after Cessation

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Background: Abuse of anabolic androgenic steroids (AAS) is suspected to increase cardiovascular morbidity and mortality. However, the effects of AAS abuse on the haemostatic balance are poorly understood and especially little is known about the long-term effects.

Aims: We assessed the effect of AAS abuse on thrombin generation and inhibitors of coagulation.

Methods: Young men 18-50 years of age and either current AAS abusers, former AAS abuser or controls were included for this cross-sectional study. Morning blood samples were collected after overnight fasting and 30 minutes rest in the supine position. Thrombin generation (lag time, time to peak, peak height and endogenous thrombin potential (ETP)) was determined in citrated plasma samples after addition of 5 pM tissue factor. Also, antithrombin, protein C, free protein S and CRP were assessed. Groups were compared by ANOVA or

Kruskal-Wallis test corrected for mass significance. Associations were evaluated by ANCOVA.

Results: ETP was increased among both current (n=37) and former AAS abusers (n=33; mean (95 % CI) duration since cessation 2.5 (1.7-3.7) years) compared with controls (n=30) (p< 0.001) (Table 1). Lag time, time to peak, protein C activity and free protein S were increased among current AAS abusers compared with former AAS abusers and controls (p< 0.001). Also, antithrombin activity was increased among current AAS abusers compared with controls (p=0.012). CRP was increased among current AAS abusers compared with both former AAS abusers and controls (p< 0.01). Among current AAS abusers, CRP was strongly associated with the ETP (β=57.5; p=0.005).

Conclusions: Current AAS abuse increases ETP and prolongs lag time and time to peak. The pro-coagulant effect of AAS appears to dominate the increase of inhibitors sustaining a pro-thrombotic condition. In current AAS abusers low-grade inflammation seems to support the phenotype. Importantly, the ETP is persistently increased more than two years after AAS cessation.

TABLE 1 Thrombin generation, coagulation inhibitors and CRP. Results are given as mean ± SD or median (25th - 75th percentiles)

	Controls (n=30)	Current AAS abusers (n=37)	Former AAS abusers (n=33)	P-value
Lag time (min)	3.04 (3.00 - 3.50)	4.00 (3.45 - 4.67)	3.33 (2.71 - 4.00)	<0.001*
Peak (nmol/l)	223 ± 45	224 ± 42	246 ± 39	0.053
Time to peak (min)	6.80 (6.17 - 8.17)	8.50 (8.13 - 10.2)	7.67 (6.33 - 8.75)	<0.001*
ETP (µmol/l x min)	1.59 ± 0.28	1.85 ± 0.23	1.81 ± 0.30	<0.001**
Antithrombin activity (IU/ml)	0.89 (0.83 - 0.93)	0.98 (0.89 - 1.10)	0.90 (0.84 - 0.98)	0.012***
Protein C activity (IU/ml	0.97 (0.88 - 1.07)	1.30 (1.07 - 2.20)	1.02 (0.96 - 1.22)	<0.001*
Free protein S (IU/ml)	0.90 ± 0.15	1.13 ± 0.21	1.00 ± 0.21	<0.001*
CRP (mg/l)	0.40 (0.22 - 0.80)	1.15 (0.67-2.24)	0.52 (0.33 - 0.89)	<0.01*

*Significant difference between current AAS abusers and controls, as well as current AAS abusers and former abusers.
**Significant difference between current AAS abusers and controls, as well as former AAS abusers as controls.
***Significant difference between current AAS abusers and controls.

PB 781 | The CHA2DS2-VASc Score Predicts Bleeding Risk in a Cohort with Implantable Cardiac Monitoring Devices without Atrial Fibrillation

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Background: Our team demonstrated in published work that the CHA2DS2-VASc score is a useful and simple tool to predict the risk of stroke and cardiovascular mortality in a population without atrial fibrillation. We investigated whether CHA2DS2-VASc score can predict bleeding risk in this cohort.

Aims: To evaluate if CHA2DS2-VASc score predicts bleeding incidence in a cohort of patients with implantable cardiac devices without atrial fibrillation.

Methods: We used the Rochester Epidemiology Project from 1/10/04 - 3/7/16 to evaluate if CHA2DS2-VASc score predicted the risk of bleeding. An implantable device was required to discern the absence of atrial fibrillation. Baseline cardiovascular risk factors (age, gender, heart failure, thromboembolism, vascular disease, and diabetes) were assessed. Log-Rank test was used for comparisons. Multivariate analysis was conducted. We applied the validated ATRIA bleeding score in the same cohort.

Results: The population (n=1,606) had mean age of 69.8 years and median follow-up of 4.8 years. CHA2DS2-VASc score was divided into 3 tertiles. Bleeding was defined as GI or other hemorrhage requiring hospitalization. The highest tertile patients were older and had more comorbidity. Those with CHA2DS2-VASc score of 2-5 and 6-9 had a bleeding hazard ratio of 2.1 (P< 0.0001) and 3.02 (P< 0.001) respectively. In a multivariate modeling age, male gender and history of CHF were associated with the highest risk of bleeding (HR 1.01, P< 0.001; and 1.6, P< 0.001, 1.2 p< 0.06) respectively. Females had lower risk of bleeding (HR 0.64 P< 0.0001). There was a linear association between CHA2DS2-VASc and the risk of bleeding (HR 1.18 per score increment, p< 0.001, or 6% increase on bleeding event at end of 5th year per score increase, p< 0.001).

Conclusions: CHA2DS2-VASc predicted bleeding risk in patients with implantable cardiac monitoring device and no evidence of atrial fibrillation. Clinicians can to use the CHA2DS2-VASc score to predict the risk of bleeding in patients with multiple comorbidities.

PB 782 | PCSK9 Promotes Arterial Thrombosis in Mice

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Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) promotes the degradation of the LDLR thereby elevating plasma low-density lipoprotein cholesterol levels and the risk of coronary heart disease. Recent study has reported that plasma PCSK9 levels are elevated with acute myocardial infarction. However, the contribution of elevated plasma PCSK9 level in arterial thrombosis remains unexplored.

Aims: The authors assessed whether PCSK9 was associated with an induction of prothrombotic effect in FeCl3 carotid artery injury model. Using pcsk9 deficient mice, the authors evaluated the roles of PCSK9 in regulation of arterial thrombosis and platelet activation.

Methods: Mice were subjected to FeCl3 carotid artery injury, and the time required to form an occlusive thrombus was measured. To study the contribution of PCSK9 in arterial thrombosis, we used *Pcsk9* deficient mice and recombinant PCSK9 protein to examine occlusion time after injury. Platelet aggregation was also measured.

Results: *Pcsk9* deficient mice exhibited slow thrombotic occlusion of the carotid artery after injury. In contrast, infusion of recombinant PCSK6 protein into C57BL mice shortened the prolonged time to carotid artery occlusion. Binding assay revealed that PCSK9 can bind directly to the LDLR in platelets. Platelet aggregation and flow cytometry analysis revealed that PCSK9 protein significantly induced platelet aggregation, and surface P-selectin exposure.

Conclusions: These findings show that PCSK6, a component of cholesterol metabolism, plays a role in hemostasis and thrombosis.

PB 783 | Plasma Lipoprotein(a) Predicts Bleeding and Long-term Adverse Events in Patients with Chronic Kidney Disease who Underwent Percutaneous Coronary Intervention

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Background: Chronic kidney disease (CKD) is associated with increased risk for cardiovascular disease. Elevated serum levels of Lp(a) are associated with an increased risk of major adverse cardiac events in patients with CAD after PCI.

Aims: We aimed to determine the role of Lp(a) on long-term clinical outcomes in patients with CKD after PCI.

Methods: Consecutive patients with CKD who underwent percutaneous coronary intervention at our institution from January 2013 to December 2013 were included in this cohort study. We divided patients into 2 groups [high (n = 214) or low (n = 213)] according to median levels of Lp(a). Outcomes included 2-year risk of major adverse cardiovascular and cerebrovascular events (MACCE) and bleeding according to Bleeding Academic Research Consortium (BARC) definitions.

Results: A total of 427 patients (41.2% women, mean age 69.6±9.1 years) were recruited. In this cohort, the prevalence of prior PCI, the level of BMI, total cholesterol, LDL-C and the level of hsCRP were higher in the group with high Lp(a) than with low Lp(a). After 2-year follow up, no significant differences were observed between the two groups in all-cause mortality, unplanned revascularization and stroke. Event rate of cardiac death (5.6% vs 1.4%, p=0.018), stent thrombosis (5.6% vs 1.4%, p=0.018) and myocardial infarction (9.4% vs 2.8%, p=0.004) were significantly higher in the high Lp(a) than in the low Lp(a) group. Unadjusted cumulative incidence of 2-year MACCE was statistically different between the two groups (23.0% vs 15.4%, p=0.047). Group with high Lp(a) had higher risk of BARC bleeding (8.9% vs 4.2%, p=0.049). Differences persisted after adjustment (hazard ratio [HR] 1.64, 95% confidence interval [CI] 1.04-2.58 for MACCE and HR 2.29, 95% CI 1.51-5.15 for bleeding).

Conclusions: Our study demonstrates that a high Lp(a) level is an independent predictor of bleeding and long-term adverse events. A high Lp(a) value is associated with a poor prognosis after PCI for patients with CKD.

PB 784 | Effects of HAART on Fibrinogen, D-dimer and Protein C Levels in Patients with HIV at the University of Benin Teaching Hospital Benin City, Nigeria

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Background: Changes in haemostatic parameters are associated with HIV infection and its therapy, which could contribute to atherothrombosis and cardiovascular diseases. However, the underlying mechanism is yet to be fully understood, particularly in the Nigerian setting.

Aims: To determine the plasma fibrinogen, d-dimer and protein C levels in HIV infected patients and to evaluate the effect of HAART on these parameters.

Methods: This is a cross-sectional study. Participants were divided into three study groups; HIV seronegative control subjects, HIV seropositive subjects therapy naïve and HIV seropositive subjects on HAART. Fibrinogen levels were assessed using Clauss modified method (GIESSA). D-dimer levels were assessed using ELISA test kits (Imunoclon). Protein C activities were assessed using chromogenic methods (Technochrom).

Results: A total of 210 participants were recruited into the study comprising of 70 HIV seronegative control subjects,70 HIV seropositive subjects therapy naïve and 70 HIV seropositive subjects on HAART. The mean plasma fibrinogen in the control, HAART naïve and HAART experienced groups were 5.0 ± 1.5g/L, 6.9 ± 2.9g/L and 6.3 ± 2.3g/L respectively. There was a significant difference between means of control and HAART naïve ($p < 0.001$). The mean d-dimer level for therapy naïve was 168 ± 41mg/L and 168 ± 17mg/L for the HAART experienced, the mean difference were statistically significant when compared with the controls (92 ± 11mg/L) $p < 0.001$. The mean

protein C level for the seropositive therapy naïve and HAART experienced subjects were 0.618 ± 0.446IU/ml and 0.34 ± 0.226IU/ml respectively (p< 0.001).

Conclusions: Fibrinogen and d-dimer levels were significantly higher in HIV infected subjects and these elevations persisted despite HAART. HIV seropositive group on HAART had a much lower protein C level compared to the other two groups. Haemostasis is grossly altered in HIV infection and HAART therapy did not correct these changes.

PB 785 | Persistent Thrombin Generation at 24 Months after Acute Coronary Syndrome

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Background: Acute coronary syndrome (ACS) is triggered by thrombosis after rupture or erosion of an atherosclerotic plaque. Thrombin generated at the site of vascular injury plays a key role in regulating thrombus formation and serves as a potent platelet activator. Antiplatelet therapy has been the cornerstone for preventing recurrent ACS.

Aims: We monitored longitudinal changes of thrombin generation (TG) indices up to 24 months in ACS patients on dual antiplatelet therapy (DAPT).

Methods: Citrated blood was obtained 48-72 hours after percutaneous coronary intervention (PCI) from ACS patients (n = 110). The 3 thrombin generation indices: rate of thrombin generation or velocity index (VI), endogenous thrombin potential (ETP) and Peak thrombin (PT) concentration were measured using Calibrated Automated Thrombography (CAT). Age-matched controls (n=25) were selected from healthy blood donors. TG measurements were repeated in ACS patients at 6, 12 and 24-month.

Results: Baseline characteristics of patients and controls are summarized in table 1. All the patients were on aspirin and a P2Y12 antagonist (Prasugrel 74.5%, Ticagrelor 14.5%, Clopidogrel 10.9%) for 1 year after the ACS event and subsequently on single antiplatelet therapy. All TG indices were significantly elevated in ACS patients when compared to controls at baseline. At 6, 12 and 24 months, the VI and PT were lower than at baseline but indices remained persistently elevated when compared to controls (figure 1). Surprisingly, ETP declined faster than VI and PT.

Conclusions: Despite treatment with new generation P2Y12 antagonists, patients with recent ACS have persistent elevation of two indices of thrombin generation at 24 months after ACS. Further studies should investigate the role of TG in guiding antithrombotic therapy after ACS.

PB 786 | A Chemical Screen for Small Molecule Modulators of Fibrinogen Production

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Background: Plasma fibrinogen levels associate with the risk of cardiovascular disease (CVD) endpoints. Despite the expectation that high fibrinogen concentrations affect fibrin clot formation, there is no direct evidence for a clinical cause-effect relationship between high plasma fibrinogen and CVD events. As agents have not been developed to decrease plasma fibrinogen levels it has not been possible to assess a possible protective effect of lowering fibrinogen on CVD endpoints.

Aims: By screening a library of defined off-patent pharmacological agents we aim to identify compounds that can lower fibrinogen production in cell culture prior to testing in vivo.

Methods: 1280 compounds were tested for their ability to alter fibrinogen production by hepatocellular carcinoma-derived HepG2 cells. Secreted fibrinogen was measured by enzyme-linked immunosorbent assay in cell-conditioned medium and normalized to total protein in cell lysates. The screen was completed three times, with technical triplicate values measured in each of the three biological replicates. Data were adjusted for medium alone on all assay plates and effects of compounds compared to vehicle (DMSO).

Results: A left-skewed frequency distribution was observed when plotting fibrinogen level (fibrinogen/total protein ratio) as the variable and normalizing to DMSO. 59 compounds reduced fibrinogen by at least 25% without reducing total protein by more than 10% (fibrinogen range -26% to -68%). Nominal p-values for fibrinogen level in compound-treated versus DMSO using paired t-tests were < 0.05 for 33 of these (n=3). These molecules will be tested for dose-response efficacy, and a selection assessed for fibrinogen modulation in zebrafish.

Conclusions: By screening components of approved off-patent medicines, we identified modulators of fibrinogen production. Such molecules could be developed for assessing a potential protective effect of lowering plasma fibrinogen in cardiovascular disease.

PB 787 | The Novel 2MACE Score Predicts Cardiovascular Events in Atrial Fibrillation Patients

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Background: Atrial fibrillation (AF) increases the risk for stroke and mortality, but also for non-embolic cardiovascular events such as acute coronary syndrome or heart failure (HF).

Aims: To investigate the incidence of non-embolic adverse events in a ‚real world' cohort of AF patients and to validate the 2MACE score [2 points (metabolic syndrome, age ≥75); 1 point (history of myocardial infarction (MI)/revascularization, congestive HF and stroke/TIA/thromboembolism)] as predictor of major adverse cardiovascular events (MACEs).

Methods: 693 consecutive AF patients were recruited and the 2MACE score was calculated. All non-embolic adverse events and MACEs (nonfatal MI/revascularization, HF and cardiovascular death) were recorded during a median of 7.2 (IQR 6.2-7.9) years. ROC curves comparison, reclassification and discriminatory analyses and decision curve analysis, were performed in order to compare predictive ability of the 2MACE score and its clinical usefulness against CHA_2DS_2-VASc. The study protocol was approved by the Ethics Committee from our institution and performed in accordance with the Declaration of Helsinki. All patients gave informed consent.

Results: Median 2MACE score was 2 (IQR 1-3). During follow-up, there were 113 MACEs (2.26% per year). Annual event rate was higher in the medium/high risk group (i.e. 2MACE score ≥3) (3.33%/year *vs*. 1.45%/year, p< 0.001). The 2MACE score ≥3 was significantly associated with MACE (HR = 2.80, 95% CI 1.90-4.10, p< 0.001). The predictive performance of 2MACE according to the ROC curve was 0.67, and was significantly higher from that of CHA_2DS_2-VASc (0.67; 95% CI 0.63-0.70 *vs*. 0.62; 95% CI 0.58-0.66, p< 0.001). Decision curve analysis demonstrates improved clinical usefulness of the 2MACE compared to the CHA_2DS_2-VASc score.

TABLE 1 Comparison of the ROC curves, IDI and NRI of the CHA2DS2-VASc and 2MACE scores

	C-index	95% CI	p*	IDI	p	NRI	p
2MACE vs. CHA2DS2-VASc	0.67 vs. 0.62	0.63-0.70 0.58-0.66	<0.001	0.02	0.002	0.22	<0.001

CI = Confidence Interval; IDI = integrated discriminatory improvement; NRI = net reclassification index. *for c-index comparison

Conclusions: In ‚real world' AF patients, the 2MACE score is a good predictor of MACE. A score ≥3 should be used to categorize patients at ‚high risk', in identifying patients at risk of MACE.

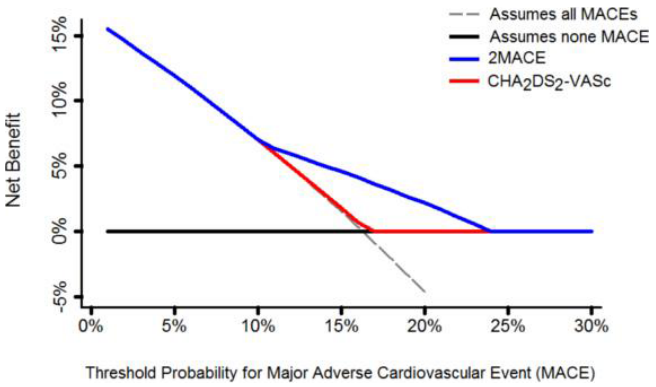

FIGURE 1 Decision curves for the 2MACE and CHA2DS2-VASc scores

PB 788 | Altered Platelet Lipidome Influences Thrombotic Disposition: Implications in Acute Coronary Syndrome and Modulation by the CXCL12-CXCR4-CXCR7 Axis

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Background: Hyperlipidaemia propagates thrombosis, while acute coronary syndrome (ACS) patients show increased platelet oxLDL binding, correlating with activation status. Besides, elevated platelet CXCR7 surface expression and altered plasma CXCL12 levels in ACS, have significant prognostic impact. Activation also influences intraplatelet lipid metabolism.

Aims: To characterize the dynamics of platelet lipidome in coronary artery disease (CAD) patients and explore the influence of CXCL12-CXCR4/CXCR7 axis on pro-thrombotic platelet-lipid association.

Methods: Flowcytometry, untargeted lipidomics analysis, confocal microscopy, impedance aggregometry, live imaging-scanning ion conductance microscopy, thrombinoscopy, *ex/in vivo* thrombosis assay.

Results: Enhanced platelet-oxLDL in CAD correlated positively with platelet CXCR7 surface expression, but inversely with CXCR4, and was further elevated in ACS with intracoronary thrombi showing oxLDL deposition in platelet-rich areas.LDL/oxLDL enhanced reactive oxygen species generation,lipid peroxidation, whereby platelets executed intracellular (per)oxidative lipid modifications. Lipidomic analysis revealed enhanced-intraplatelet oxidized phospholipids denoting redox stress, cholesteryl-esters, triglycerides despite normal plasma levels, sphingomyelin-ceramides denoting increased sphingolipid me tabolism,acylcarnitine,PLA2(lysoPC), PLC(diacyglycerol) metabolites in ACS(STEMI). LDL/oxLDL induced degranulation, $\alpha_{IIb}\beta_3$-integrin activation, aggregation, apoptosis, thrombin generation, dynamic shape change, also thrombus formation *ex vivo* and *in vivo*. LDL-oxLDL

enhanced CXCL12 release, decreased CXCR4, while enhanced CXCR7 expression. CXCL12-CXCR4-CXCR7 prompted LDL/oxLDL uptake by enhancing availability of scavenger receptors, also synergistically augmented LDL/oxLDL-induced oxidative and thrombogenic functions.

Conclusions: Pro-oxidative platelet lipidome favours thrombotic disposition, potentially modulated by CXCL12-CXCR4-CXCR7 axis to influence progression and prognosis in CAD.

PB 789 | A Time-course Analysis of Activated Factor VII-antithrombin Complex Plasma Concentration in the Follow-up of Patients with Angiographically-demonstrated Coronary Artery Disease

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Background: activated factor VII-antithrombin (FVIIa-AT) complex reflects tissue factor exposure to the blood, thus being a biomarker of prothrombotic diathesis, and has been associated with mortality in patients with coronary artery disease (CAD).

Aims: to evaluate FVIIa-AT plasma concentration in CAD patients in a two-points (baseline and during the follow-up) time-course analysis.

Methods: within the original prospective study showing baseline levels of FVIIa-AT as a predictor of cardiovascular mortality, in 178 survived CAD patients (85.4% males, mean age 58.2±8.5 years) a second blood drawn for FVIIa-AT evaluation was performed in occasion of an ambulatory visit (after a median follow-up of 63 months). FVIIa-AT plasma levels were measured by ELISA.

Results: during the follow-up 58 subjects reported angina symptoms, 7 had myocardial infarction (MI), and 35 underwent new coronary revascularization. FVIIa-AT levels at second control were significantly correlated with those at baseline (R=0.262, P< 0.001) and were significantly higher than those at baseline (P< 0.001), except for patients having begun anticoagulant therapy with warfarin. Neither baseline nor second control FVIIa-AT levels were associated with angina, MI, or coronary revascularization. On the other hand, subjects with angina had a higher increase of FVIIa-AT levels from baseline to the second control (50.1±55.9 *versus* 31.4±54.4 pM, P=0.035), as well as subjects with MI (79.4±52.2 *versus* 35.8±55.1 pM, P=0.040), while no significant difference was found for coronary revascularization (49.9±48.8 *versus* 34.4±56.6 pM, P=0.139). Subjects with an increase higher than the median value (>36.4 pM) had an about two-fold increased risk of angina/MI after adjustment for sex, age, time of follow-up, and warfarin therapy (OR 2.05 with 95%CI 1.05-4.02).

Conclusions: in the setting of secondary prevention of CAD a larger time-related increase of FVIIa-AT levels during follow-up is associated with angina and non-fatal MI.

PB 791 | Up-Regulation of HO-1 by CORM-2 Attenuates Thrombin-Induced COX-2 Expression and Hypertrophy in Human Cardiomyocytes

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Background: Heme oxygenase (HO)-1 and exerts anti-inflammatory action in various models. However, the detailed mechanisms underlying CO-induced HO-1 expression in primary human cardiomyocytes remain largely unidentified.

Aims: We used primary human cardiomyocytes as a model and applied CO releasing molecule (CORM)-2 to investigate the relationship of CO and HO-1 expression and its protecting effects.

Methods: COX-2 and HO-1 expression were determined by Western blotting, real time-PCR, and promoter analyses. The signaling molecules were investigated by pretreatment with respective pharmacological inhibitors or transfection with siRNAs. The interaction between COX-2 promoter and transcription factors was determined by chromatin immunoprecipitation (ChIP) assay. Finally, the effect of CORM-2 on COX-2 expression and PGE_2 synthesis induced by thrombin was determined by Western blot and EIA to investigate the role of HO-1 expression protecting against thrombin-mediated responses.

Results: We found that thrombin-induced COX-2 expression, PGE_2 release and cardiomyocyte hypertrophy markers (increase in ANF/BNP, α-actin expression and cell surface area) was attenuated by pretreatment with CORM-2 which was partially reversed by hemoglobin (Hb) or ZnPP (an inhibitor of HO-1 activity), suggesting that HO-1/CO system may be of clinical importance to ameliorate heart failure through inhibition of inflammatory responses. CORM-2-induced HO-1 protein expression, mRNA and promoter was attenuated by pretreatment with the inhibitors of Pyk2 (PF431396), PDGFR (AG1296), PI3K (LY294002), Akt (SH-5), p38 (SB202530), JNK1/2 (SP600125), FoxO1 (AS1842856) and Sp1 (mithramycin A). The involvement of these signaling components was further confirmed by transfection with respective siRNAs, consistent with those of pharmacological inhibitors.

Conclusions: These results suggested that CORM-2-induced HO-1 expression is mediated through a Pyk2/PDGFR/PI3K/Akt/FoxO1/Sp1-dependent manner and exerts a cytoprotective effect in human cardiomyocytes.

PB 792 | High Antithrombin Levels in Women Are Associated with a Lower Risk of Recurrent Arterial Thromboembolism or Death. Results from the Observational ATTAC-Study

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Background: Inherited thrombophilia is associated with venous and arterial thromboembolism (ATE). Antithrombin deficiency is a strong risk

factor for venous thromboembolism, but data on antithrombin levels and the risk of ATE are sparse. An association is not clearly established. A role of antithrombin levels in recurrent ATE is largely unknown.

Aims: First we aimed to investigate if antithrombin levels are associated with first ATE-events at a young age. The second aim was to investigate if antithrombin levels are associated with recurrent ATE or death.

Methods: In a case-control study, 620 patients with a recent premature first event of coronary heart disease, cerebrovascular disease or peripheral artery disease and 461 community controls were included. Antithrombin activity levels were measured. 323 subjects of this study that had presented with a cardiac event in the case-control study participated in a prospective cohort study. Time to a recurrent ATE or death was recorded.

Results: No differences in antithrombin levels were found between the cases and controls. Only after correction for all for classical risk factors for ATE risk factors logistic regression showed a relationship between antithrombin levels and ATE (HR 1.35, 95%CI 1.01-1.81). When comparing the three subgroups and controls also no differences were found. In the follow-up study no increased risk for recurrent ATE or death was found in subjects with low versus high antithrombin levels overall. However women with lower antithrombin levels had a higher risk (HR4.53, 95%CI 1.25-16.34) than women with levels above median antithrombin levels. In men, no such difference was found, and the risk was similar to the risk in women with low antithrombin levels.

Conclusions: Antithrombin levels are not clearly associated with first ATE at a young age. Women with high antithrombin levels have a lower risk of recurrent ATE or death than women with low antithrombin levels, whereas in men antithrombin levels do not influence risk of recurrent ATE or death.

PB 793 | The Role of Fasting Glucose Levels and Self-reported Diabetes in the Risk of a First Event of Venous Thrombosis: The MEGA Case-control Study

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Background: Conflicting results have been reported on the risk of venous thrombosis (VT) for diabetes mellitus (DM) and whether individuals with hyperglycaemia in the non-diabetic population are at an increased risk of a first event of VT.

Aims: To assess whether self-reported DM and hyperglycaemia in a non-diabetic population are associated with an increased risk of a first event of VT.

Methods: Analyses were performed in the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis (MEGA) case-control study. Pregnant women and individuals with malignancy were excluded. In the analysis of glucose levels and the risk of VT, participants with self-reported DM (n=145) were further excluded. Fasting glucose levels were categorized based on the World Health Organization (WHO) criteria (< 6.1 mmol/L [reference], 6.1-7.0 mmol/L, ≥7.0 mmol/L) as well as using the fasting glucose distribution of the control subjects. Logistic regression models were performed, after adjusting for age, sex, body mass index, statin use, estrogen use, and C-reactive protein levels.

Results: In the analysis of fasting glucose and the risk of VT, 1,888 patients (median age, 49 years; 51% women) and 2,531 controls (median age, 50 years; 50% women) were included. Using the lowest category of the WHO classification as the reference, the odds ratios for VT in the second and third categories of fasting glucose levels were 0.98 [95% CI 0.69-1.37] and 0.97 [95% CI 0.58-1.63]. Similar results were observed from the classification by glucose distribution in the controls. In the analysis of self-reported DM and the risk of VT, 1,930 patients (147 self-reported diabetes cases) and 3,331 controls (184 self-reported diabetes cases) were included. The odds ratio for VT in the diabetic population compared with the non-diabetic population was 1.10 [95% CI 0.78-1.56].

Conclusions: Neither increased levels of fasting glucose in non-diabetic individuals nor self-reported diabetes was associated with risk of a first event of VT.

PB 794 | Urinary 11-dehydrothromboxane B2 Measurement Assays and their Clinical Correlation

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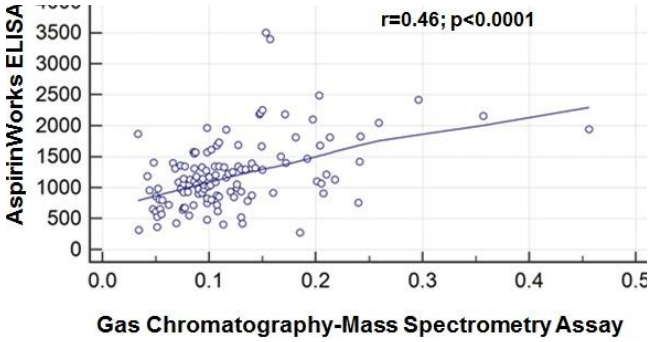
Background: High urinary 11-dehydrothromboxane B_2 (UTB) is linked with adverse outcomes in cardiovascular (CV) disease patients on aspirin therapy. We correlated UTB levels determined by AspirinWorks ELISA and gas chromatography-mass spectrometry (GC/MS) and determined their association with CV risk factors; hypercoagulability (HC), high platelet reactivity (HPR), and inflammation.

Aims: To compare UTB levels measured by AspirinWorks ELISA and gas chromatography-mass spectrometry (GC/MS) and to determine their relation to CV risk factors.

Methods: Blood and Urinary samples (n=122) were collected multiple times from Type 2 diabetes patients (n=37) on 81-325mg aspirin. HC was defined as >69mm platelet-fibrin clot strength by TEG. HPR was defined as any 2: 2mM AA-induced aggregation ≥ 10%, 4mg/ml collagen-induced aggregation ≥ 70%, AU*min ≥ 30 by Multiplate Aspitest, and aspirin reaction units (ARU) ≥550 by VerifyNow. Inflammation was defined as hs-CRP >3 mg/L.

Results: UTB levels measured by ELISA weakly correlated with GS/MS (r=0.46, p< 0.0001) Figure).



FIGURE

HC patients (41%) had higher UTB by ELISA (p=0.013) and GS/MS (p=0.041). Patients with HPR (51%) had higher UTB by ELISA (p< 0.05) but not for GS/MS (p≥0.05 for all). Patients with inflammation (54%) had higher UTB by ELISA (p=0.014). By INOVA, UTB increased in a stepwise fashion with the number of risk factors more closely by ELISA (p< 0.001) than GS/MS (p=0.041).

Conclusions: Identification of hypercoagulability, high platelet reactivity, and inflammation by UTB is dependent on the assay with better identification by ELISA. Our findings are relevant for future investigations of UTB as a high cardiovascular risk marker.

PB 795 | The Value of sPESI for Risk Stratification in Patients with Pulmonary Embolism

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Background: Various risk stratification methods exists for patients with pulmonary embolism (PE).

Aims: Use the simplified Pulmonary Embolism Severity Index (sPESI) as a risk stratification method to understand the PE population within the Veterans Health Administration (VHA).

Methods: Retrospective review using VHA dataset. Entry criteria were age > 18 with ≥1 inpatient diagnosis for PE (index date) between October 2011- June 2015, continuous enrollment for ≥12 months pre- and 3 months post-index date. We defined a sPESI score of 0 as low risk, and all others as high risk. Hospital-acquired complications (HACs), 90-day follow-up PE-related outcomes, and health care utilization and costs were compared between high-risk (HRPE) and low-risk (LRPE) patients.

Results: Of 6,746 PE patients, 95.4% were men, 67.7% were white, 22.0% were African American; LRPE occurred in 28.4%, and HRPE 71.6%. Versus HRPE, LRPE patients had lower Charlson Comorbidity Index scores and other baseline comorbidities, fewer HACs (11.37% vs 19.97%, p< 0.0001), less bacterial pneumonia (10.58% vs 22.33%, p< 0.0001), and shorter inpatient length of stay (8.77 vs 11.15 days, p=0.0050) during the index hospitalization. During follow-up, LRPE patients had fewer PE-related outcomes of recurrent venous thromboembolism (4.38% vs 6.03%, p=0.0077), major bleeding (1.20% vs 1.93%, p=0.0382), death (3.70% vs 16.16%, p< 0.0001). LRPE also had fewer inpatient, outpatient, and pharmacy visits per patient, and lower total health care costs ($12,021 vs $16,911, p< 0.0001) (Figure 1).

Conclusions: Using the sPESI score identifies a PE cohort with a lower clinical and economic burden.

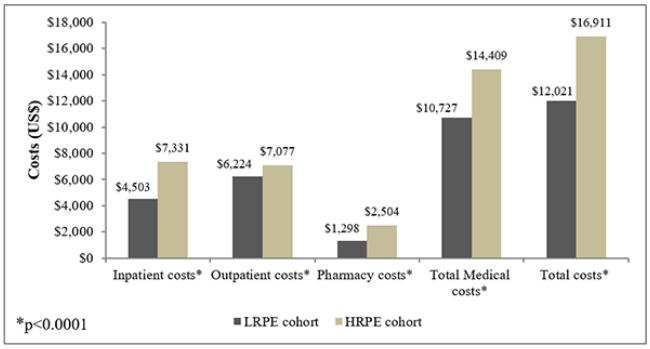

FIGURE 1 Health Care Costs among LRPE vs. HRPE Patients

PB 796 | D-Dimer but Not Platelet Function Testing Improves Risk Stratification in Patients Treated with Elective PCI

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Background: Integration of anatomical and clinical variables enhanced risk stratification following PCI.

Aims: Aim of the study was to assess the potential role of D-Dimer and platelet reactivity (PR) for improvement of conventional scoring algorithms in all-comer patients treated with elective PCI.

Methods: Single center data from 188 consecutive patients (76% males, age 61±10,7 yrs) undergoing successful PCI with new-generation drug-eluting stent implantation and receiving 1 year dual antiplatelet therapy were prospectively collected. Baseline D-Dimer level and on-treatment PR (VerifyNow P2Y12) were assessed in addition to conventional MACE scores (SYNTAX, clinical SYNTAX, SYNTAX Score-II (SS-II), ACEF).

Results: The frequency of major adverse cardiac events (MACE : vascular death, acute coronary syndrome, stroke/transient ischemic attack) at a median time of 3.2 years was 15,4% (4,8/100 patient years). Among all scores only SS-II was predictive of MACE. Age and sex adjusted Cox relative risk (RR) for high SS-II (cutoff ≥40) was 3.9 (95% CI 1.7-9.1, p=0.001). D-Dimer and PR were tended to be higher in upper

vs lower quintiles of SS-II distribution: 777,1±100,7 vs 379±48,1 ng/ml and 178,7±14,6 vs 159±11,9 PRU respectively. PR has no prognostic impact. D-Dimer (cutoff ≥783 ng/ml) per se has weak association with MACE: age and sex adjusted RR = 2.2; 95% CI 0.76-6.58, p=0,1. Adding D-Dimer improves the prognostic capacity of the SS-II. Frequency of MACE in pts with simultaneous elevations of both variables was 27,3% compared with 10,6% in pts with low SS-II and low D-Dimer (RR = 5.9, 95% CI 1.7-19.7, p=0.004).

Conclusions: D-Dimer may be considered as a valuable prognostic biomarker to improve the prediction of MACE in addition to well-known scoring systems in patients after elective PCI.

PB 797 | Increased Extracellular Nucleosome Levels, Biomarkers of Cell Death, in Atrial Fibrillation Patients Compared to the Normal Population

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Background: Atrial fibrillation (AF) is currently the most common cardiac arrhythmia encountered in clinical practice and a major cause of morbidity and mortality among adults. Extracellular plasma nucleosomes (PNs) are complexes of DNA and histones that are released during cell death. Histones have been shown to function as DAMPs when they are translocated from the nucleus to the extracellular space. These nucleosomes mediate inflammatory and thrombotic responses and could serve as biomarkers in atrial fibrillation.

Aims: The primary aim of this study was to demonstrate that PN levels are increased in atrial fibrillation and may contribute to the pathogenesis of this syndrome.

Methods: The concentration of PNs in plasma samples of atrial fibrillation patients (n=43), aged normals (n=35), and young normals (n=29) were measured using the Cell Death Detection ELISA PLUS assay (Roche Diagnostics, Mannheim, Germany). For this study, the baseline plasma samples of atrial fibrillation patients were analyzed.

Results: In comparison to the plasma from aged normals (11.42 ± 9.15 Arbitrary Units (AU)) and young normals (6.42 ± 0.87 AU; p < 0.0001), the plasma collected from atrial fibrillation patients (19.42 ± 20.5 AU; P = 0.0015) demonstrated much higher levels of PNs. When comparing the concentration of nucleosomes in plasma, the atrial fibrillation patients (306.1 ± 328.86 µg/mL) had much higher levels than the aged normals (177.9 ± 146.7 µg/mL; p = 0.0015).

Conclusions: PNs were elevated in atrial fibrillation when compared to both aged and young normals, indicating an increased release of nucleosomes, suggesting increased cell death. Due to the involvement of inflammation and thrombosis in the pathology of atrial fibrillation, the increased circulating nucleosome level in patients with atrial fibrillation implies that PNs may activate multiple pathogenic responses including immunologic, thrombotic and vascular responses.These results also suggest that PNs may provide a new biomarker for atrial fibrillation.

PB 798 | vWF Thr789Ala Genetic Variants Correlates with the Type of Myocardial Ischemia in Egyptian Patients

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Background: von Willbrand factor antigen (vWF: Ag) levels were shown to contribute to the risk cardiovascular disease. Single nucleotide polymorphism (SNP) at codon 789 results in replacement of amino acid threonine by alanine (Thr789Ala) and is thought to affect factor level and activity.

Aims: The aim was to adress the impact of vWF Thr789Ala gene polymorphism on the risk of critical myocardial ischemia.

Methods: The study included 112 patients, 31 unstable angina (UA) and 81 myocardial infarction (MI), and 118 healthy controls. vWF :Ag level was measured by ELISA (Diapharma, West Chester, USA). The gene analysis was carried out by polymerase chain reaction using restriction fragment length polymorphism (RFLP-PCR) principles (Bioline, Toronto, Canada).

Results: There was significant correlation between the vWF genetic variant and the type of ischemia as Ala789 homozygous was seen in 25 patients with UA (80.6%), in 28 MI patients (34.6%) compared to 56 controls (47.5%). Thr789Ala heterozygous was seen in 39 MI patients (48.1%) and in only 2 (6.5%) of those with UA compared to 39 of the controls (33.1%). The genotype distribution was significantly different among the 3 groups, p < 0.001, and between the groups with UA and MI, p < 0.001. Our data showed the AA genotype to be an independent risk factor for UA with an estimated risk of 3.7 per minor allele (p < 0.001). Interestingly, T/A genotype was associated with 13.46 folds estimated risk of MI compared to UA (p < 0.001). vWF :Ag levels were significantly higher in patients compared to controls (111.29 ± 24.43 Vs 71.13 ± 13.72 respectively, p < 0.0001).

Conclusions: vWF Thr789Ala genotype is independent risk factor for UA and has significant impact on disease phenotype in Egyptian patients with myocardial ischemia. It should be incorporated in a risk assessment model to identify individual patient risk and guide the management plan.

PB 799 | The Incidence and Prognosis of Venous Thromboembolism (VTE) and Arterial Thromboembolism (ATE) Associated with Oral Contraceptives by Age Groups in Japan

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Background: Thromboembolism is an unavoidable adverse event of combined oral contraceptives (COCs), and the reported events increased year by year in Japan.

Aims: We analyzed the incidence and prognosis of thromboembolism associated with COCs by age groups in Japan.

Methods: 394 venous thromboembolism (VTE) events and 154 arterial thromboembolism (ATE) events associated with COCs were analyzed from the Pharmaceuticals and Medical Devices Agency database from 2004 to 2013. In a statistical analysis, a good-prognosis group included recovery cases and a poor-prognosis group involved unrecovered cases with some sequela or fatal cases definitely caused by thromboembolism. The significant difference between both groups was calculated by Pearson´s chi-square test, and the age-specific tendency was examined by Cochran-Armitage test. Statistical analysis was done using SPSS version 20. The study was approved by the Ethics Committee of Hamamatsu University School of Medicine.

Results: 548 events involved DVT only, cerebral infarction, PE with DVT, PE only, cerebral vein thromboses, other venous thromboses, coronary heart diseases, and other arterial thromboses in that order. Thromboembolic events occurred mostly in the 40s, followed by the 30s, 20s, 50s and teens by age. Judging from whole VTE and ATE events by age, the VTE rate decreased whereas the ATE rate significantly increased with advancing age (p=0.0041). Recovery cases were the most common, followed by unrecovered cases and fatal cases. In VTE and ATE, recovery cases were 86.4% and 70.9%; unrecovered cases were 10.1% and 28.2%; and fatal cases were 3.6% and 0.9%, respectively. All ATE cases had a significantly much poorer prognosis in comparison with all VTE cases (p< 0.0001).

Conclusions: Thromboembolic events were the most frequent in the 40s, followed by the 30s. The VTE rate decreased whereas the ATE rate significantly increased with advancing age. All ATE cases had a significantly much poorer prognosis in comparison with all VTE cases.

PB 800 | Implementation of CHA2DS2-VASc and HAS-BLED Scoring E-tool for Aatrial Ffibrillation Patients into Routine Clinical Practice in North Estonia Medical Centre

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Background: European Society of Cardiology (ESC) guidelines recommend to estimate stroke and bleeding risk for all atrial fibrillation (AF) patients (1). Simple and clinically applicable stroke risk score CHA2DS2-VASc and bleeding risk score HAS-BLED are recommended tools for selecting AF patients who would benefit from oral anticoagulation and in whom usage of anticoagulants is inappropriate because of the high risk of bleeding. Earlier in our hospital these scores were possible to calculate manually and then results were reported in patients' records. However, due to inconvenience of scoring, it was frequently not performed and could lead to incorrect treatment decisions. Since January 1st 2017 a new e-tool for risk scoring and reporting was implemented into e-record of the North Estonia Medical Centre (NEMC) and made mandatory for all patients with AF.

(1) European Heart Journal (2016) 37, 2893-2962

Aims: Aim for this study was to describe first results of implementing e-tool for CHA2DS2-VASc and HAS-BLED scoring of AF patients.

Methods: All patients (incl patients discharged from hospital or treated as outpatients) with diagnosis of AF were selected from the hospital database from January 1st 2016 to January 30th 2017. Data about age, gender, risk scores reporting, physician and departments/clinics were collected. Data analysis was performed by MS Excel 2010.

Results: There were total 12386 cases of AF during the period studied. Baseline characteristics of patients are listed in table 1 and risk scores reporting in table 2.

TABLE 1 Baseline characteristics of the patients

	2016	January 2017
Patients (n)	11659	727
Female sex (n) (%)	5865 (50.3)	365 (50.2)
Male sex (n) (%)	5794 (49.7)	362 (49.8)
Age- years (SD)	71,8 (11.7)	73,3 (11.6)
Female age- years (SD)	75,4 (9.7)	77,3 (9.5)
Male age- years (SD)	68,2 (12.5)	69,2 (12.0)

TABLE 2 Risk scores reporting in AF patients

	2016		January 2017	
	Patients (n)	Risk scores reported (n) (%)	Patients (n)	Risk scores reported (n) (%)
Anaesthesiology clinic	3042	70 (2.3)	225	12 (5.3)
Internal medicine clinic	7337	1564 (21.3)	448	369 (82.4)
Other clinics	1280	29 (2.3)	54	40 (74.1)
Total	11659	1663 (14.3)	727	421 (57.9)

Conclusions: The e-tool increases the rate of reporting risk scores and could improve patient management among the population of patients with AF. However, recognising of AF diagnosis, scoring and reporting in non-internal medicine clinics should be further evaluated, too.

PB 801 | Heightened Thrombogenicity in Women with Myocardial Infraction No Obstructive Coronary Artery Disease: The Difference between the Sexes

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Background: Sex differences exist in the prevalence and severity of myocardial infraction no obstructive coronary artery disease (MINOCA). Symptomatic women are less likely to have obstructive CAD than men with similar symptoms, and tend to have a significantly increased risk of heart attack or death.

Aims: The aim of the study is to compare thrombogenicity, lipid profile, and urinary 11-dehydrothromboxane B_2 ($dTxB_2$) among men and women with MINOCA

Methods: Consecutive patients (n=139) undergoing elective cardiac catheterization with ANOCA were analyzed. Patients with prior percutaneous coronary intervention and coronary artery bypass grafting were excluded. Blood and urine samples for thromboelastography, lipid profile, $dTxB_2$ and oxidized-LDL were obtained prior to cardiac catheterization.

Results: Women with MINOCA (n=76) had a higher prevalence of hypertension, and higher total cholesterol, total LDL, LDL subtypes1 and 2 , total HDL, HDL subtypes 2 and 3, and ApoA1 compared to men with MINOCA ($p < 0.05$ for all). Women also had greater thrombin-induced platelet fibrin strength (TIP-FCS), greater fibrinogen activity, and clotting index (Table 1). On linear regression, only TIP-FCS remained significantly higher in women (p=0.0002). Urinary $dTxB_2$ and oxidized-LDL were similar between the two groups.

TABLE 1

	Men (n=63)	Women (n=76)	p value
TIP-FCS (mm)	64.6+/-6.2	67.9+/-4.5	0.001
Clotting Time, R (min)	7.3+/-2.4	7.1+/-2.1	0.64
Clotting Index, CI	-0.62+/-2.71	0.30+/-2.22	0.05
Fibrinogen activity (degrees)	63.2+/-7.4	66.7+/-7.1	0.01

Conclusions: Heightened thrombogenicity with a greater platelet response to thrombin may play an important mechanistic role in the poorer clinical outcomes observed in women with angina and ANOCA compared to men. Further larger translational studies potentially targeting the thrombin pathway are warranted.

PB 802 | Role of Matrix Metalloproteinase-2 and Correlation with the Anticoagulant System in Diabetes

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Background: Diabetes mellitus is a metabolic disorder frequently associated with vascular complications that worsen the life expectancy and quality of life of many patients worldwide. Matrix metalloproteinase-2 (MMP-2) is a gelatinase with multiple functions including proteolysis, tissue remodeling, angiogenesis and cell-cycle control. Circulating MMP-2 is increased in diabetes but its relationship with the anticoagulant system is unknown.

Aims: To evaluate the role of MMP-2 in diabetes mellitus and its relationship with the anticoagulant system.

Methods: Experimental model of diabetes mellitus was developed by injection of streptozotocin in transgenic mice overexpressing MMP-2 and in wild type mice. Blood glucose and insulin levels were measured after induction of diabetes. The grade of pancreatic β-cell apoptosis was evaluated. The mechanistic action of MMP-2 was evaluated using MIN6 β cells. Blood samples from 30 patients with type 2 diabetes and 37 healthy volunteers were available to measure circulating MMP-2 and total protein S. All subjects gave written informed consent, the study was approved by the Institutional Ethics Committee and performed following the Helsinki declaration.

Results: Compared with wild type mice with diabetes, the blood glucose level was significantly decreased and the grade of insulin secretion was significantly enhanced in hMMP-2 transgenic mice with diabetes. The number of insulin-positive cells in pancreatic islet was significantly preserved in diabetic hMMP-2 transgenic mice compared to their wild type counterparts.

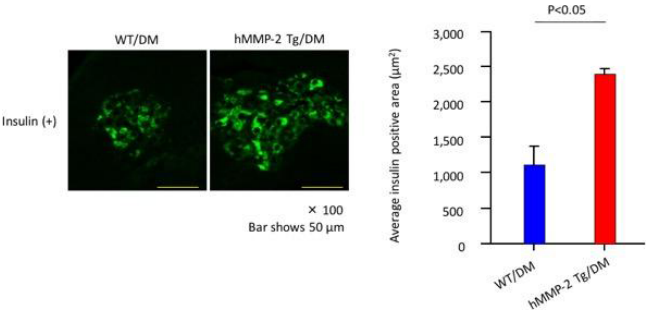

FIGURE 1 The number of insulin-positive cells in pancreatic islet was significantly preserved in diabetic hMMP-2 transgenic mice compared to controls

MMP-2 showed apoptotic activity by stimulating the activation of the AKT pathway in pancreatic cells. Circulating MMP-2 was significantly increased in diabetic patients compared to healthy controls and showed a strong tendency (p=0.05) to correlate with circulating protein S.

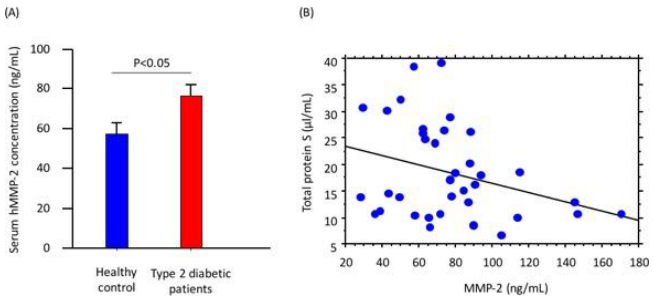

FIGURE 2 (A) Circulating MMP-2 levels in diabetic patients and healthy controls. (B) Correlation between MMP-2 and protein S in diabetic patients

Conclusions: Human MMP-2 is protective in experimental diabetes by activating the AKT pathway and correlates with protein S in diabetic patients.

PB 804 | Extracellular Nucleosomes, Markers of Cell Death, are Elevated in End-Stage Renal Disease Independent of Circulating Microparticle-associated Tissue Factor

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Background: Extracellular nucleosomes in plasma (PNs) are complexes of DNA and histones that are released during cell death. In acute kidney injury, there is an increased release of nucleosomes with decreased nucleosome clearance. Microparticle-associated tissue factor (MP-TF) are released during cell death and mediate thrombotic responses.

Aims: The objective of this study is to determine the interdependance of nucleosomes and microparticles in ESRD.

Methods: The concentrations of PNs in ESRD patients (n = 90) and healthy volunteers (n = 50) were measured using the Cell Death Detection ELISA PLUS assay (Roche Diagnostics, Mannheim, Germany). MP-TF levels were measured using the ZYMUPHEN MP-TF kit (Hyphen BioMed, Neuville-sur-Oise, France).Th elevels of both PNs and MP-TF were also correlated with WBCs, RBCs and platelets to determine the origin of measured PNs.

Results: In comparison to the plasma from healthy volunteers (6.74 ± 13.7 Arbitrary Units (AU)), the levels of PNs in ESRD patients were higher (15.5 ± 14.1 AU; $p < 0.0001$). Similarly, MP-TF levels were elevated in ESRD patients (3.00 ± 1.42 pg/mL; $p < 0.0001$) compared to normal (0.363 ± 0.263 pg/mL). There was no correlation between PNs and MP-TF in ESRD patients ($r = 0.077$; $p = 0.501$). Moreover, there was no correlation between PNs and platelets ($r = 0.067$; $p = 0.543$) and RBCs ($r = 0.083$;
$p = 0.447$). However, the PNs showed a positive correlation with WBCs ($r = 0.223$; $p = 0.042$). There was no correlation between MP-TF and WBCs ($r = -0.057$; $p = 0.632$) and RBCs ($r = -0.042$; $p = 0.722$), but a positive correlation was observed between MP-TF and platelets ($r = 0.237$; $p = 0.042$).

Conclusions: PNs were elevated in ESRD patients, suggesting increased cell death. The observed correlation between PNs and WBCs suggests that the detected PNs are derived from WBCs. A lack of correlation between PNs and MP-TF suggests that the MP-TF increase is independent of the pathophysiologic processes responsible for abnormal PN generation in ESRD patients.

PB 805 | Stroke Prevalence in Patients with Different Patterns of Atrial Fibrillation: Cross-sectional Study of Moscow Registry

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Background: 2016 ESC guidelines for the atrial fibrillation (AF) patients management states that stroke risk is equal in all AF patterns, paroxysmal, persistent or permanent. But some recent studies´ve shown weak evidence, that AF pattern may influence stroke risk.

Aims: To estimate the prevalence of stroke in patients with different patterns of AF.

Methods: Cross-sectional study of Moscow AF registry data, 1624 adult patients of 2 hospitals and 3 out-patient clinics, observed at 2009-2015.

Results: Median age was 73,0 (64,0-79,0), women prevailed (61,4%). 39,1% of patients had permanent, 31,4% - paroxysmal and 29,4% - persistent AF. The age of patients with permanent AF was significantly higher (75 (66,9-81,0) years), than with non-sustained forms (71 (62,5-78) years), as well as CHA_2DS_2-Vasc (6 (4-7) vs 4 (3-5)) and HAS-BLED scores (3 (2-3) vs 2 (2-3)), $P < 0,05$ for all comparisons.
29,2% of the registry patients had a stroke or a transient ischemic attack (TIA) (21,7% - one, 7,4% - repeated). The patients, who had stroke, were significantly older, than the patients without stroke. 29,9% of patients with permanent AF had a stroke, 11,7% - repeated strokes. In patients with paroxysmal or persistent AF these rates comprised 21,4; 7,7% and 11,6; 6,6% respectively ($P < 0,05$, X^2 method).
Warfarin use rates in out-patients with permanent AF was 8,4%, with paroxysmal - 4,9%, with persistent - 13,0%. In hospital these rates increased to 28; 17,3 and 68,9% respectively. New oral anticoagulants (NOAC) use rates was very low: 0,8; 0,6; 9,6% vs 6,4; 3,9; 23,1% respectively. The patients, who had stroke, especially repeated, were prescribed both warfarin and NOAC less likely.

Conclusions: Permanent AF was associated with higher prevalence of stroke, that can be considered while making a decision of anticoagulants prescription to the patients with intermediate risk (CHA_2DS_2-Vasc=1).

PB 806 | Validation of a Patient-completed Caprini Risk Assessment Tool in a Spanish-speaking Population

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Background: Personalized risk assessment for venous thromboembolism (VTE) using the Caprini risk score (CRS) coupled with targeted prophylaxis based on the score is effective in reducing the post-operative VTE. Critics contend that the tool is time-consuming for healthcare providers and is limited to the English language.

Aims: To develop and validate a patient-completed CRS for Spanish-speaking (SS) population.

Methods: A focus group was held with family members and patients to determine areas of misunderstanding in the original score. Based on these interviews a patient-friendly form was created and completed by hospitalized patients. A CRS trained, blinded SS physician scored 33 patients during the pilot study. Patients found it challenging to calculate BMI,

which we excluded from the final Patient-CRS form due low agreement in the interim analysis. The study was approved by our local Institutional Review Board. We calculated the sample size to be 37 assuming power of 80% and an alpha of 0.05. We calculated the individuals' questions and categorized scores using SPSS version 23 to estimate Kappa, linear correlation and Bland Altman test. A kappa value over 0.8 was defined as "almost perfect agreement", 0.61-0.79 was "substantial agreement".

Results: We recruited 50 native SS patients (average age (47yo), men (56%), less than college education (77.5%)). Of the 30 individual questions 1 had substantial agreement and the rest had almost perfect agreement comparing physician and patient results. We report a high correlation (r= 0.99) for the overall score and the Bland Altman plot did not show any trend for extreme values.

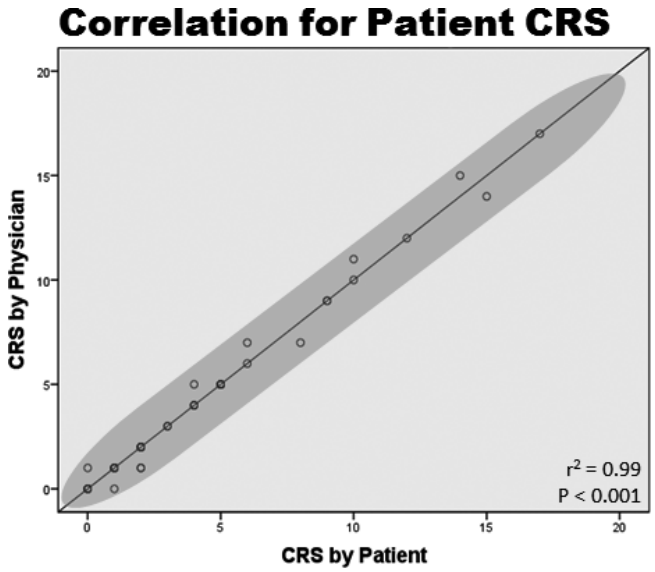

FIGURE 1 Correlation of Patient CRS

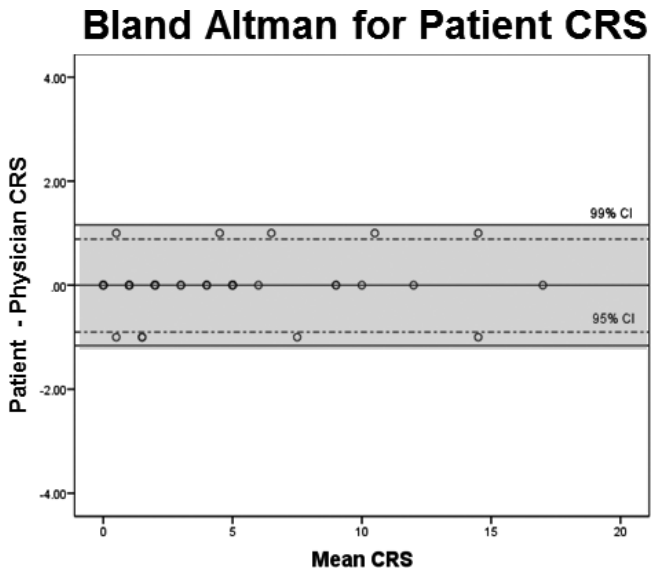

FIGURE 2 Bland Altman for Patient CRS

Conclusions: We created and validated a self-reported CRS form to assess peri-operative thrombotic risk in SS patients. The new version has an almost perfect agreement between patient and physician completed scores and substantial agreement for immobilization during the past 2 days. Based on these results, the physician only needs to calculate the BMI. Completing the form was not time-consuming.

PB 807 | The Procoagulant Contribution of Tissue Factor Expressing Platelets in Hypertension

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Background: Platelets (PLT) express Tissue factor (TF), the main activator of the blood coagulation cascade. Cardiovascular risk factors, such as hypertension and diabetes, affect TF expression both in human and in rat PLT. However, the relative contribution of TF+ PLT, compared to that of the other circulating TF-expressing cells, in thrombin-generating events has not been addressed.

Aims: To analyze the procoagulant potential of TF+ PLT compared to that of TF+ monocytes (Mo) and microvesicles (MV).

Methods: Hypertension was induced by stenosis of renal artery in Wistar Kyoto rats (Hyp; n=7). Sham operated rats were used as normotensive controls (Norm, n=4). Blood was drawn into CTI tubes and intracellular TF expression was evaluated by flow cytometry in PLT, Mo and MV. Their thrombin generation (TG) capacity was measured by CAT assay, taking into account their relative number in 1µl blood volume.

Results: Compared to Norm, Hyp had an increased number of TF+ PLT and Mo (PLT: 7.5±3.2% vs 11.1±2.9%; p=0.035; Mo: 10.3±9.4% vs 34.8±30%; p=0.04) as well as of TF+/Ann+ MV (157±86 /µl vs 428±24 /µl; p=0.04). However, the amount of TF+ PLT in 1µl blood volume was more than 500- and 100-fold higher compared to that of Mo and MV, both in Norm and Hyp. CAT assay indicated that while PLT from Hyp had a higher kinetic rate of TG compared to Norm (Lag Time: 8.6±3.1 vs 10±0.7 min; p=0.036) and generated greater amount of thrombin (Peak: 32.5±21 vs 23.1±6.6 nM, p=0.04), no differences between Norm and Hyp were found in the TG of Mo and MV. Moreover, a double amount of thrombin was generated by PLT compared to that of Mo and MV. After preincubation of samples with a neutralizing anti-TF antibody, a significant delay in the TG was observed only for PLT.

Conclusions: Despite in hypertension an increase of the amount of TF+ PLT, Mo and MV occurs, the number of TF+ PLT, in one unit of blood, far exceeds that of Mo and MV and results in a significantly higher TF-dependent TG, thus supporting a role for TF+ PLT as coagulation trigger.

PB 808 | Patients with Multiple Cardiovascular Risk Factors Show a Characteristic Decrease in Circulating MAIT Cells

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Background: Mucosal-associated invariant T (MAIT) cells regulate natural immunity and exhibit antimicrobial activities in infectious diseases. Inflammation is involved in the development of atherosclerosis and thrombosis. However, little is known about cardiovascular risk factor-related changes in circulating MAIT cell level in healthy Japanese subjects.
Aims: We aimed to determine the level of circulating MAIT cells in plasma in healthy adults and the potential relationships between cardiovascular risk factors and circulating MAIT cell levels.
Methods: Healthy subjects were enrolled (n = 20, age 20 - 60 years). MAIT cells were measured by flow cytometry.
Results: Circulating MAIT cell levels extensively varied (0.4% to 9.5%) among subjects. A linear regression analysis revealed that circulating MAIT cell levels declined significantly with aging (R = -0.86, P < 0.01). No significant difference was found in the circulating MAIT cell levels between male and female subjects (P = 0.43). MAIT cell levels were significantly lower in obese subjects (BMI > 25) than in non-obese subjects (BMI < 25, P < 0.01). In addition, the number of representative cardiovascular risk factors (male gender, age over 55 years, history of smoking, obesity, family history of cardiovascular diseases, hypertension, dyslipidemia, hyperuricemia) closely correlated with the decrease in circulating MAIT cells (P < 0.05), suggesting a close link between MAIT cells and progression of early atherosclerosis.
Conclusions: Our data strongly suggest that aging and accumulation of cardiovascular risk factors are closely associated with a reduction in circulating MAIT cells in Asians. The precise roles of circulating MAIT cells as biomarker of early atherosclerosis need further investigation.

PB 809 | Protein Z Deficiency: A Reliable Risk Factor for Acute Coronary Syndrome

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Background: Acute coronary syndrome (ACS) comprises a range of life-threatening thrombotic coronary artery diseases. Understanding the pathophysiology and identifying the risk factors are crucial for prevention of ACS. The protein Z (PZ)/PZ-dependent protease inhibitor (ZPI) is a natural anticoagulant system, with a presumptive role for PZ deficiency in the pathogenesis of ACS.
Aims: We aimed at investigating the role of plasma PZ level as a risk factor for ACS.
Methods: Hundred patients with history of ACS and 60 matched healthy controls were enrolled. Patients with recent ACS, on oral anticoagulants or with liver diseases were excluded. ACS patients were subdivided into 3 clinical subgroups [ST-segment elevation myocardial infarction (STEMI), non-STEMI (NSTEMI) and unstable angina (UA)], and 2 age subgroups (Group A ≤55 years and Group B >55 years). Plasma PZ levels were assayed using enzyme linked immunosorbent assay.
Results: PZ levels were lower in ACS patients' group and clinical subgroups (STEMI, NSTEMI, UA) compared to controls (p< 0.01), however; showed no difference among the ACS clinical subgroups (p= 0.783). PZ levels decreased with increasing age (r= -0.739, p< 0.01) and were lower in female versus male patients (p< 0.05). Lower PZ levels were found in hypertensive ACS patients in both age groups (p< 0.05). Smokers and patients with family history of ACS in Group A showed lower PZ levels (p< 0.05), while Group B patients showed lower PZ levels among diabetics (p< 0.05). In Group A, the more the number of ACS conventional risk factors, the lower the PZ levels (p< 0.01). Receiver operating characteristic (ROC) curve assigned the PZ level 3.7 µg/mL as the best cut-off value for predicting ACS. Uni/multi-variate logistic regression analyses denoted PZ level as an independent risk factor for ACS (p< 0.01).
Conclusions: PZ deficiency is a reliable risk factor for ACS. Assay of PZ level, especially in older age, female patients, and in the subset of patients lacking ACS conventional risk factors, is worthwhile.

PB 810 | Cardiac and Inflammatory Biomarkers and their Role in the Pathogenesis of Heart Failure in End-Stage Renal Disease

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Background: Heart failure (HF) is highly prevalent in patients with End-Stage Renal Disease with a presence of approximately 40 %. The purpose of this study was to determine the role of cardiac and inflammatory biomarkers in the pathogenesis of HF in ESRD patients.
Aims: The primary aim of this study is to investigate the role of inflammatory mediators in heart failure.
Methods: Ninety blood samples from maintenance hemodialysis patients were retrospectively collected and stored at -70° C. Twenty-five male and twenty-five female plasma samples from healthy individuals were purchased from a biobank as a control. The samples were used to profile KIM-1, NT-pro BNP, NGAL, IL-18, PDGF, Vitamin D, PTH, Endothelin, Endocan, MPTF, Heparin anti Xa, Lp(a) using commercial sandwich and competitive ELISA kits and assays.
Results: All plasma biomarkers, except PDGF, Endothelin and Vitamin D, were statistically increased in patients with ESRD compared to normal (P < 0.05). HF patients with ESRD, as compared to non-HF patients with ESRD, had significantly elevated NT-pro-BNP (P = 0.0194 | % change = 52.9) and KIM-1 (P = 0.0485 | % change = 58.5%). There were no statistical differences found between age groupings, except < 60 y.o KIM-1 vs 60-69 y.o KIM-1. NT-pro-BNP in ESRD patients with HF was found to correlate

with K+ (P = 0.023 | R = -0.39), Ca+ (P = 0.029| R = -0.38), and Heparin anti Xa (P = 0.045 | R = 0.35). KIM-1 in ESRD patients with HF was found to correlate with Creatinine (P = 0.0175 | R = -.41), EGFR (P = 0.008 | R = 0.45), Phosphate (P = 0.002 | R = -0.51), Intact PTH (P = 0.043 | R = -0.36), Calcium Phosphate Product (P = 0.002 | R = -0.52), and Vitamin D (P = 0.037 | R = 0.36).

Conclusions: Elevated plasma NT-pro-BNP and KIM-1 in all of the ESRD patients and ESRD patients with HF suggest that natriuretic peptides and KIM-1 may contribute to the pathogenesis of HF in ESRD patients. Elevated NT-pro-BNP further supports previous studies demonstrating NT-pro-BNP's potential diagnostic and prognostic utility.

PB 811 | Risk Factors for Recurrent Venous Thromboembolism in the Elderly

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Background: Although recurrent venous thromboembolism (VTE) is an important cause of morbidity and mortality among the elderly, limited data written about risk factors predisposing to recurrent VTE in the elderly.

Aims: To identify the risk factors associated with recurrent VTE in the elderly. Incidence of recurrence rate was also assessed.

Methods: A retrospective cohort observational study was conducted between January 2014 and December 2016. A cohort of 458 VTE elderly patients aged 65 and above who were referred to VTE clinic were included. Univariate and multivariate Cox proportional hazards model were used to calculate the hazard ratios for the recurrence of VTE.

Results: A total of 72 elderly patients (16%) developed a recurrent VTE over the study period. The annual rate of recurrence was 1.0% in patients with trauma, 2.0% in patients with active cancer and 3.2% in patients with primary unprovoked VTE. Hazards ratio (HR) for recurrence was 0.34 (95% CI 0.21-0.62) in elderly with VTE provoked by trauma while elderly with active cancer associated VTE had a HR of 0.60 (95% CI 0.39-1.15) compared to elderly with unprovoked VTE.

Conclusions: Our findings suggest elderly patients had lower risk of developing recurrent VTE. Elderly with active cancer associated VTE had a lower risk of recurrence than elderly with unprovoked VTE, but not as low as trauma provoked VTE.

PB 812 | Increased von Willebrand Factor Platelet Interactions in Patients with Chronic Pain

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Background: Von Willebrand factor and factor VIII are acute phase proteins. Besides, they are associated with arterial thrombosis in coronary heart diseases and ischemic stroke (Sonneveld M.A.H. et al. 2014).

Aims: To solve the crucial question whether patients with chronic pain show elevated levels of Von Willebrand factor, factor VIII and low ADAMTS 13 activity which may contribute to arterial thrombosis.

Methods: Our clinical study was carried out in the departments of anaesthesiology and hemostaseology. It was approved by a recognized ethic committee. A blood sample was taken from all participants in order to determine the Von Willebrand factor, factor VIII and the Von Willebrand factor cleaving protease ADAMTS 13. In addition, the blood groups of the participants were identified.

Results: 52 patients, mean age 52,1 years, with chronic pain (57,7% suffering for > 5 years; 26,9% for 2-5 years; 11,5% for 1-2 years; 3,8% for ½-1 year) and 53 pain-free probands, mean age 51,96 years, were included after an informed consent. Patients with chronic pain had significant higher levels of Von Willebrand factor activity and by trend higher levels of Factor VIII and lower levels of ADAMTS13 compared to pain free probands matching by age and sex.

Conclusions: According to our survey, patients with chronic pain may be a risk group for arteriosclerotic diseases, particularly due to the permanent higher levels of Von Willebrand factor and factor VIII and the lower levels of ADAMTS13. However, our results cannot be explained with the age or blood group of the patients. The influence of AB0-system was adjusted in our testing methods.

PB 813 | Routine Care Data in Prediction Research

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Background: Data from routine care is becoming increasingly available, also in the field of thrombosis research. While having great potential, their use for research inherently has its limitations. For instance, misclassification of both risk factors and the outcome under study may strongly affect inferences, though to what extent is currently unclear.

Aims: To quantify the amount of misclassification in routine care data and its influence on studies evaluating risk prediction models.

Methods: In a prospective cohort in general practice in the Netherlands, we used routinely collected data from the electronic medical records of patients with known atrial fibrillation (AF) and compared these to data collected after manually scrutinizing all complete medical files. As an example, we used the CHA2DS2-VASc clinical decision rule to predict the outcome, all-cause mortality. Using our manually obtained data as a reference standard, we assessed misclassification of individual predictors and compared hazard ratios (HR) and predictive performance from Cox survival analyses using either routine care data or manually collected data.

Results: In total 2363 AF patients were included. After a median follow-up of 2.7 years (IQR 2.3 - 3.0 years), all-cause mortality occurred in 368 patients (incidence rate 6.2 deaths per 100 person-years). Misclassification in individual predictors ranged from substantial (Cohen's kappa 0.56 for heart failure) to minor (kappa 0.90 for diabetes), as did differences in HRs in the Cox models (univariate HRs for heart failure 2.1 using routine care data versus 1.7 in

manually collected data; HRs for diabetes 0.81 versus 0.83, respectively). However, overall model performance was not affected by misclassification (c-statistic for CHA2DS2-VASc as a continuous score of 0.684 for routine care data vs. 0.681 for manually collected data, predicted probabilities were similar).

Conclusions: While misclassification was substantial in several variables, this did not influence apparent overall model performance.

PB 814 | Markers of Plasma and Platelet Hemostasis Function as the Risk Factors for Leg Amputation in Patients with Critical Limbs Ischemia

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[1]Clinic of Vascular and Internal Medicine, Dr Jan Biziel University Hospital No. 2, Bydgoszcz, Poland, [2]Department of Pathophysiology, Faculty of Pharmacy, Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum, Bydgoszcz, Poland

Background: Identification of factors linking the risk of failure of endovascular treatment might help in better patients qualification and individualization of pharmacotherapy and in decrease in risk of amputation.

Aims: The aim of the study was to determine the importance of chosen hemostatic factors in prediction of leg amputation after endovascular procedure.

Methods: One hundred twenty five patients treated endovascularly due to critical limbs ischemia were enclosed to the analysis, 39 patients in whom leg amputation was performed 192 ± 257 days after procedure, and 86 patients with salvaged extremities.

Results: Patients with leg amputation, in comparison to patients with rescued limb, had: greater INR (1.14 ± 0.2 vs. 1.03 ± 0.11, p=0.003) and platelet count (356.4 ± 143.5 vs. 272.9 ± 110 G/l, p=0.001). In progressive stepwise regression method the time duration between the first revascularization procedure and the amputation was significantly determined by many various factors, including clinical (e.g. age, history of atrial fibrillation, stroke, hypertension, diabetes mellitus), periprocedural (e.g. number of implanted stents), angiographical (e.g. atherosclerotic lesion advancement, inflow, outflow in relation to target lesion), biochemical (LDL cholesterol, leukocytes count, C-reactive protein), and hemostatic (number of platelets, mean platelets volume, INR, aPTT).

Conclusions: Simple hemostatic tests were the ones of independent factors affecting the risk of leg amputation after endovascular procedure. The further studies are needed to determine their predictive values and confirm their clinical usefulness.

PB 815 | Validation of a Patient-completed Caprini Risk Assessment Tool

H. Fuentes[1], L. Paz[1], A. Al-Ogaili[1], D. Oramas[2], C. Acob[1], A. Tafur[3], J. Caprini[3]

[1]John Stroger Hospital Cook County, Internal Medicine, Chicago, United States, [2]University of Illinois, Chicago, United States, [3]NorthShore University HealthSystem (University of Chicago Pritzker School of Medicine), Evanston, United States

Background: Individual risk assessment for Venous Thromboembolism (VTE) using the Caprini Risk Score (CRS) coupled with targeted prophylaxis based on the score is effective in reducing the post-operative VTE. Critics contend that the tool is time-consuming for healthcare providers.

Aims: To compare scores calculated by a patient to scores in the same patient calculated by a blinded physician.

Methods: A focus group interview was held with family members and patients to determine areas of misunderstanding in the original CRS. Based on these interviews a patient-friendly form was created and completed by hospitalized patients. A CRS trained, blinded physician scored 20 patients during the pilot study. Patients found it challenging to calculate BMI, which we excluded from the final Patient-CRS form due to low agreement in interim analysis. The study was approved by our local Institutional Review Board. We calculated the sample size to be 37 assuming power of 80% and an alpha of 0.05. We calculated the individuals' questions and categorized scores using SPSS version 23 to estimate Kappa, linear correlation and Bland Altman test. A Kappa value over 0.8 was defined as "almost perfect agreement."

Results: We recruited 42 patients (average age (55yo), female (45%), less than college education (62%)) who completed the CRS form. There was almost perfect agreement both for individual questions and for the overall score comparing physician and patient results. We report a high correlation (r=0.97) and the Bland Altman did not show any trend for extreme values.

Conclusions: We created and validated a self-reported CRS form to assess peri-operative thrombotic risk. The new version has an almost perfect agreement between patient and physician completed scores except for BMI. Based on these results, the physician only needs to calculate the BMI. Completing the form was not time-consuming.

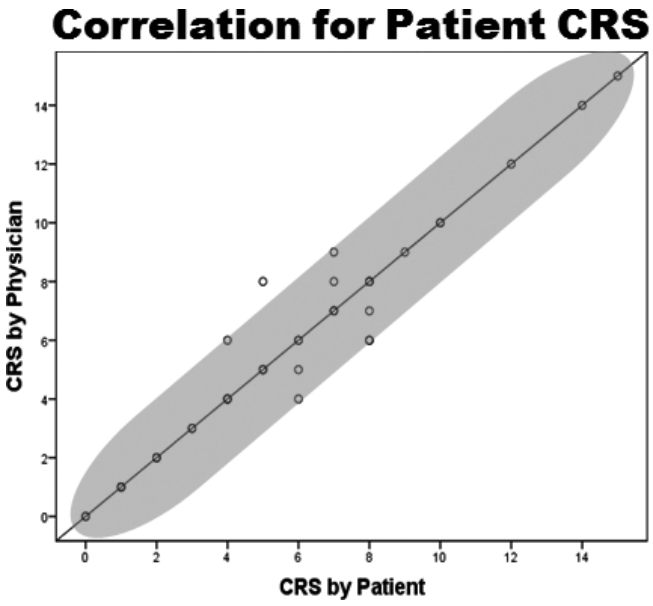

FIGURE 1 Correlation of Patient CRS

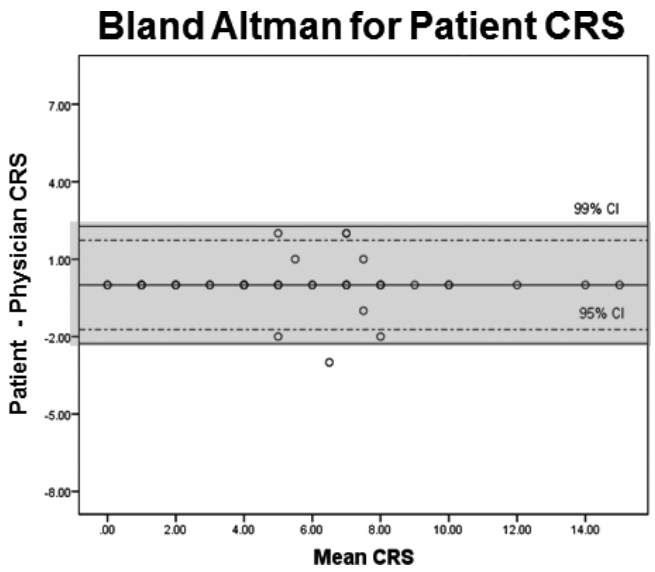

FIGURE 2 Bland Altman for Patient CRS

PB 816 | Patients with Diabetes Mellitus 2 on Treatment, with or without Previous Cardiovascular Events, Have Delayed Clot Lysis, but Not Hyperactive Platelets Nor Increased Thrombin Generation

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Background: The increased cardiovascular risk (CVR) in patients with diabetes mellitus 2 (DM-2) is well known (65-80% of deaths). Glycemic control has not decreased significantly the CVR in 3 Clinical Trials. Thus, other factors must play a role in it, such as the prothrombotic abnormalities described in DM-2.

Aims: We studied hemostasis variables in 2 groups of 20 DM-2 patients with (CV+) and without (CV-) previous CV events, and compared them with 20 age and sex-matched Controls (Co), all of them without aspirin.

Methods: Both patient groups had increased BMI (means of 29 and 28 vs 26 Kg/m^2 in Co), glycosylated hemoglobin (8.0 and 7.6 vs 5.6%) and triglycerides (187 and 149 vs 130 mg/dL). Most DM-2 patients received statins and had total and LDL cholesterol lower than Co ($p< 0.01$). Blood pressure, creatinine and smoking habits were similar in the 3 groups. We also measured sCRP, advanced oxidation protein products (AOPP) and VWF. Clot Lysis Time (CLT) in ristocetin stimulated PRP was also measured (Panes et al Platelets 2012).

Results: Basal P-selectin exposure was slightlyly higher in CV+ patients than in Co ($p=0.03$). CV+ patients had less aggregation and 5-HT secretion to sub-threshold 0.5µM ADP (but not to epinephrine) concentration than CV- and Co ($p< 0.01$). Platelet tissue factor-dependent FXa generation was similar in all groups. Thrombin generation in PPP and PRP, and plasma F_{1+2} and TAT complexes in CV+, CV- and Co, as well as inflammatory, oxidative stress and endothelial dysfunction markers did not differ significantly in all the groups. However, PAI-1 was lower in Co compared with CV+ ($p=0.003$) and CV- ($p< 0.05$); and CLT-PRP was significantly prolonged in CV+ ($p< 0.001$) and CV- ($p< 0.001$) patients compared with Co.

Conclusions: We did not confirm platelet hyperfunction (aggregation, secretion and procoagulant activity) nor increased thrombin generation potential in our DM-2 cohorts. Hypofifrinolysis may be major contributor of CVR in DM-2, but the mechanisms involved need to be studied.

PB 817 | Haemorrheologic and Fibrinolytic Activities in Diabetic Patient with Hypertension in Calabar, Nigeria

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Background: Cardiovascular disease is the leading cause of morbidity and mortality among persons with diabetes with smoking, hypertension, poor glycaemic control etc. as risk factors.

Aims: This study is aimed at determining the plasma viscosity, euglobulin lysis time, and fibrinogen levels in diabetics and diabetics patients with hypertension.

Methods: A total of fifty (50) diabetic subjects aged between 35-75 years attending the diabetic clinic of University of Calabar Teaching Hospital, and fifty age-matched non-diabetic apparently healthy volunteers (controls) were selected for the study. Diabetes and hypertension in this study was defined according to WHO 1999 guidelines. 7ml of venous blood was collected from each subject. It was dispersed to the appropriate bottles for analysis. Standard methods of Haugie, (1986), Reid and Ugwu, (1987), Ingram's and Hills (1976) and Nelson (1944) were employed for the determination of euglobulin lysis time, relative plasma viscosity, plasma fibrinogen concentration and fasting blood sugar levels in duplicates respectively. The results were expressed as mean ± standard deviation and students't test for paired means was used for statistical comparison.

Results: The fasting blood sugar of diabetic patients, RPV, PFC and ELT (10.23mmol/l, 1.65, 5.22g/L and 268.4mins) was significantly higher than that of the control subjects. There was no statistical difference in RPV, PFC and ELT ($P>0.05$) for both diabetic subjects and the controls based age and gender. Fibrinogen levels (6.27g/l) showed significant increase ($P< 0.05$) in diabetics with hypertension when compared with those who had no hypertension while no statistical difference was observed in RPV and ELT.

Conclusions: This study shows values of RPV, PFC and ELT in diabetics, control subjects and diabetics with hypertension in this locality. it indicate that the subjects are prone to developing thrombosis. For better management of diabetic patients in this locality, it may be necessary to include RPV, PFC and ELT as routine tests.

PB 1638 | Platelet α-granule Deficiency Protects Local and Distal Organs in Intestinal Ischemia Reperfusion Injury

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Background: Intestinal I/R has been associated with distal organ injury, in particular the lungs. A deficiency of α-granules in *Nbeal2*^{KO} platelets has been previously reported to reduce brain infarct size in transient cerebral ischemia. A functional role for platelets and their α-granules in tissue damage after intestinal I/R injury remains unknown.

Aims: Investigate the role of platelet α-granules in the pathological process of intestinal ischemia reperfusion injury.

Methods: *Nbeal2*^{KO} and *Nbeal2*^{WT} mice were subjected to 1 hour of intestinal ischemia by clamping of the superior mesenteric artery using a small arterial clip. The arterial clip was removed, followed with a 3-hour period of reperfusion. During the reperfusion period, platelets and neutrophils in the mouse mesentery were monitored in real time by fluorescent microscopy. At the end of reperfusion, mice were euthanized and their tissue was harvested.

Morphological changes in the small intestine and lungs were determined by H&E staining. Histopathology of the small intestine and lungs was assessed by randomly selecting 8 images per mouse/ tissue section and scored in individual categories.

Results: During reperfusion, platelet-leukocyte aggregates in the mesentery were significantly decreased in the *Nbeal2*^{KO} mice. Intestinal I/R in *Nbeal2*^{WT} mice caused severe damage, including distortion and shortening of villi, disruption of villi epithelium, infiltration of leukocyte cells and hemorrhage. Similarly, in *Nbeal2*^{WT} mice, lung injury was characterized by the congestion of lung vessels, thickening of the alveolar wall, mild atelectasis, leukocyte cell infiltration and hemorrhage in the air space. *Nbeal2*^{KO} mice displayed statistically significant less severe morphological changes in their intestinal mucosa and lung architecture.

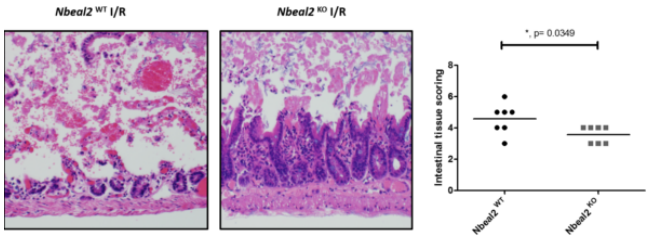

FIGURE 1 Representative images of H&E stained sections of small intestine after 1hr ischemia and 3hrs reperfusion and tissue injury scoring

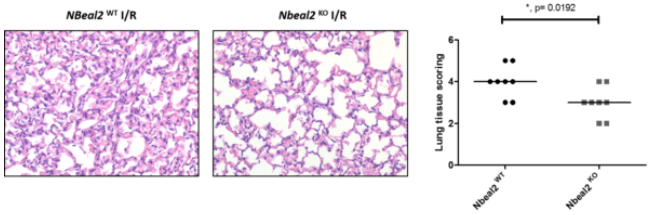

FIGURE 2 Representative images of H&E stained sections of mouse lung after 1hr ischemia and 3hrs reperfusion and lung tissue injury scoring

Conclusions: Local intestinal and distal lung tissue damage was reduced in *Nbeal2*^{KO} mice after intestinal ischemia reperfusion underscoring the role of platelet α-granules in thromboinflammation and tissue injury.

PB 1639 | Structure-function Relationships of Krait Natriuretic Peptide: Design of Natriuretic Peptide Analogues with Vasodilatory or Renal Activities for Personalized Care of Heart Failure Patients

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Background: Heart failure (HF) or congestive heart failure is a common, costly, and frequently fatal condition. Current treatments for acute decompensated HF, including combinations of renin-angiotensin-aldosterone system inhibitors, diuretics, vasodilators, inotropes, opiates and ion channel blockers, are effective in short-term restoration of function but are associated with higher readmission rates. HF patients, classified as „dry/wet“ and “cold/warm” depending on their hemodynamic status based on perfusion vs. congestion, need specific treatments.

Aims: To understand the structure-function relationships of ‚ring' of krait natriuretic peptide (K-Ring) and to design distinct classes of natriuretic peptide analogues (NPAs) with exclusive vasodilatory or diuretic effects.

Methods: We designed and synthesized a number of analogues of K-Ring and human ANP and evaluated their hemodynamic and diuretic properties in anesthetized rats.

Results: By systematic mutations and functional studies of various mutants, we identified residues in K-Ring responsible for vasodilatory and/or diuretic functions of NPs. In the process, we also identified the residues affecting heart rate and pulse pressure, sustained effect on vasodilatory function, vasodilatory-diuresis balance and forceful diuresis switches. Based on the results, we designed human ANP analogues with predominant vasodilatory effects without diuresis and others with exclusive diuretic effects without vasodilation.

Conclusions: Through systematic evaluation of K-Ring and human ANP, we have delineated the key molecular switches responsible for vasodilatory or diuretic effects of NPs and further design NPAs with exclusive functions. Thus, this study has led to the development of a unique set of ligands that alter blood pressure-volume status independently, thereby creating a platform for personalised care of heart failure patients.

PB 1640 | Development of a Novel Strategy to Target CD39 Antithrombotic Activity to the Endothelial-platelet Microenvironment in Kidney Ischemia-reperfusion Injury

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Background: Kidney ischemia-reperfusion injury (IRI) is characterised by inflammation and thrombosis. P-selectin and its ligand PSGL-1 control leukocyte-endothelial and leukocyte-platelet interactions under inflammatory conditions. CD39 is the dominant vascular nucleotidase that facilitates adenosine generation via extracellular ATP/ADP-phosphohydrolysis. Adenosine signalling is protective in renal IRI, but CD39 catalytic activity is lost with exposure to oxidant stress. We designed a new therapeutic targeting CD39 to P-selectin to improve renal IRI.

Aims: To improve outcomes from renal IRI with a novel therapeutic rsol.CD39-PSGL-1

Methods: Production of rsol.CD39-PSGL-1: Production in CHO cells that stably express core 2 O-linked B1-6-N-acetylglucosaminyltransferase (C2GnT) and α1-3 fucosyltransferase activity (FucTVII), followed by concentration by immunoaffinity with anti-FLAG antibody.

P-selectin ELISA, aggregometry, flow cytometry, tail bleeding time, NTPDsase activity, serum urea and creatinine: conventional techniques

Warm renal IRI model: Unilateral kidney IRI with nephrectomy of the second kidney with varying doses of rsol.CD39-PSGL-1, or clopidogrel, 30 min before IRI.

Renal Histology and Scoring: Kidney tissue sections were stained with hematoxylin-eosin and a semi-quantitative scale was used to evaluate the degree of tubular necrosis.

Results: The rsol.CD39-PSGL-1 displayed ADPase activity and inhibited platelet aggregation ex vivo, as well as bound with high specificity to soluble P-selectin and platelet surface P-selectin. Importantly, mice injected with rsol.CD39-PSGL-1 and subject to renal IRI showed significantly less kidney damage both biochemically and histologically, compared to those injected with untagged solCD39. Furthermore, the equivalent dose of rsol.CD39-PSGL-1 had no effect on tail template bleeding times.

Conclusions: Targeting CD39 to the injured vessel wall resulted in substantial preservation of renal function and morphology after IRI without toxicity.

PB 1641 | Trif-deficiency Impairs Leukocyte Adhesion and Netosis in Mesenteric Ischemia-reperfusion

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Background: Vascular responses to injury depend on Toll-like receptor (TLR) signaling that requires the adapter molecules myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF).

Aims: To analyze the role of MyD88 and TRIF for leukocyte and platelet adhesion to mesenteric venules and the formation of neutrophil extracellular traps (NETs) in ischemia-reperfusion injury, as it occurs during mesenteric infarction.

Methods: By clamping the *A. mesenterica superior*, WT, *Myd88* $^{-/-}$, and *Trif* $^{-/-}$ mice were subjected to 1 hour of ischemia. Then, reperfusion of the small intestine was allowed and the number of rolling leukocytes, firmly adherent leukocytes, and platelet-leukocyte conjugates per viewing field was quantified in mesenteric venules by fluorescence intravital microscopy. NETs were stained with SYTOX orange and the number of NETs per viewing field was quantified prior to and directly after the onset of mesenteric ischemia-reperfusion injury. Ex vivo, LPS-induced formation of NETs from cultured bone marrow neutrophils was compared.

Results: In contrast to WT and *Myd88* $^{-/-}$ mice, *Trif* $^{-/-}$ mice were protected against the ischemia-induced increase in leukocyte rolling, adhesion, and conjugate formation in mesenteric venules. In contrast, platelet adhesion to mesenteric venules was diminished in *Myd88* $^{-/-}$ mice. Neutrophil extracellular traps (NETs) in mesenteric venules could be detected prior to ischemia induction, with reduced numbers in *Myd88* $^{-/-}$ mice. Interestingly, the ischemia-induced increase in NETosis was not observed in mesenteric venules of *Trif* $^{-/-}$ mice. In isolated bone marrow derived neutrophils, LPS-induced NETosis of *Myd88* $^{-/-}$ or *Trif* $^{-/-}$ neutrophils was unchanged relative to WT.

Conclusions: Our results demonstrate the involvement of TLR adaptors in balancing heterotypic cell interactions and NET formation in the post-ischemic mesenteric microvasculature.

PB 1642 | Inhibition of Coagulation Factor Xa Attenuates Myocardial Ischemia Reperfusion Injury in Mice

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Background: Ischemic/reperfusion (I/R) injury affects the outcome of myocardial infarction (MI). Current reperfusion therapy does not sufficiently prevent microvascular thrombo-inflammatatory injury. Coagulation proteases mediate inflammation via protease activated receptors (PARs).

Aims: We aim to elucidate FXa's role in I/R injury after MI by inhibiting FXa with rivaroxaban.

Methods: Male WT BL/6 mice underwent surgical ligation of the left anterior descending coronary artery 7days pre-experimentation. Next, the ligature was tightened for 1h to induce ischemia and loosened either for 4h (early), or 4 weeks (late), to allow reperfusion. The intervention consisted of 2 rivaroxaban (1.6 mg/kg) i.v.-injections or placebo (0.9%NaCl) after 15min ischemia and 5min reperfusion. In the early model the area at risk (AAR) was visualized with Evans blue and differentiated from the area of infarction (AOI) through triphenyl tetrazolium chloride. Plasma cardiovascular markers were quantified using Luminex Multiplex. In the late model, left ventricle ejection fraction (LVEF) was measured 10min pre-ischemia and 4 weeks' post-reperfusion.

Results: The rivaroxaban treatment group showed attenuated myocardial damage indicated by reduced AOI/AAR (41%[IQR34-48]) vs. control (62%[IQR52-67]) (Fig1). This was supported by preliminary data indicating a better preserved LVEF after 4 weeks' reperfusion in the treatment group (25%[IQR19-31]) vs. control (16%[IQR12-21]) (Fig2). Plasma E-Selectin, PECAM-1, PAI-1, proMMP9, and thrombomodulin levels tended to be increased upon rivaroxaban treatment.

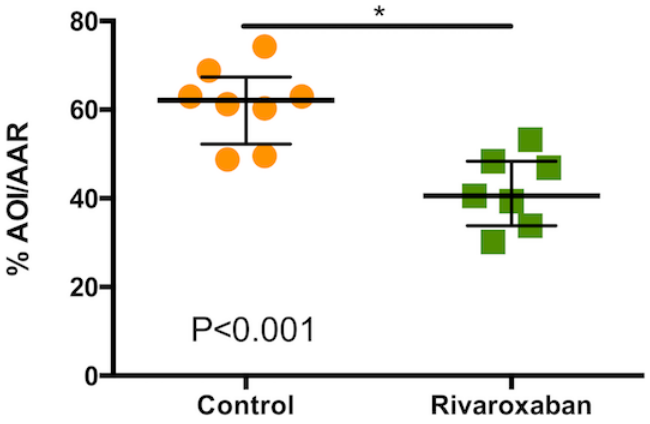

FIGURE 1 Area of Infarction. Median infarction area was reduced in the rivaroxaban treated group (n=7) vs. control (n=8)

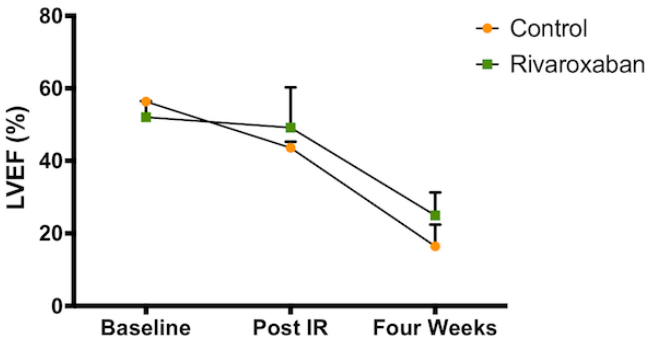

FIGURE 2 Left Ventricle Ejection Fraction. Median left ventricle ejection fraction of control mice (n=2) vs. rivaroxaban treated mice (n=4) NS

Conclusions: FXa inhibition by rivaroxaban reduces myocardial I/R injury in mice and may provide long term preservation of LVEF. Raised cardiovascular markers suggest increased tissue remodeling and phenotypical alteration of endothelial cells after rivaroxaban treatment. These results suggest that coagulation proteases play a role in I/R injury during MI, most likely by activating PARs.

PB 1643 | Biomarkers of Oxidative Stress in Venezuelan Sickle Cell Patients: Association with the Clinical Severity

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Background: Sickle cell Disease (SCD) includes a group of hemolytic anemia associated to the presence of hemoglobin S, which is characterized by acute pain episodes and progressive damage of different organs. In Venezuela SCD frequency ranges between 0 and 7%. The overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are part of the pathophysiology and induce a state of oxidative stress that is associated with endothelial dysfunction and tissue damage in SCD.

Aims: This study evaluated biomarkers of oxidative stress in Venezuelan sickle cell patients and their association with the clinical severity.

Methods: The present study was conducted in 22 Venezuelan sickle cell patients that were classified according to their genotype (Hb SS, Hb SC and Hb Sβtal) and phenotype (Mild, Moderate and Severe) and 23 apparently healthy individual. Biomarkers related to oxidative stress (serum nitrites, conjugated dienes, thiobarbituric acid reactive substances (TBARS) and serum homocysteine) were evaluated and their associated with the clinical severity.

Results: In reference to the lipid peroxidation markers, a significant increase of TBARS was observed in patients with Hb SS and Severe phenotype ($p < 0.05$).Also was exhibited a significant increase of conjugated dienes in Hb SC patients with Mild and Severe phenotype ($p < 0.05$). Homocysteine levels were significantly lower in patients with Hb SS and Moderate and Severe phenotypes ($p < 0.05$). Nitrite values did not showed statistically significant changes.

Conclusions: Our results evidence an increase in biomarkers related to oxidative stress in Venezuelan sickle cell patients with Hb SS and SC genotypes, especially in severe phenotypes. This study suggests the importance of evaluating different oxidative stress biomarkers in patients with SCD, in order to prevent endothelial damage and complications of the disease.

PB 1644 | Contribution of Neutrophil Extracellular Trap (NET) Components to the Structure of Thrombi from Therapeutic Intervention in Ischemic Coronary, Peripheral and Cerebral Artery Disease

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Background: The ultrastructure and cellular composition of thrombi profoundly affect the therapeutic outcome in myocardial infarction (AMI), stroke and peripheral artery disease (PAD). In thrombi, citrullination of histones in activated white blood cells (WBC) initiates the release of NETs composed primarily of DNA and histones that modify the stability and lysibility of fibrin.

Aims: To investigate the interrelations of structural properties of thrombi and routinely available clinical data.

Methods: Thrombi extracted from AMI (n=65), PAD (n=64) or stroke (n=78) patients were processed for scanning electron microscopy, (immune)stained for fibrin, citrullinated histone H3 (cH3) and extracellular DNA. Fibrin fiber diameter, relative occupancy of fibrin, cellular components, DNA and cH3 were measured. Correlations between these structural features and clinical parameters were analyzed.

Results: The relative occupancy of platelets is generally higher in AMI and stroke compared to PAD thrombi. The presence of certain comorbidities, risk factors (atherosclerosis, thrombophilia, cancer and smoking) usually lowered the platelet content of thrombi (Table 1). In stroke thrombi, the diameter of fibrin fibers shows an inverse correlation with the plasma level of C-reactive protein in the range of 0-8 mg/L, but this trend is reversed in more severe inflammation. There is no significant difference in the fibrin/cH3 (FHr) and fibrin/DNA ratio (FDr) between samples from the three localizations. In AMI, FDr correlates inversely with plasma fibrinogen level and WBC count (up to 10 G/L). FHr correlates positively with WBC count, but in malignancy the relationship is reversed despite elevated fibrinogen levels.

TABLE 1 The relative occupancy of platelets (median, lower and upper quartile, %) in thrombi in different subgroups (*: p<0.05, n.a., not applicable)

Subgroup		AMI	PAD	Stroke
All		3.1 (0.7-8.75)	2.2 (0.8-6.025)*	3.9 (1.5-9.6)
Athero-sclerosis	No	3.15 (0.625-8.4)	3 (1.05-6.325)	5.25 (1.975-13.2)
	Yes	2.3 (0.9-9.35)*	2 (0.7-5.7)	2.7 (1.3-7.525)*
Smoking	No	3.6 (1.15-9.625)	3.2 (1.3-6.9)	5 (1.65-12.83)
	Yes	1.6 (0.5-7.1)*	1.6 (0.6-4.3)*	2.4 (0.9-10.6)
Thromb-ophilia	No	3.2 (0.8-8.9)	n.a.	3.85 (1.5-9.675)
	Yes	0.6 (0.275-2.75)*	n.a.	5.4 (1.4-9.6)
Malignancy	No	2.5 (0.625-8.25)	2 (0.7-5.7)	4.4 (1.7-10.4)
	Yes	3.9 (1.2-10.1)	3.4 (1.6-10)	1.9 (0.8-6.5)*

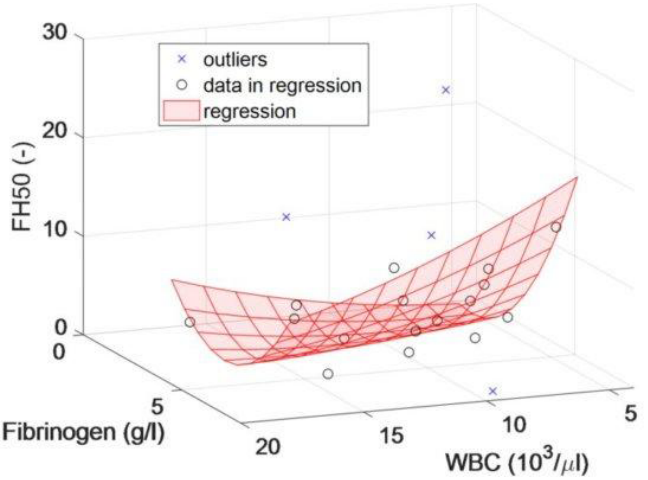

FIGURE 1 Joint impact of WBC and fibrinogen on fibrin/histone ratio (FH50) of thrombi in patients with malignancy

Conclusions: Structural variations (fibrin composition, cellular and NET content) of thrombi from different locations are related to systemic signs of inflammation and comorbidities. The established relationships could help to optimize interventional therapy using routinely available laboratory and clinical data.

PB 1645 | A Novel Easy-to-Perform Thromboembolic Stroke Model in Mice: Application to the Study of the Thrombolytic Effect of N-acetylcysteine

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Background: Several experimental models of ischemic stroke are currently available. Among the most clinically relevant, models involving occlusion of a cerebral artery by a blood clot allow investigation of thrombolytic and combined strategies. However, most of these models are technically challenging, limiting their large adoption.

Aims: To developed an original model of thromboembolic stroke in mice mimicking large vessel atherothrombotic stroke in humans.

Methods: Stroke was induced by intracranial embolization of an endogenous thrombus formed on the surface of the common

carotid artery using topical application of ferric chloride. We characterized this model using 7 Tesla magnetic resonance imaging, immunohistology and behavioral testing. Then, using this model, we performed a double-blinded randomized and controlled trial of N-acetylcysteine
(NAC, a von Willebrand Factor targeted thrombolytic drug) combined with non-peptidic anti-GpIIb/IIIa inhibitor (GR).

Results: This model led to occlusion of intracranial arteries by platelet-rich thrombi formed under high shear rates. Interestingly, it showed all the common features of human stroke: a thromboembolic nature, a diffusion-perfusion mismatch, a low mortality rate (< 10%) and significant neurological deficits. Importantly, this model is straightforward to perform, do not require expensive reagents/material and can be performed in less than 20 minutes. In the randomized trial involving 25 animals per group, we observed a beneficial effect of NAC+GR on stroke outcome (lesion size, recanalization rate, neurological outcome) without significant increase in the risk of hemorrhagic transformation.

Conclusions: We developed a new thromboembolic stroke model in mice with potential for a large diffusion in laboratories interested in brain ischemia. Using this model, we demonstrated the efficacy of NAC combined with an anti-GpIIb/IIIa inhibitor as a treatment for acute ischemic stroke.

PB 1646 | Human Platelet Alloantigens and P-Selectin Gene Polymorphisms in Pediatric Arterial Ischemic Stroke

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Background: Pediatric arterial ischemic stroke (AIS) is a heterogeneous multifactorial disorder, with a wide range of identified inherited and acquired risk factors. Genetic risk factors are incompletely characterized with only FV Leiden being consistently associated with pediatric AIS. We have also demonstrated that inherited genetic risk factors for perinatal and childhood AIS are not the same (Coen Herak et al *in press*).

Aims: The study aimed to find out if individual gene polymorphisms or haplotypes of: a) human platelet alloantigens (HPA) and b) P-selectin (P-SEL) gene alone and combined with FV Leiden are risk factors for perinatal and childhood AIS.

Methods: Study group comprised 110 children with childhood (N=61) and perinatal AIS (N=49), and 100 age- and sex-matched controls. Genotyping of HPA-1, -2, -3 and PSEL-S290N, -N562D, -V599L, -T715P were performed using allele-specific PCR.

Results: Carriers of at least one HPA-3b allele had a 2-fold lower risk of AIS (OR: 0.48, 95% CI: 0.26-0.89, P=0.018) and perinatal AIS (OR: 0.45, 95% CI: 0.21-0.97, P=0.041), but not of childhood AIS (P=0.074). Increased risk for AIS was not found for any single P-SEL gene polymorphism, but carriers of PSEL-562DD genotype had a 2.37-fold (95% CI: 1.07-5.23, P=0.034) increased risk for perinatal AIS. The presence of P-SEL-599LL genotype was significantly associated with childhood AIS (P=0.048). Haplotype ANDVT (FV Leiden/P-SEL-S290N/N562D/V599L/T715P) was identified more frequently in perinatal AIS (0.053) compared to controls (0.005), but the difference was not statistically significant (P=0.075). On contrary, lower, although nonsignificant HPA-1a/2a/3b haplotype frequency (P=0.078) was found in childhood AIS (0.242) compared to controls (0.365).

Conclusions: Identified association of HPA-3b allele with perinatal AIS and different P-SEL polymorphisms with perinatal and childhood AIS corroborate our previous finding that perinatal and childhood AIS do not share the same genetic risk factors.

PB 1647 | MicroCT Imaging of Cerebral Thromboemboli *In vivo*: The Effects of Tissue Plasminogen Activator

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Background: Since tPA was first introduced in 1996, no study has quantitatively assessed a detailed spatial and temporal evolution of cerebral thromboemboli before, during, and after tissue plasminogen activator (tPA) treatment.

Aims: To quantify the evolution of cerebral thromboemboli using fibrin-targeted gold nanoparticles (fib-GC-AuNPs) and micro-computed tomography (mCT), with/without tPA-therapy.

Methods: We injected thrombi into the distal internal carotid artery in mice (n=50). Fifty-five minutes later, we injected fib-GC-AuNPs, and five minutes after that, treated animals with tPA or not (25mg/kg). We acquired serial mCT images for 24 hours post-treatment.

Results: Thrombus-burden at baseline was $784\pm59\times10^3\mu m^2$ for the tPA-group (n=42) and $655\pm103\times10^3\mu m^2$ for the saline-group (n=8, P=0.37). Thrombus shrinkage began at 0.5-1 hour after tPA-therapy, with a maximum initial Rate-of-Change (RoC) at $4603\pm957\mu m^2$/minute. The RoC lowered to ~61% level of the initial in hours 1-2, followed by ~29% and ~1% in hours 2-3 and 3-24, respectively. Thus, 85% of total thrombolysis over 24 hours (about $500\mu m^2$, equivalent to 64% of the baseline thrombus-burden) occurred within the first 3-hours of treatment. Thrombus-burden at 24 hours could be predicted at around 1.5-2 hours. Saline treatment was not associated with significant changes in the thrombus-burden. Infarct size was smaller in the tPA-group vs. saline-group ($18.1\pm2.3mm^2$ vs. $45.8\pm3.3mm^2$, P< 0.01). Infarct size correlated to final thrombus-burden (r=0.71, P< 0.01). Time to thrombolysis, completeness of thrombolysis, and tPA-therapy were independent predictors of infarct size.

Conclusions: Thromboembolic burden and the efficacy of tPA-therapy can be assessed serially, non-invasively, and quantitatively using high-resolution mCT and a fibrin-binding nanoparticle imaging-agent.

PB 1648 | Incidence Rates and Case Fatality Rates of Cerebral Vein Thrombosis: A Population-based Study

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Background: The incidence of cerebral vein thrombosis (CVT) is estimated at 0.2- 0.5/100000/year. These data derived from autopsy studies performedl decades ago. Little is known about mortality rates in patients with CVT during the acute phase of the disease.

Aims: To estimate, in a large epidemiologic study on hospital admissions in Northwestern Italy, the contemporary incidence of hospital admissions for pyogenic (PCVT) and non pyogenic CVT (NPCVT) in Italy, to assess in-hospital mortality rates and factors associated with mortality, and to assess trends in the incidence of this disease from 2002 to 2012.

Methods: Primary and secondary discharge diagnoses of PCVT and NPCVT were identified using ICD-9 codes in Lombardy and Piedmont. Recurrent events were excluded. Age, gender, vital status at discharge, duration of hospitalization, up to 5 secondary discharge diagnoses and concomitant intracranial hemorrhage (ICH) were registered. Comorbidity was evaluated using the Charlson comorbidity index (CCI).

Results: 1718 patients were hospitalized for CVT (1147 females, 66.7%; 810 PCVT and 908 NPCVT), 134 patients (7.8%) had a concomitant ICH. The overall gender-specific incidence rates for CVT were 17.3 (95% CI 16.3, 18.4)/1000000 in females and 8.3 (95% CI 7.6, 9.1)/1000000 in males. The incidence was highest in women aged 45-50 years (27 cases/1000000) and increased in women from 2002 to 2012. In-hospital case fatality rate (CFR) was significantly higher in patients with concomitant ICH (7.46% vs 2.65%; p< 0.001) and progressively increased with CCI (2.27% in CCI=0, 14.29% in CCI>1; p< 0.001). Age and CCI were independently associated with in-hospital mortality after stepwise regression analysis.

Conclusions: Incidence of CVT is higher than previously reported. The incidence appeared to increase in women during the period of observation. In-hospital CFR was not negligible in patients presenting with a concomitant ICH.

PB 1649 | Biomarkers for the Symptomatic Carotid Atheroma

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Background: Unstable carotid plaque is an important source of extracranial atheroembolism and stroke. Current guidelines for stroke-preventive carotid endarterectomy are based on surrogate markers for stroke risk and no precise diagnostic tools are available for identification of either individuals or lesions at risk.

Aims: Our aim is to identify biomarkers for the unstable atheroma, which could offer new diagnostic tools to improve selection of patient for intervention.

Methods: We analyzed transcriptional profile of plaques from patients treated for symptomatic (S) and asymptomatic (AS) carotid stenosis, collected within the Biobank of Karolinska Endarterectomies (BiKE) using microarrays. Proteomic profiling of peripheral and ‚local' plasma, obtained during carotid clamping prior to arteriotomy, was performed by suspension antibody-bead assays using over 10,000 antibodies as part of the Human Protein Atlas project.

Results: Plasma analysis identified 150 candidates with p< 0.05 based on comparisons in local vs. peripheral plasma, as well S vs. AS patients in both plasma sources. Among the identified candidates, Biliverdin reductase B (BLVRB) was significantly enriched in local plasma and plaque tissue. Upregulation of BLVRB in plasma was validated by dual binder assay in both the discovery and an extended validation sample set. Moreover, transcriptomic levels of BLVRB in plaques from S patients were verified by qPCR and BLVRB was localized in CD68+ macrophages by immunohistochemistry.

Conclusions: The high-throughput analysis of plasma and tissue samples from patients undergoing carotid surgery permitted identification of BLVRB as a potential biomarker for unstable carotid atherosclerosis. Further validation in independent, extended cohorts of patients is necessary in order to determine predictive power of BLVRB for the identification of individuals at risk or development of targeted bioimaging for lesion detection.

PB 1650 | Neutrophil Extracellular Traps (NETs) in Patients with Acute Stroke Are Associated with Stroke Severity and Progression

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Background: NETs are considered a link between immunity, inflammation and thrombosis. Data on arterial thrombosis is scarce, particularly in patients with acute stroke, and there is no data on the NET-specific marker, citrullinated histone 3 (CitH3) in stroke.
Aims: To study NETs in the plasma of patients with acute stroke, and their potential association with stroke severity, risk factors, and clinical outcomes at one-year.
Methods: Patients (n=243) had suffered stroke < 24h before admission to hospital and treated according to stroke guidelines. The study was approved by the ethical Committee of the Hospital La Fe. All patients or their proxy gave their written informed consent. Clinical and demographic data were registered, including scores of neurological severity (NIHSS, mRs) at the onset and at discharge. Patients were followed-up for one-year. A control group of normal subjects was used for comparison. Cell free DNA (cfDNA), nucleosomes and CitH3 were determined in plasma as markers of NETs.
Results: All the NET markers were significantly elevated in patients as compared with controls, and positively associated with the clinical scores of stroke severity at entrance and discharge. CitH3 was elevated particularly in older patients (> 65 years of age), patients with higher fasting glucose, and in patients with history of atrial fibrillation (AF). In multivariate analysis including risk factors, medications and NIHSS, elevated CitH3, the most specific marker of NETs, was independently associated with AF and all-cause mortality at one-year follow-up.
Conclusions: Conclusions. NETs participate in the pathopysiology of ischemic stroke and are associated with the stroke severity and mortality. NETs could be a potential therapeutic target in patients with acute stroke, and especially CitH3 may be of prognostic value in those patients. Grants. FIS13/00016. ACIF/2016/465. RETICS networks INVICTUS (RD12/0014/0004) and INVICTUS+ (RD16/0019/0008) Instituto de Salud Carlos III.

PB 1651 | Case-fatality after Venous Thromboembolism and Ischemic Stroke in Subjects without and with Atrial Fibrillation. The Tromsø Study

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Background: Atrial fibrillation (AF) is associated with increased risk of ischemic stroke (IS) and stroke severity. Recent studies have also demonstrated that patients with AF have increased risk of venous thromboembolism (VTE), but data on VTE-related mortality in AF is lacking.
Aims: To investigate the impact of atrial fibrillation on mortality rates after incident VTE and ischemic stroke in a population-based cohort.
Methods: Patients with a first, objectively confirmed VTE (n=735) or IS (n=1250) were recruited among participants in the Tromsø study (1994-2008) and deaths were recorded up to the end of 2012. Information on prevalent AF and potential confounders was obtained at baseline. Crude mortality rates (per 100 person-years) according to AF status were calculated among patients with VTE and IS, respectively, and Cox proportional hazards regression models were used to obtain hazard ratios (HR) for death with 95 % confidence intervals (CI).
Results: There were 337 (45.9%) deaths among the VTE patients and 682 (54.6%) deaths among the IS patients during a mean follow-up of 4.6 years. In VTE patients, the mortality rate was 20.6 (95% CI 15.9-26.7) in those with AF and 9.0 (95% CI 8.0-10.2) in those without AF. In IS patients, the corresponding rates were 19.8 (95% CI 17.1-23.1) and 10.1 (95% CI 9.3-11.1), respectively. AF was associated with a 1.7-fold (HR 1.73, 95% CI 1.29-2.30) increased risk of death after VTE, and a 1.5-fold risk of death after IS (HR 1.45, 95% CI 1.22-1.73) in analyses adjusted for age and sex. Multivariable adjustment for cardiovascular risk factors (BMI, hypertension, total cholesterol, smoking, diabetes and a history of myocardial infarction) barely altered the risk estimates.
Conclusions: We found that atrial fibrillation aggravated the mortality rate to a similar extent in patients with VTE and stroke. Our findings suggest that special medical attention should be given to VTE and stroke patients with AF.

PB 1652 | Role of ARHGEF10 in Platelet Function and Thrombosis

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Background: ARHGEF10, a member of Rho guanine nucleotide exchange factor (GEF) family, stimulates the Rho GTPases. Rho GTPases have been reported to regulate a variety of cellular behaviors such as cell polarity, cytoskeletal organization, and gene transcription. GEFs are responsible for the activation of Rho GTPase and ARHGEF10 is especially for RhoA.
Aims: ARHGEF10 is expressed in several tissues in human and murine, however, its role and impact on platelet function are not well understood.
Methods: We generated ARHGEF10 knockout mice and examined the in vitro and in vivo effect of ARHGEF10 knockout on the platelet function and thrombosis formation.
Results: ARHGEF10 knockout mice had normal platelet counts but displayed altered aggregation in response to collagen, ADP, PAR4 peptide and U46619 stimulation. Knockout of ARHGEF10 influenced platelets spreading on fibrinogen - coated surface and formed less lamellipodia-like extension than wild-type platelets. ARHGEF10 knockout also inhibited platelet clot retraction induced by thrombin stimulation. Knockout of ARHGEF10 resulted in prolonged tail bleeding time and

inhibited the stable thrombus formation induced by $FeCl_3$ in the carotid artery. Furthermore, the ischemia/reperfusion-induced infarct volume was markedly reduced in ARHGEF10 knockout mice.

Conclusions: These results demonstrate the role of ARHGEF10 in platelet function in homeostasis and thrombosis.

PB 1653 | The Efficacy and Safety of a Laser Thrombolytic System in Animal Thrombosis Models

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Background: Currently, the thrombolytic agent rt-PA is used for treating acute cerebral infarction in the world. However, the risks of cerebral bleeding and other side effects result in the limited application. In addition, endovascular interventions (e.g., Merci retriever, Penumbra system and Solitaire FR) are also applied to treating acute cerebral infarction. Yet, those devices are not completely free from clinical problems, e.g., risk of vessel injury, indicating the need for safer, quicker, and more reliable recanalization approaches. We therefore developed a laser thrombolytic system with the second harmonic generation of microsecond Nd:YAG laser.

Aims: We investigated its effectiveness, safety and mechanisms in animal thrombosis models.

Methods: The dynamics of laser-induced thrombolysis in a gelatin phantom model was investigated with a high-speed camera. In vivo thrombolytic efficacy was investigated using several animal thrombosis models. Thrombi in the vena cava inferior of rats or in the carotid artery of rabbits were induced by an application of ferric chloride ($FeCl_3$). Laser irradiation was then carried out through an optical fiber inserted from the femoral vein or artery.

Results: The dynamic observation revealed that laser irradiation generated a bubble in the gelatin phantom. Laser irradiation induced significant thrombolysis in the rat thrombosis model. Laser irradiation also resulted in recanalization in the rabbit thrombosis model. One day after the recanalization, neurological disorders, cerebral ischemia and cerebral hemorrhage were not observed. No vascular endothelial damage evaluated by Evans blue staining after laser irradiation was observed.

Conclusions: The irradiation of the pulsed green laser can induce bubbles that fragment thrombi without vessel or brain damage. Recently, we have started investigator-initiated clinical trials in Japan.

PB 1654 | Thrombi Retrieved from Patients Suffering Acute Ischemic Stroke Show both Active and Mechanical Interaction between Stent Retrievers and Thrombus, and is Associated with Specific Thrombus Surface Characteristics

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Background: Multiple randomized controlled trials have confirmed the positive effects of intra-arterial treatment (IAT) by means of retrievable stent based thrombectomy on clinical outcome for patients suffering acute ischemic cerebral stroke (AIS). Although a pure mechanical interaction between clot and stent-retriever is suspected, no study has focused on the direct interaction between stent-retriever and thrombus.

Aims: To study the interaction between stent and thrombus following thrombectomy for AIS.

Methods: From the MR CLEAN registry, seven stents were collected directly after IAT. The stent-retriever with thrombus was carefully rinsed in non-heparinized saline and fixed in buffered formaldehyde-glutaraldehyde in preparation for micro CT, scanning electron - and light microscopy (SEM, LM). All stent-thrombus interactions and associated thrombus surfaces were studied by SEM. In total 85 interactions were identified by two independent raters. Then, specimens were prepared for LM (methacrylate embedding), sectioned and stained using Hematoxylin-Eosin as an overview stain, Resorcin-Fuchsin as an elastin stain, and Okajima as a hemoglobin stain.

Results: Retrieved thrombi were heterogeneous in composition as determined by LM, consisting of erythrocyte rich and fibrin rich areas. Atheromatous plaque was not observed. Stent-thrombus interaction as determined by SEM consisted of mechanical interaction in 47% of interaction sites, showing thrombus wrapped around wires. Active adhesion was observed in 53% of interaction sites, showing mostly thrombus coalescing around the wires. While mechanical interaction did not show an association with a specific thrombus surface, active interaction between thrombus and stent retriever was characterized by a dense thrombus surface, p=0.003 (Chi-square)).

Conclusions: The interaction between stent retrievers and thrombus as revealed by SEM identifies both active and mechanical interaction, with active adhesion being associated with specific surface characteristics.

PB 1655 | The Effect of Recanalization on Long-term Neurologic Outcome after Cerebral Vein Thrombosis

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Background: Only few studies with small sample sizes have investigated the long-term recanalization rates after cerebral venous thrombosis (CVT) and their association with neurologic outcome.

Aims: To evaluate the recanalization rate in a large population of patients with CVT and to assess the prognostic role of recanalization on the long-term neurologic outcome in these patients.

Methods: Patients with an acute first episode of CVT with at least one available imaging test during follow-up were included. Patency status of the vessels was categorized as completely, partially or not recanalized. Neurologic outcome was defined using the modified Rankin Scale (mRS) as excellent by a score of 0-1 or poor by a score of 2-6. Predictors of recanalization and residual disability were assessed with a follow-up of up to three years.

Results: Five-hundred and sixteen patients (median (IQR) age, 39 (28.5-49) years; 74% female) were included. Complete or partial recanalization was detected in 81.2% of patients at scans performed between 28 days and 3 months; in 75.2% of patients at scans performed between 3 and 6 months; in 80.4% of patients at 6-12 months; 74.5 of patients at 1-3 years (p=0.452). At multivariate analysis, single site involvement (OR 1.78, 95% CI 1.27-2.50) and pregnancy or puerperium at the time of index event (OR 2.16, 95% CI 1.23-3.77) were associated with recanalization, whereas age>39 years was associated with no recanalization (OR 0.54, 95% CI 0.38-0.77). mRS at the time of follow-up imaging was available in 491 patients; 454 of them (92.5%) had a mRS of 0-1. CVT recanalization (OR 2.30, 95% CI 1.47-3.60), cancer (OR 0.28, 95% CI 0.09-0.84), and personal history of venous thromboembolism (OR 0.36, 95% CI 0.14-0.91) were independently associated with a favourable outcome at follow-up.

Conclusions: Most patients with a first CVT had complete or partial recanalization at follow-up. Recanalization was independently associated with a good neurologic outcome.

PB 1656 | Hyperglycemia Precipitates Infarct Growth and Hemorrhagic Transformation in Ischemic Stroke Though Exacerbation of Thromboinflammation

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Background: Admission hyperglycemia is associated with a poor outcome in acute ischemic stroke (AIS). How hyperglycemia impacts the pathophysiology of AIS remains largely unknown.

Aims: We investigated how preexisting hyperglycemia increases ischemia/reperfusion cerebral injury.

Methods: Normoglycemic (NG) and streptozotocin-treated hyperglycemic (HG) rats were subjected to transient middle cerebral artery occlusion (tMCAO). Infarct growth and brain perfusion were assessed by perfusion/diffusion MRI sequences. Markers of platelet, coagulation, and neutrophil activation were measured in plasma samples and in brain homogenates. tMCAO-associated thromboinflammation was investigated by intravital microscopy.

Results: HG rats had an increased infarct volume with an increased blood-brain barrier (BBB) disruption and hemorrhagic transformation rate compared to NG rats. MRI scans during occlusion and after recanalization revealed that hyperglycemia enhanced and accelerated diffusion lesion growth, and was associated with hemorrhagic transformation (HT) originating from non-reperfused downstream territories. Intravital microscopy showed that downstream microvascular thromboinflammation (DMT) was initiated immediately after MCA occlusion and was exacerbated by hyperglycemia. Analysis of plasma samples indicated that neutrophils and platelets were primed in HG rats. Neutrophils from HG diabetic patients showed increased adhesion to endothelial cells as compared to neutrophils from NG control donors in flow chamber experiments.

Conclusions: We show that hyperglycemia primes the inflammatory cascade, thus amplifying MCAO-induced DMT. DMT exacerbation in HG rats impaired reperfusion and precipitated neurovascular damage, BBB disruption and hemorrhagic transformation. Importantly, we show that HT occurred in non-reperfused areas, thus challenging the current paradigm that HT is a consequence of reperfusion. Our results designate DMT as a possible target for reduction of the deleterious impact of hyperglycemia in AIS.

PB 1657 | The Association between Ring Finger Protein213 (RNF213) Polymorphisms and Ischemic Stroke Risk in Korean Patients

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Background: Ring finger protein 213 (RNF 213) encodes RNF motif that functions as an E3 ubiquitin involved in angiogenesis. Therefore, RNF213 may be relevant to vascular disorder of intracranial arteries including ischemic stroke and moyamoya disease.

Aims: Aim of this study was four RNF213 polymorphisms that RNF213 4448G>A, 4810G>A, 4863G>A, and 4950G>A association with ischemic stroke susceptibility.

Methods: We analyzed the associations with RNF213 polymorphisms in 529 ischemic stroke patients and 424 controls. DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. Genotyping was performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Results: The RNF213 4950G>A (GA: AOR, 1.547; 95% CI, 1.025-2.335, P=0.038; GA+AA: AOR, 1.637; 95% CI, 1.089-2.461, P=0.018) was significantly associated with ischemic stroke. Also, the genotype combination analysis of RNF213 was shown that RNF213 4448GG/4950GA (AOR, 5.327; 95% CI, 2.482-11.434, P< 0.001), RNF213 4448GA/4950GG (AOR, 1.769; 95% CI, 1.028-3.047; P=0.040) combined genotypes were increased risk of ischemic stroke. Whereas RNF213 4448GA/ 4950GA (AOR, 0.216, 95% CI, 0.091-0.514, P=0.001) was decreased ischemic stroke risk.

Conclusions: Our data demonstrated that RNF213 4950G>A polymorphism associated with ischemic stroke susceptibility.

PB 1658 | Effects of Phosphodiesterase 3A Modulation on Murine Cerebral Microhemorrhages

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Background: Cerebral microbleeds are MRI-demonstrable cerebral microhemorrhages (CMH), which commonly coexist with ischemic stroke. A strategy that simultaneously protects the vessel wall and provides anti-thrombotic activity is an attractive potential therapeutic approach. Phosphodiesterase 3A (PDE3A) inhibition is known to have vasculoprotective and anti-thrombotic effects.

Aims: To test the hypothesis that inhibition of the PDE3A pathway is protective against CMH development.

Methods: PDE3A pathway inhibition was studied in the inflammation-induced mouse model of CMH. 3mg/kg lipopolysaccharide (LPS) was given intraperitoneally (IP) at 0, 6, and 24 hours, and brains are harvested at 48 hours. The PDE3A pathway was examined using PDE3A knockout mice (KO) (9/15 LPS-treated) and wildtype (WT) littermates (16/20 LPS-treated), and effects on hematoxylin & eosin (H&E)-positive CMH development; BBB function (IgG, claudin-5, and fibrinogen); inflammation of brain endothelium (ICAM-1), astrocytes (GFAP), and microglia (Iba-1); and serum TNF-α were investigated.

Results: Robust development of CMH was observed following LPS treatment. LPS induced significant increases in all markers of BBB dysfunction and inflammation. Deletion of the PDE3A gene did not alter CMH number or size. Compared to WT littermates, PDE3A KO mice had significantly reduced markers of endothelial and astrocyte activation and peak TNF-α levels following LPS treatment. Indices of BBB function and microglial activation did not significantly differ between PDE3A KO and WT littermates.

Conclusions: Genetic deletion of PDE3A does not alter CMH development in an inflammation-induced mouse model of CMH. Comparison of PDE3A KO versus WT mice using this model showed that microglial activation was associated with CMH development and BBB dysfunction. The role of microglial activation in CMH development warrants further investigation.

PB 1659 | Coding Variants in ADAMTS Genes Derived from Next Generation Sequencing Associated with Pediatric Stroke

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Background: Extracellular matrix components like members of the ADAMTS gene family in combination with misbalanced coagulation signals appear to play an important role in pediatric stroke etiology after postnatal vascular injuries. Recently, we have reported results of a family-based genome wide association study (GWAS) pointing our attention to ADAMTS2 and ADAMTS12.

Aims: Identification of coding variants in ADAMTS genes contributing to risk for pediatric stroke.

Methods: ADAMTS2 and ADAMTS12 were sequenced in 48 affected children and 48 unaffected siblings to identify putative causative variants using next generation sequencing (NGS). Sequencing reads (>50x coverage) were mapped by using BWA and analyzed by GATK. We identified 8 non-synonymous single nucleotide polymorphisms (SNPs) in ADAMTS2 and 6 in ADAMTS12 potentially influencing the respective protein function. Promising variants were genotyped within a cohort of 270 affected offspring trios using Taqman genotyping assays. Association with pediatric stroke was calculated using the Transmission Disequilibrium Test (TDT) as implement in PLINK. No adjustment for covariates was applied.

Results: While no significant association was found for variants residing in ADAMTS2, we observe the ADAMTS12 variant rs77581578 to be significantly under-transmitted (p = 6.26E-3) to pediatric stroke patients. This variant resides within a thrombospondin 1 domain of ADAMTS12 and leads to replacement of proline by threonine at amino acid position 1329 (NP_001311441). This change is predicted as damaging to the protein according to the respective SIFT score of 0.029 and potentially influences the protein's function and adhesion properties.

Conclusions: A protective variant in ADAMTS12 likely influences the function of ADAMTS12 protein and thereby decreases the risk for pediatric stroke. To validate this result, future studies in independent replication cohorts as well as functional studies to assess its biological properties are warranted.

PB 1660 | Long-term Stroke Risk Prediction in 'Real World' Atrial Fibrillation Patients: A Comparison of the ABC-stroke and CHA_2DS_2-VASc Scores

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Background: The ABC-stroke score (age [A], cardiac biomarkers (N-terminal fragment B-type natriuretic peptide, troponins high-sensitivity) [B], and clinical history (prior stroke/transient ischaemic attack) [C])

has recently been proposed to predict stroke in atrial fibrillation (AF). This score was derived and validated in two clinical trial cohorts, where AF patients are often highly selected and carefully followed-up. ‚Real world' patients tend to be older, with associated comorbidities and polypharmacy.

Aims: Given that the ABC-stroke score was derived from a trial cohort with a median follow-up of 1.9 years, we aimed to investigate its long-term predictive performance in comparison with CHA_2DS_2-VASc.

Methods: We recruited 1125 consecutive AF patients stables on Vitamin K Antagonists (INR 2.0-3.0) and followed-up for a median of 6.5 years. We calculated ABC-stroke and CHA_2DS_2-VASc scores. All ischaemic strokes were recorded.

Results: The median CHA_2DS_2-VASc and ABC-stroke scores were 4 (IQR 3-5) and 9.1 (IQR 7.3-11.3), respectively. There were 58 ischaemic strokes (1.50%/year) at 3.5 years (IQR 3.1-3.6) and 114 (1.55%/year) at 6.5 years (IQR 4.3-7.9). The c-index of ABC-stroke at 3.5 years was significantly higher than CHA_2DS_2-VASc (0.66 vs. 0.60, p=0.046) but at 6.5 years the c-indexes of ABC-stroke and CHA_2DS_2-VASc were similar (both approx. 0.6), with no difference in their predictive performances.

IDI showed a small improvement (< 2%) in sensitivity at 3.5 and 6.5 years with ABC-stroke. NRI demonstrated a non-significant positive reclassification at 3.5 years and a negative reclassification at 6.5 years of the ABC-stroke compared with CHA_2DS_2-VASc. Decision curves analyses did not show a substantial improvement in clinical usefulness of the ABC-stroke over CHA_2DS_2-VASc.

TABLE 1 C-indexes, IDI and NRI of the ABC-stroke score in comparison with CHA2DS2-VASc score for predicting ischaemic stroke

	C-index	95% CI	p	IDI	p	NRI	p
At 3.5 years							
ABC-stroke score vs. CHA2DS2-VASc	0.600 vs 0.663	0.567-0.625 0.634-0.690	<0.001 <0.001	0.019	0.002	0.002	0.903
At 6.5 years							
ABC-stroke score vs. CHA2DS2-VASc	0.620 vs 0.662	0.590-0.648 0.633-0.690	<0.001 <0.001	0.019	0.002	-0.053	<0.001

CI = confidence interval; IDI = integrated discriminatory improvement; NRI = net reclassification index

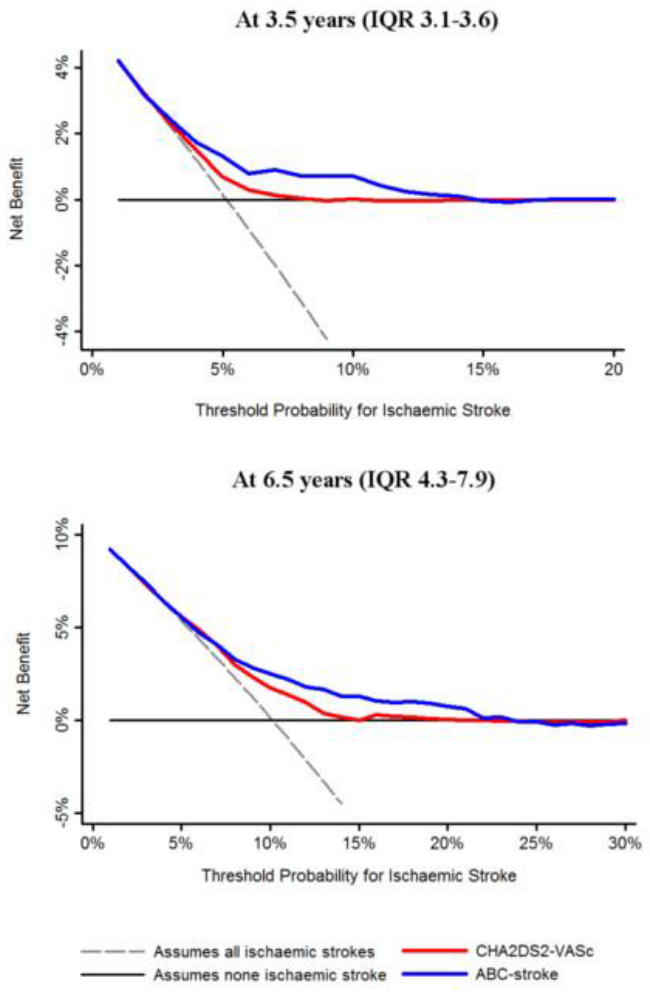

FIGURE 1 Decision curves for the ABC-stroke and CHA2DS2-VASc scores.

Conclusions: In 'real world' anticoagulated AF patients followed-up over a long-term period, the novel ABC-stroke score has not better predictive performance compared to the CHA_2DS_2-VASc score.

PB 1661 | Time-restricted Feeding Disturbs Circadian Expression of Thrombomodulin in the Mouse through Peripheral Clock Molecules

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Background: Cardiovascular diseases are closely related to circadian rhythm, which is under the control of the internal biological clock, and it has been shown that most of cardiovascular diseases including stroke occurred in the morning to late morning. A biological clock exists not only in the hypothalamus but also in peripheral tissues and desynchronization between the central and peripheral clocks by altered timing of food intake can induce development of cancer, metabolic syndrome, and obesity.

Aims: However, whether the timing of food intake influences the thrombin formation is obscure. In this study, we measured the expression of thrombomodulin (TM), an important regulator of thrombin production on the endothelium, in the mice fed on normal or high-fat diets under the regular and/or time-restricted feeding patterns.

Methods: Under the condition of constant light-dark cycle, male mice were received a normal or a high-fat diet containing 1% cholesterol in the night- or day-time for 2 to 30 weeks, and measured mRNA and antigen levels of TM in lung which is abundant in microendothelium. To examine interaction of TM protein with the promoter region of the gene, cultured human cells were used.

Results: A circadian variation of TM mRNA level as well as that of the circadian clock gene *per2*, in the lung of mice fed a normal diet was observed, though the expression rhythms were obviously damped in *clock* mutant mice. The mRNA and antigen levels of TM were reduced by feeding of a high-fat diet containing 1% cholesterol, and the circadian oscillation of the TM expression was shifted by temporal restriction feeding of the both diets.

Conclusions: These results demonstrate that the peripheral clocks in vascular endothelial cells modulate TM gene expression and that the regularly eating of breakfast is quite important to prevent thrombosis formation through maintaining the daily normal oscillation of TM expression.

PB 1662 | Reduced ADAMTS13 Levels in Patients with Acute and Chronic Cerebrovascular Disease

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Background: Von Willebrand Factor (VWF) plays a major role in thrombosis and hemostasis and its thrombogenicity is controlled by ADAMTS13. Whereas increasing evidence shows a clear association between VWF levels and acute ischemic stroke, less is known about a correlation with ADAMTS13.

Aims: To compare plasma levels of ADAMTS13 between acute ischemic stroke patients, patients with a chronic cerebrovascular disorder (CCD) and healthy volunteers (HV).

Methods: In this case-control study, ADAMTS13 antigen levels from 104 ischemic stroke patients, 112 patients with CCD and 85 HV were measured by ELISA. VWF levels, measured previously, were used to calculate VWF:ADAMTS13 ratios. ADAMTS13 levels and VWF:ADAMTS13 ratios were correlated with key demographic and clinical parameters.

Results: Acute stroke patients had significantly reduced ADAMTS13 levels (82.6% ± 21.0%) compared with HV (110.6% ± 26.9%; $p < 0.0001$). Interestingly, also CCD patients had significantly lower ADAMTS13 levels compared with HV (99.6% ± 24.5%; $p < 0.03$), however this was still higher compared with the acute stroke patients ($p < 0.0001$). In particular, the combination of high amounts of VWF with low levels of ADAMTS13 could be harmful. We therefore also assessed the VWF:ADAMTS13 ratio in our cohorts. A significantly higher VWF:ADAMTS13 ratio was found in stroke patients (2.7 ± 1.9) compared with HV (1.1 ± 0.5; $p < 0.0001$) and patients with chronic cerebrovascular disease (1.7 ± 0.7; $p < 0.0001$). Interestingly, the VWF:ADAMTS13 ratio was significantly associated with stroke severity (NIHSS: $p = 0.048$; mRS: $p = 0.015$ and BI: $p = 0.004$) and stroke modality (AIS or TIA; $p = 0.023$).

Conclusions: In both acute and chronic cerebrovascular disease patients, ADAMTS13 levels were significantly decreased, with the lowest ADAMTS13 levels found in acute stroke patients. This difference was even more distinct when the ratio of VWF:ADAMTS13 was considered. These results demonstrate the possible important involvement of the VWF/ADAMTS13 axis in ischemic stroke.

PB 1663 | High Level of von Willebrand Factor Ristocetin Cofactor Activity is Associated with Poor 2-years' Prognosis in Cerebral Infarction

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Background: Cerebral infarction (CI) is the most common type of stroke, and it also generates quantitative and qualitative changes of von Willebrand factor (VWF).

Aims: To investigate the clinical value of VWF ristocetin cofactor activity (VWF:Rco) in predicting the 2-years' prognosis of CI patients.

Methods: 61 CI patients and 104 controls (CTL) were measured for VWF antigen (VWF:Ag) and VWF:Rco by ELISA. CI patients were divided into three subgroups: < 40, 40-60 and > 60 years old. The modified Barthel index and Rankin score were used to evaluate the followed up 2-years' prognosis of activities of daily life and neurological recovery.

Results: Plasma VWF:Ag and VWF:Rco in CI patients (mean ± SD, 172.5% ± 10.4 and 225.0% ± 14.6) were significantly higher than those in controls (72.8% ± 4.1 and 65.4% ± 3.4, all $P < 0.0001$), and VWF:Rco in CI patients was positively correlated with VWF:Ag (Figure 1A, $r = 0.996$, $P < 0.0001$). VWF:Rco in CI patients was increased with age, and had significant differences in the three subgroups (162.3% ± 29.5, 214.4% ± 17.6 and 315.6% ± 25.5, $P < 0.01$). There were no significant differences between incipient and relapsed CI patients for VWF:Ag and VWF:Rco. VWF:Rco in CI patients was positively correlated with both high-sensitivity C-reactive protein (Figure 1B, $r = 0.382$, $P < 0.01$) and the 2-years' recovery of neurological function (Figure 1D, $r = 0.269$, $P < 0.05$) in the firstly diagnosed CI patients, however, VWF:Rco in CI patients was negatively correlated with 2-years' activities of daily life (Figure 1C, $r = -0.293$, $P < 0.05$). The plasma ultra-large VWF was increased in CI patients.

Conclusions: CI patients on admission had significantly higher VWF:Ag and VWF:Rco than those in controls. VWF:Rco level was associated with the severity of CI, and high VWF:Rco level was associated with poor 2-years' prognosis in CI patients. VWF:Rco may be used as a marker to predict the prognosis of CI.

PB 1664 | Evaluation of the Thrombolytic Potential of N-acetyl Cysteine in a Mouse Model of Thrombotic Stroke

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Background: The mainstay of ischemic stroke treatment is aimed at achieving fast restoration of blood flow. We recently demonstrated a

thrombolytic effect of ADAMTS13 on von Willebrand Factor (VWF)-rich thrombi that were resistant to t-PA mediated thrombolysis. N-Acetylcysteine (NAC) is a mucolytic drug that was shown to also disassemble large VWF multimers.

Aims: The aim of this study was to assess the effect of NAC in stroke, with a particular focus on its thrombolytic potential.

Methods: The therapeutic potential of NAC was assessed in a mouse model of $FeCl_3$-induced thrombotic stroke, characterized by a VWF-rich occlusive thrombus, and a mouse model of transient middle cerebral artery occlusion (tMCAO). Cerebral blood flow was monitored, and twenty-four hours after stroke mice were neurologically scored and brain infarct sizes were calculated.

Results: When NAC was administered 5 min before vessel wall injury, occlusive thrombus formation was significantly delayed or did not occur within the experimental timeframe of 40 min ($p< 0.01$). As expected, this effect of NAC on thrombus formation was associated with a reduced stroke brain damage ($p< 0.001$). However, when NAC was administered 5 min after thrombotic occlusion of the MCA, NAC was unable to restore blood vessel patency, and infarct sizes were similar in vehicle and NAC treated animals. Despite the lack of a thrombolytic effect, the anti-thrombotic properties of NAC could be beneficial in the reperfusion phase after ischemic stroke. Indeed, in a model of cerebral ischemia/reperfusion injury, NAC reduced cerebral infarct sizes when administered immediately after reperfusion was allowed ($p< 0.01$).

Conclusions: We have shown that NAC is protective when given before thrombotic stroke and during cerebral reperfusion injury. However, NAC was unable to dissolve an already formed VWF-rich thrombus when treatment was initiated after occlusion. These results implicate distinctive effects of NAC on circulating VWF and VWF incorporated in a thrombus.

PB 1665 | Is There a Difference in Inherited Prothrombotic Polymorphisms between Arterial Ischemic Stroke and Cerebral Sinus Venous Thrombosis in Children?

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Background: Cerebral sinus venous thrombosis (CSVT) in children is a rare, but serious multifactorial disorder, associated with significant morbidity and mortality. The etiology of CSVT is not completely clarified. The role of inherited prothrombotic risk factors remains to be elucidated. To date, no difference in investigated inherited prothrombotic polymorphisms between arterial ischemic stroke (AIS) and CSVT in children was demonstrated.

Aims: The aim was to investigate whether CSVT and AIS in children share the same inherited prothrombotic polymorphisms.

Methods: The study included 14 children with CSVT (7 boys and 7 girls) and 41 children with AIS (20 boys and 21 girls). Sixteen polymorphisms (FV Leiden, FV HR2, FII G20210A, MTHFR C677T, MTHFR A1298C, FXIII-A Val34Leu, PAI-1 4G/5G, EPCR haplotype, eNOS -786T>C, eNOS G894T, LTA C804A, ACE I/D, HPA-1, β-fibrinogen -455G>A, and apoE ε2-4 were genotyped using a multilocus CVD Strip assay (ViennaLab, Austria).

Results: Similar genotype distribution frequencies for majority of investigated polymorphisms (FV Leiden, FV HR2, FII G20210A, MTHFR C677T, FXIII-A Val34Leu, PAI-1 4G/5G, eNOS -786 T>C, eNOS G894T, ACE I/D, β-fibrinogen -455G>A and apoE ε2-4) were found in both patient groups. Higher frequencies of MTHFR 1298CC genotype, although nonsignificant (P=0.270), were identified in CSVT (0.14) compared to AIS (0.05). On contrary, LTA 804AA genotype (4/41) and EPCR A3 haplotype (7/41) were found only in children with AIS, but the associations were not significant (P=0.120 and P=0.172, respectively). Statistically signicant difference of genotype distributions were obtained for HPA-1 (P=0.009), resulting in a 5.50-fold increased risk for CSVT (95% CI: 1.48-20.39).

Conclusions: Although a relatively small number of children with CSVT was investigated, obtained results indicate that AIS and CSVT mostly share the same inherited prothrombotic polymorphisms. Results suggest that HPA-1, not yet identified as risk factor, could be involved in the etiology of CSVT in children.

PB 1666 | Alpha-2-antiplasmin Arg407Lys Polymorphism and Cryptogenic Ischemic Cerebrovascular Events: Association with Neurological Deficit

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Background: Studies on the genetic background of ischemic stroke (IS) and transient ischemic attack (TIA) have yielded inconsistent results. Alpha-2-antiplasmin (α2AP) Arg407Lys polymorphism has been previously examined in patients with abdominal aortic aneurysm (AAA) and showed a different distribution in AAA patients compared with healthy controls (Arg/Arg: 71% vs. 65%; Arg/Lys: 27% vs. 33%; Lys/Lys: 2% vs. 2%, respectively).

Aims: We investigated associations of α2AP Arg407Lys polymorphism with the occurrence of IS and TIA and neurological deficits during follow-up.

Methods: We studied 199 Caucasian patients aged below 70 years who experienced cryptogenic IS (n=149) or TIA (n=50). Neurological outcomes were assessed using the modified Rankin Scale (mRS) as

the excellent recovery (mRS score 0-1) or poor outcome (mRS score 2-6) on admission in the acute phase of IS and then at 8 (6-12) months after the index episode. Patients were genotyped for α2AP Arg407Lys polymorphism (rs1057335) using real time PCR technique.

Results: The genotypes distribution of Arg407Lys polymorphism was as follows: Arg/Arg: 136 (68%), Arg/Lys: 57 (29%), Lys/Lys: 6 (3%). Interestingly, 407Lys allele was more frequent in TIA patients compared to the IS group (0.29 vs. 0.13, p=0.004). Moreover, in the whole group, as well as in IS and TIA patients analyzed separately, possession of the Arg/Arg genotype was associated with worse outcomes during follow-up (p=0.04), but at not in the acute phase of ischemic events. Possession of Arg/Lys genotype was associated with excellent outcomes a few months after the event (p< 0.001).

Conclusions: We found that the presence of 407Lys allele in cerebral ischemia patients is associated with better prognosis in both IS and TIA patients. The α2AP Arg407Lys polymorphism could be involved in the pathogenesis of cerebrovascular events and contribute to neurological outcomes within months since the episode, which highlights a role of α2AP in cerebral ischemia.

PB 1667 | Association between Ring Finger Protein213 Polymorphisms (*RNF213* 4448 G>A, 4810G>A, 4863G>A, 4950G>A) and Moyamoya Disease (MMD) in Koreans

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Background: Moyamoya disease (MMD) is a chronic occlusive cerebrovascular disease characterized by progressive stenosis at the terminal portion of the internal carotid artery and an abnormal vascular network at the base of the brain. Although its etiology is unknown, recent genetic studies have identified *RNF213* in the 17q25-ter region as an important susceptibility gene of MMD among East Asian populations. Ring finger protein 213 (RNF 213) encodes RNF motif that functions as an E3 ubiquitin involved in angiogenesis. Therefore, RNF213 may be relevant to vascular disorder of intracranial arteries including moyamoya disease.

Aims: The aim of this study was four RNF213 polymorphisms that *RNF213* (4448G>A, 4810G>A, 4863G>A, and 4950G>A) association with moyamoya disease (MMD) susceptibility.

Methods: We analyzed the associations with RNF213 polymorphisms in 117 moyamoya disease (MMD) patients and 310 controls. Genotyping was performed by polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) method.

Results: Consequently, the *RNF213* 4810GA type (AOR: 92.84, P< 0.001) and dominant type (AOR: 93.61, P< 0.001) was significantly associated with moyamoya disease. The *RNF213* 4950GA type (AOR: 2.440, P=0.003) and dominant type (AOR: 2.440, P=0.003) was significantly associated with moyamoya disease. Also, the GA/GG (*RNF213* 4810/4950; AOR: 87.91, P< 0.001) and the GA/GA (*RNF213* 4810/4950; AOR: 260.9, P< 0.001) were significantly increased risk of moyamoya disease in combined genotype analysis.

Conclusions: In conclusion, our study demonstrated that *RNF213* 4810G>A and *RNF213* 4950G>A polymorphisms were associated with moyamoya disease susceptibility.

PB 1669 | Early Plasma Fibrinogen Level after Thrombolytic Therapy in Acute Ischemic Stroke Patients Cannot Predict Intracranial Hemorrhage

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Background: Thrombolytic therapy (ThLy) in acute ischemic stroke (AIS) makes impressive outcome, however intracranial hemorrhage (ICH) as a serious complication is of concern.The prevalence of ICH is 2-10%. Intra-luminal fibrin clot as well as plasma fibrinogen are dissolved by thrombolytic agents.Whether plasma fibrinogen level during ThLy predicts ICH is not known.

Aims: We aimed to study the prevalence of ICH and predictive factors of ICH after recombinant tissue-plasminogen activator (rt-PA) administration in AIS patients.

Methods: During July 2014 to June 2016, AIS patients with the onset of ≤ 4.5 hours (hr) and without contraindication of rt-PA were enrolled prospectively. Repeated CTscan of brain was performed in every patient after ThLy for one day or immediately if ICH was suspected. Demographic data, NIH stroke scale (NIHSS), and plasma fibrinogen level at 0, 6 and 24 hr after rt-PA administration were recorded. The clinical and laboratory data were compared between ICH and non-ICH group.

Results: A total of 102 patients had a median age of 67 years old (range 24-88). Median duration from the onset of AIS to rt-PA administration (time to injection, TTI) was 90 minutes (range 10-270 minutes). Seven patients (6.8%) developed ICH. No significant difference of age, sex, concomitant use of anticoagulants or antiplatelets, systolic and diastolic blood pressure, blood sugar and NIHSS were found between two groups. The TTI of ICH group was significantly shorter than that of non-ICH group (60 VS. 120 minutes, p=0.04). Mean plasma fibrinogen at 0 hr of ICH group did not differ from that of non-ICH group (292±79 VS. 318±85 mg/dL, p=0.4). Mean plasma fibrinogen level of ICH and non-ICH group at 6 hr was 148±110 and 189±91 mg/dL, respectively (p=0.3). 22/102 patients (21.5%) had plasma fibrinogen level lower than 100 mg/dL but only three patients (13.6%) developed ICH.

Conclusions: The prevalence of ICH after thrombolysis was 6.8%. Plasma fibrinogen level at 6 hr did not predict ICH.

PB 1670 | Prediction of Venous Thromboembolism and Ischemic Stroke by Modified CHA_2DS_2-VASc Score in Patients with Atrial Fibrillation. The Tromsø Study

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Background: Recent studies have demonstrated that patients with atrial fibrillation (AF) have increased risk of venous thromboembolism (VTE). While the CHA_2DS_2-VASc score is an established risk stratification scheme for ischemic stroke (IS) in AF patients, data on its predictive ability for VTE is scarce.

Aims: To investigate the association between modified CHA_2DS_2-VASc scores and risk of first-ever VTE and IS in subjects with AF recruited from a general population.

Methods: Patients with confirmed AF (n=1668) were recruited among participants in the Tromsø study (1994-2008), and incident VTE and IS events were recorded to the end of 2012. Information on the CHA2DS2-VASc score components (congestive heart failure (CHF), hypertension, age ≥75 (doubled), diabetes, stroke (doubled), vascular disease, age 65-74 and sex category (female)) was registered at baseline. As CHF data was only available for a subset of the study population, the CHA2DS2-VASc score was modified by omitting CHF from the model. Incidence rates (per 1000 person-years (PY)) for VTE and IS were calculated, and Cox proportional hazards regression models were used to obtain hazard ratios (HR) for VTE and IS by CHA2DS2-VASc score categories with 95% confidence intervals (CI).

Results: The mean age of the AF patients was 73 (range 28-99) years. There were 75 VTEs (IR 7.7 (95% CI 6.1-9.6) per 1000 PY) and 229 IS (IR 24.8 (95% CI 21.8-28.2) per 1000 PY) during a mean follow-up of 5.7 years. Increasing CHA_2DS_2-VASc score was associated with both VTE (HR per 1-point increase 1.24, 95% CI 1.05-1.47) and IS (HR per 1-point increase 1.54, 95% CI 1.39-1.70). When grouping patients by CHA_2DS_2-VASc score, a CHA_2DS_2-VASc score >1 increased VTE risk 2.7-fold (HR 2.70, 95% CI 1.38-5.28) and IS risk 3.1-fold (HR 3.10, 95% CI 2.10-4.56).

Conclusions: Increasing modified CHA_2DS_2-VASc score was associated with both VTE and IS. The utility of the score for VTE prediction in AF patients merits further study in larger cohorts.

PB 1671 | Safety of Aspirin for Secondary Stroke Prevention: POoled Data Reanalysis of Aspirin for CErebraL InfArction PreventIoN: PORCELAIN Study

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Background: Although aspirin is globally known as a first-line drug for secondary stroke prevention, differences of its safety among patients are not well established.

Aims: To clarify the individual risk for aspirin therapy, we combined and reanalyzed the aspirin treatment arms from three large scale randomized double-blinded antiplatelet trials conducted in Japan.

Methods: S-ACCESS study (2008; aspirin vs sarpogrelate), CSPS 2 study (2010; aspirin vs cilostazol) and JASAP study (2011; aspirin vs Aggrenox) were included in this analysis. All trials are randomized double-blinded prospective studies and enrolled only patients with non-cardioembolic stroke. The dosage of aspirin was 81mg/day. The patients' profiles from aspirin arms of each trial are combined and compared between patients with intracranial hemorrhage or intracerebral hemorrhage and those without.

Results: Total 2726 patients (752, 1315, 639, respectively; mean age 64.5 y/o, 28.5% female) were included. The average follow-up period was 715 days. The average aspirin initiation was 60 days from initial stroke onset. The intracranial hemorrhage and intracerebral hemorrhage occurred in 56 and 46 patients (1.05 and 0.86 %/patient-year) respectively. No pre-specified clinical background profile was identified as a risk factor for intracranial hemorrhage whereas the lacunar stroke was the significant risk factor for intracerebral hemorrhage (2.12% vs 0.85*%; p=0.046).

Conclusions: For secondary non-cardioembolic stroke prevention, the intracerebral hemorrhage risk of aspirin is prominent in patients with lacunar stroke.

PB 1672 | Abnormal Red Cell Indices and the Risk of Arterial Ischemic Stroke in Children

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Background: High red cell distribution width (RDW) and low hemoglobin (Hb) levels have been reported to increase risk of cardiovascular diseases, arterial ischemic stroke (AIS) and venous thromboembolism (VTE) mainly in case controlled studies, mostly in adults. The mechanisms related to the diseases were unknown.

Aims: To determine the risk of abnormal red cell indices and AIS in children and demonstrate the correlation of red cell indices with the endothelial and coagulation markers.

Methods: Children age 1-18 years with AIS and children without underlying medical illnesses were informed to enroll to the study. Blood from healthy controls was collected and tested for complete blood count, thrombomodulin (TM), E-selectin (CD62E), prothrombin fragment (F1+2), thrombin-antithrombin complex (TAT), and D-dimer levels. The red cell indices in patients were recorded. The Chi-square, Wilcoxon-Mann-Witney tests and Pearson's correlation coefficient were used for statistical analysis.

Results: A total of 240 subjects, 97 were AIS patients and 143 were controls. The mean (SD) for ages in patients and controls was 9 (4.2) and 11.4 (1.8) years respectively. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Hb, and RDW showed significant correlation with TM level (r, -0.36, 0.37, 0.2, and -0.24, $p< 0.001$, < 0.001, 0.03 and 0.04 respectively). RDW level had significant correlation with D-dimer level (r 0.2, $p= 0.01$). Hb < 11 g/dL, RDW >15% and MCV < 80 fL had higher levels of TM when compared to those with Hb ≥ 11 g/dL, RDW ≤ 15% and MCV ≥ 80 fL, p =0.007, 0.004 and < 0.001 respectively. Patients with Hb < 11 g/dL, RDW >15% and MCV < 80 fL increased ORs for AIS at 4.8 (95% CI 2.3-10.0), $p< 0.001$, 3.8 (95% CI 2.0-7.4), $p< 0.001$ and 3.6 (95% CI 2.0-6.3), $p< 0.001$ respectively.

Conclusions: Low Hb and MCV, and high RDW levels may cause endothelial activation and increased risk of AIS in children. High RDW level may contribute to the coagulation stimulation.

PB 1673 | Remote Platelet Function Testing Using Platelet-bound P-selectin as a Marker of Platelet Activation. Findings in Patients with Stroke or TIA Treated with Clopidogrel

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Background: Remote assessment of platelet activation is available and involves quantification of surface expression of P-selectin. Other platelet assays have suggested that ‚resistance' to clopidogrel may be common, e.g. in 30-40% of patients.

Aims: To study platelet function in patients studied 4 weeks after acute stroke or TIA receiving a standard daily dose of clopidogrel.

Methods: Kits to assess the effects of aspirin and clopidogrel on platelet activation were obtained from Platelet Solutions Ltd (Nottingham, UK). Patients with recent non-cardioembolic ischaemic stroke or TIA were enrolled. Only clopidogrel was prescribed. Activating agents included arachidonic acid (AA) to assess effect of aspirin), adenosine diphosphate (ADP) to assess effects of clopidogrel), and thrombin receptor activating peptide (TRAP) to assess overall platelet reactivity. Data are number (%) or mean Median Fluorescence (MF, standard deviation); aspirin non-response was defined as mf >=500, and clopidogrel non-response as mf >=860, levels used in previous studies.

Results: Data were analysed from the first 50 patients enrolled (24 males) with stroke (29) or TIA (21), mean age 64 (13), range 40-88. Platelet testing was performed at least one month after starting clopidogrel. The mean MF for the Aspirin Test was 627 (335, range 82-1462); 30 (60%) of patients had a level exceeding 500. The mean MF for the Clopidogrel Test was 457 (238, range 138-1083); 3 (6%) patients exceeded 860. Significant correlations were observed: aspirin and clopidogrel (r=0.329, 2p=0.020), aspirin and TRAP (r=0.412, 2p=0.003).

Conclusions: Remote measurement of platelet function assessed by platelet surface expression of P-selectin is feasible. Few patients taking clopidogrel showed resistance. 40% of patients demonstrating suppressed levels in the Aspirin Test; this may reflect non-prescribed aspirin use or the effects of other drugs or foods. Assessment of thrombotic outcomes in this patient group is warranted.

PB 1674 | Mechanical Endovascular Therapy Plus Intravenous Tissue Plasminogen Activator versus Intravenous Tissue Plasminogen Activator Alone for the Management of Acute Ischemic Stroke: An Adjusted Indirect Treatment Comparison Meta-analysis

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Background: Randomized controlled trials (RCTs) have compared mechanical endovascular therapy (MET) in addition to intravenous tissue plasminogen activator (IVtPA) to IVtPA alone for the management of acute ischemic stroke (AIS). Direct comparative studies between individual METs+IVtPA vs. IVtPA alone are not available.

Aims: To perform an adjusted indirect treatment comparison (ITC) meta-analysis to assess the efficacy and safety of different METs+IVtPA vs. IVtPA alone in AIS patients.

Methods: A systematic literature search was conducted in MEDLINE and CENTRAL from 1/2005-10/2016 for RCTs in AIS patients comparing a single MET+IVtPA to IVtPA alone and reporting shift in ordinal modified Rankin Scale (mRS) score at 90 days. Secondary endpoints included 90 day mortality and early symptomatic intracerebral hemorrhage (sICH). Endpoints were pooled using traditional random effects meta-analysis methods, producing odds ratios and 95% confidence intervals. Adjusted ITCs using pooled estimates were then performed.

Results: Three studies (SWIFT PRIME, EXTEND-IA, THERAPY) were included; 2 evaluating the Solitaire stent retriever and 1 the Penumbra system.

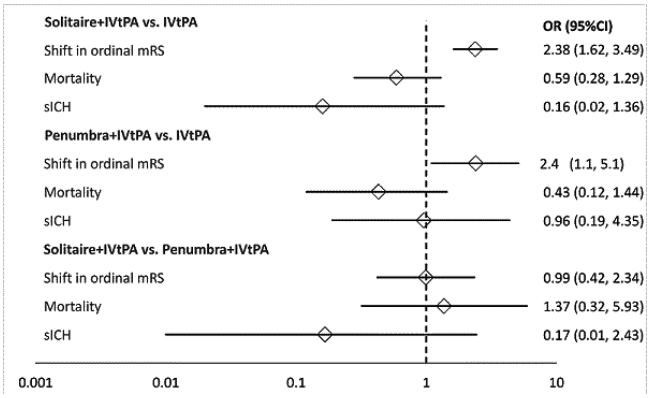

FIGURE 1 PRISMA Diagram for Traditional and Indirect Treatment Comparison Meta-Analyses]

Traditional meta-analysis demonstrated each MET+IVtPA resulted in an increased odds of improving ordinal mRS score vs. IVtPA alone, but did not alter the odds of death or sICH. Adjusted ITC showed no significant difference between the METs for any outcome.

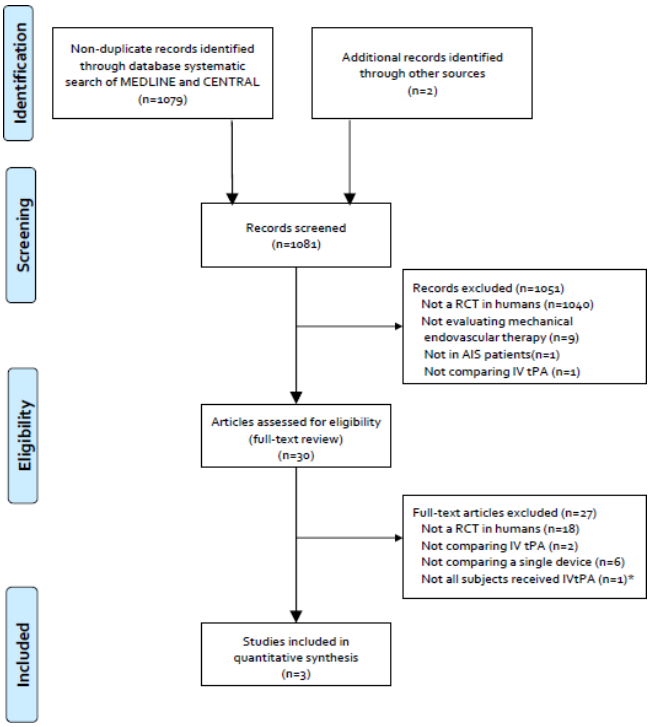

FIGURE 2 Results of Traditional and Indirect Treatment Comparison Meta-Analyses

Conclusions: No significant difference in efficacy or safety between the Solitaire and Penumbra devices were observed.

PB 1676 | Unhelpful Use of Hereditary Thrombophilia Tests in Young Patients with Ischemic Stroke. Final Results of the AISYF's Trial

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Background: Between 5-10% of ischemic strokes occur in young patients (pts). A hereditary thrombophilia (HTF) panel is frequently performed, but the value of such screening is still controversial. AISYF is a national, prospective, multicenter study for detection of Fabry disease in Argentina. It enlisted 293 pts between 18-55 years old that had ischemic or hemorrhagic stroke in the previous 180 days. All patients signed informed consent.

Aims: To investigate HTF in a prospective young population with ischemic stroke.

Methods: We evaluated 236 pts with ischemic stroke confirmed by images that participated in the AISYF study. Cardioembolic pts (n=20) were excluded. Antithrombin (AT) and Protein C were performed by chromogenic assay, Free Protein S (FPS) by immunoturbidimethric method, Anticardiolipin Antibodies IgG-IgM by ELISA. Lupus Anticoagulant according ISTH recommendations. Factor V Leiden (FVL), Prothrombin G20210A (P20210) and PAI 4G/5G gene mutation by PCR.

Results: mean age 43 years (53% male). Molecular TF tests were performed in 161 of 236 pts (68%). Just 7 pts were FVL positive (one Homozygous, 2 combined). P20210 was found only combined (one AT deficiency, one antiphospholipid syndrome (AFLS). Tree pts had a confirmed inhibitor deficit: PC (24%), FPS (40%) and AT (28%). The PAI polymorphism was 19% homozygotes 4G/4G and 46% heterozygotes 4G/5G which was similar to the average frequency in Argentine population (21% 4G/4G and 43% 4G/5G). Only one patient had Fabry disease. On the other hand 9 pts (4%) had AFLS, 2 combined with other TF. Only the AFLS and the AT deficient patient were anticoagulated.

Conclusions:

a) Our study showed alterations in HTF studies in 12/161 (7%) pts; 3 of them were combined with other TF.
b) HTF seems to be unhelpful as part of the screening work up in young patients with stroke, even in patients with an idiopathic event, since only a few required anticoagulation.
c) AFLS remains the most frequent TF in this population.

PB 1677 | Association of PAI-1 4G/5G Polymorphism with Ischemic Stroke in Young Indians

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Background: A serine protease inhibitor that has an important role in the regulation of fibrinolytic system is Plasminogen activator inhibitor-1 (PAI-1). PAI-1 is crucial in the regulation of the conversion of plasminogen into plasmin by inhibiting the action of tissues plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Diverse role of PAI-1 is known to be in metabolic and vascular diseases. It has a role in the progression of brain damage and recuperation after stroke; and also modulates the activity of tPA and uPA. Also both clinical and experimental studies have clearly suggested an increased risk for thrombosis with high PAI-1 levels while deficiencies are associated with accelerated fibrinolysis and bleeding.

Aims: To find the association of higher PAI-1 plasma levels and the prevalence of the 4G/5G polymorphism in PAI-1 gene promoter region in young Ischemic stroke (IS) patients.

Methods: Our study included subjects of young age from 18-45 years. Seventy patients with ischemic stroke and equal number of age- and

sex-matched controls were studied. We genotyped 4G/5G polymorphism in the study population through allele specific PCR. Commercial Kit was used to evaluate plasma PAI-1 levels.

Results: We observed that Pai-1 levels were significantly higher in patients as compared to controls. (p=0.04).The variant 4 G allele for the PAI-I 4G/5G polymorphism showed both genotypic (p = 0.02, Chi-square = 8.32; O.R = 2.60) and allelic association (p = 0.004, Chi-square = 10.54; O.R = 1.80) with ischemic stroke. The homozygous variant 4G/4G was also found to be associated with the higher PAI-1 levels. (0.001).

Conclusions: There is an association of the variant allele 4G with higher PAI-1 levels as well as with ischemic stroke. We thereby suggest in the light of our findings that PAI-1 gene polymorphism may be a risk factor for ischemic stroke in young Asian Indians.

PB 1678 | Serotonin and von Willebrand Factor Serum Content under Ischemic Stroke with or without Type 2 Diabetes Mellitus

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Background: The incidence of stroke among patients with diabetes of the 2 type can be 3 times higher, comparing to the general population. Besides it is known that platelets in patients with the 2 type of diabetes adhere and aggregate more frequently than the ones of healthy people so activated platelets release serotonin and therefore, changes of serotonin levels and enzymes activity responding of serotonin metabolism (i.e. monoamine oxidases) in blood could be a signalling biomarker of the risk of cerebrovascular occurrences in the future under diabetes mellitus.

Aims: Thus to examine of coagulation von Willebrand (vW) factor, serotonin and tryptophan contents, monoamine oxidase (MAO) activity in ischemic stroke (IS) patient blood with or without 2 type diabetes were the aim of the present study.

Methods: The experimental group consisted of 65 IS individuals including 34 persons with type 2 diabetes. All patients or their relatives gave written agreement to participate. Von Willebrandt factor was determined by Elisa. Serum tryptophan and serotonin content assay included ion-exchange chromatography with fluorescence spectrophotometry. Determination of monoamine-oxidase serum activity was spectophotometry.

Results: The investigation showed an increase of tryptophan, serotonin and vW factor blood content in both group of IS patients independently from diabetes present comparing with the healthy donors values. Moreover, a larger deviation of serotonin - 65% from the control was observed in the blood of patients with IS alone, meanwhile the higher serum concentrations of tryptophan and von Willebrandt factor were determined of IS patients with diabetes. The monoamine-oxidase activity was reduced in diabetes patients' blood against to the values of the healthy donors and ischemic stroke alone patients.

Conclusions: These obtained data suggested a significant imbalance of different elements of hemostasis under the ischemic stroke and amplification of negative changes in case the 2 type diabetes present under the stroke.

PB 1679 | Incidence and Outcome of Childhood Stroke in Thailand

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Background: Since the development of Stroke fast track in Thailand in 2008, the incidence rate of stroke in adult group is increasing. Incidence of stroke in Thai children has not yet been reported. In addition, there is still lack of information of the outcome.

Aims: A retrospective descriptive study was to identify the incidence rate of stroke in the Pediatric group.

Methods: Neonates and children age less than 18-year diagnosed with stroke during 2004-2015 were identified through the National Health Security Office (NHSO) using International Classification of Diseases for codes G08 (cerebral sinovenous thrombosis;CSVT), codes I60-I69 (arterial ischemic strok;AIS). The first diagnosis of either AIS or CVST was recruited as subjects. The NHSO data was limited only patients under health insurance gold card that includes 75% of all patients' data in Thailand. Data of patients those parents work for government was not included in NHSO data which accounted for 25% of patients' data.

Results: The number of children with CSVT is 11 [M:F=3:8] with age < 1 year =2, 1-5 years =3 and >10 years =6. The number of children with AIS was 1499 [M:F=768:731] with age of diagnosis < 1 year =186, 1-5 years =335, 6-10 years =241 and >10 years =737. Incidence rate of CSVT was 0.006/100,000 children-yr. Trends of incidence rate of AIS between 2004- 2015 increased from 0.5 to 0.9/100,000 children-yr. The most common condition associated with stroke is obesity followed by heart diseases, sepsis, autoimmune diseases, and hematologic disorders. Among of 478 subjects died from AIS, death at < 30 days was 196, 31-90 days: 62, 91-180 days: 44, 181-1 year: 40 and > 1 year: 140 subjects.

Conclusions: Incidence of AIS and CSVT in Pediatric group was lower than the incidence reported from China, Europe and North America. Trend of diagnosis has been increasing since 2007 to date. The mortality rate of AIS was high, as a result, the improvement of education to medical personnel should be provided to early diagnosis and appropriate treatment.

PB 1681 | Another Indication to DOACs

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Background: Carotid and vertebral artery dissection (CAD) is an important cause of stroke in the young, up to 25% in patients below 45 years. Antiplatelets or anticoagulants are the conventional treatment, but the superiority of either approach has not yet been estabilished.
Aims: Anticoagulants represent the treatment of choice expecially in severe stenotic or occluded vessels and in case of luminal thrombosis or cerebral microembolism. Given the safety profile of direct oral anticoagulants (DOACs) and their efficacy in stroke prevention, we evaluated their use in young patients with CAD.
Methods: We treated with Rivaroxaban 20 mg daily three young patients (mean age 42 years). Oral anticaogulation was preferred to antiplatelets for the dissection features: cerebral embolization in two and severe vertebral stenosis in one. The choice of DOACs over Warfarin was justified by the difficult monitoring of Warfrain and by the patients compliance.
Results: After 60 days from DOAC start Doppler Sonography (TSA e TCCD) and brain MR documented signs of recanalization.The clinical symptoms improved as well and no major bleeding or clinically significant event was reported, a part from a slight metrorrhagia.
Conclusions: Althought there are only few retrospective case reports on the use of DOACs in CAD, these drugs, easier to use and with a fastest and predictable action, could represent a suitable alternative to conventional anticoagulation, expecially in young patients with an active life.

COAGULANT & ANTICOAGULANT MECHANISMS

PB 071 | The Chimeric Monoclonal Antibody MHCSZ-123 against Human von Willebrand Factor A3 Domain Inhibits High Shear Arterial Thrombosis Model in Rhesus Monkeys

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Background: SZ-123, a murine monoclonal antibody targeting the human von Willebrand factor (VWF) A3 domain and blocking the binding of collagen, is a powerful antithrombotic. In Rhesus monkeys, this was without side effects such as bleeding or thrombocytopenia. Recently, we developed a mouse/human chimeric version of SZ-123, MHCSZ-123, which maintains its inhibitory capacities *in vitro* and *ex vivo* after injection in monkeys. We selected CHO-S cells stably expressing MHCSZ-123 with G418 and adapted cell clones to serum-free suspension culture.
Aims: To investigate the antithrombotic properties, the effect on bleeding time and blood loss and initial pharmacokinetics of MHCSZ-123 in monkeys.
Methods: The antithrombotic effect of MHCSZ-123 on acute platelet-mediated thrombosis was studied in Rhesus monkeys. The thrombus formation is induced at an injured and stenosed site of the femoral artery, allowing for cyclic flow reductions (CFRs) which were measured in the femoral artery of anesthetized monkeys before and after intravenous administration of MHCSZ-123. *Ex vivo* VWF binding to collagen, platelet agglutination, platelet count, and template bleeding time were used as measurements of antithrombotic activity. In addition, plasma VWF and VWF occupancy were measured by ELISA. Statistical analysis was performed using GraphPad Prism 5 (San Diego, CA) using Student's *t* test (paired) and one-factor ANOVA followed by Fisher's test. $P < 0.05$ was considered significant.
Results: Injection of 0.1, 0.3, and 0.6 mg/kg MHCSZ-123 significantly reduced the CFRs by 29.4%, 57.9%, and 73.1%, respectively. When 0.3 and 0.6 mg/kg MHCSZ-123 were administered, 46.6% - 65.8% inhibitions of ristocetin-induced platelet agglutination were observed from 15 min to 30 min. Finally, minimal effects on bleeding time and blood loss, no spontaneous bleeding and no thrombocytopenia were observed.
Conclusions: We conclude that the VWF-A3 inhibitor MHCSZ-123 significantly reduced thrombosis in Rhesus monkeys and appeared to be safe and well tolerated.

PB 072 | Effects of Two Plant Protein Inhibitors on Haemostasis and Thrombosis

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Background: The purpose of antithrombotic therapy is the prevention of thrombus formation and/or its extension with a minimum risk of bleeding. The inhibition of a variety of proteolytic processes, especially those of the coagulation cascade, has been reported as a property of plant protease inhibitors.
Aims: In the present study, the role of DrTI and AsTI, from *Delonix regia* and *Acacia schweinfurthii*, respectively, being members of the Kunitz family of protease inhibitors, inhibiting trypsin in the nM range, were investigated in blood coagulation.
Methods: The inhibitory activity of DrTI and AsTI with bovine trypsin, human plasma kallikrein (huPK), and factors XIIa and XIa (FXIIa and FXIa, respectively) was analysed against specific chromogenic and fluorogenic substrates. The effect of DrTI and AsTI on prothrombin time (PT) and activated prothrombin time (aPTT) was determined. The

in vivo and *ex vivo* effect of the protein inhibitors on haemostasis and arterial thrombus formation was investigated.

Results: DrTI and AsTI increased the aPTT in human plasma due to the inhibition of huPK. DrTI differs from AsTI by inhibiting FXIa, with a K_{iapp} of 1.3 nM, indicating the dynamic nature of their reactive sites. DrTI and AsTI inhibited ex-*vivo* mice platelet aggregation induced by ADP and significantly prolonged the time for total carotid artery occlusion in mice compared to the NaCl control. In contrast to heparin, the bleeding time in mice treated with the two inhibitors did not differ from that of the control group.

Conclusions: DrTI and AsTI effectively prevented arterial thrombus formation in mice without increasing the bleeding time, possibly by interfering with the activity of the huPK and FXIa blood coagulating contact factors associated with platelet inhibition. Support by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [grant number 23038.0077762/2014-32, AUPE 140/2015].

PB 073 | Slounase, a Modified Snake Venom Hemocoagulase Combined with FXa, Enhances Hemostasis and Limits Bleeding in Both Normal and Hypocoagulant Conditions

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Background: Uncontrolled post-traumatic bleeding is the leading cause of preventable death. Hemocoagulase, a thrombin-like serine protease from snake venom, converts fibrinogen to fibrin and thus may reduce blood loss in hemorrhagic medical conditions. A modified hemocoagulase combined with activated factor X (FXa), may potentially improve the hemostatic effect of hemocoagulase. However, the *in vivo* hemostatic effect of these reagents is not well characterized due to lack of reliable *in vivo* hemostasis models.

Aims: To determine the efficacy of slounase *and* hemocoagulase on hemostatic clot formation and bleeding at the site of vascular injury i*n vivo*.

Methods: Effect of slounase, hemocoagulase, and FXa on human platelet function was assessed *in vitro*. *In vivo* hemostatic efficacy was determined in mice using intravital microscopy laser ablation hemostasis models and liver puncture hemostasis model.

Results: Both slounase and FXa, but not hemocoagulase, enhanced human platelet aggregation induced by thrombin. As expected intravenous injection of 25U of heparin into WT mice prevented the hemostatic clot formation at the site of vascular injury and FXa treatment did not restore clot formation *in vivo*. Hemocoagulase treatment in heparin-treated hypocoagulant mice resulted in detectable fibrin formation but no notable change in platelet recruitment in the clot. Interestingly, slounase treatment resulted in a significant enhancement of both fibrin and platelet content in control WT mice and restored the clot formation in heparin-treated hypocoagulant mice. Similar results were also observed in laser-ablated saphenous vein hemostasis model. The bleeding time was shorter in slounase-treated hypocoagulant mice both in vascular injury models and using liver pricking injury model.

Conclusions: Slounase enhances hemostasis in normal mice and restores hemostasis in hypocoagulant conditions via both enhancing fibrin formation and platelet activation while hemocoagulase only enhances fibrin formation *in vivo*.

PB 074 | Platelet Defects Upon Surgical Liver Disease Models iin Mice

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Background: Acute and chronic liver diseases are characterized by alterations in primary hemostasis including thrombocytopenia and platelet function defects. Moreover, different studies show that hypercoagulability play a prominent role in liver diseases. However, the consequences of liver failure on platelet function are not well defined.

Aims: Functional analysis of platelet activity, thrombus formation and hemostasis in liver injury models.

Methods: *In vitro* and *in vivo* experiments using Bile Duct Ligation (BDL) and Partial Hepatectomy (PHx) in mice.

Results: 24 h and 3 days after BDL a moderate decrease of platelet counts occurred, whereas the platelet counts increased to normal levels after 7 and 21 days. Platelet counts after PHx were slightly reduced after 3 days and fully recovered at later time points. However, intrinsic platelet activation was strongly reduced upon CRP activation at early time points in both BDL and PHx operated mice compared to sham controls. 7 days after BDL platelets displayed a complete activation defect with all agonists tested, whereas in the PHx model acute platelet activation defects were measured in the first 3 days. This activation defects resulted in strongly reduced thrombus formation under flow in both models as well as reduced platelet adhesion to fibrinogen. In vivo platelet defects resulted into bleeding complications, predominantly in BDL mice as measured by tail bleeding experiments. The analysis of platelet activation defects revealed a contribution of altered glycoprotein expression to defective signal transduction. Moreover, high plasma levels of NO, PG-E and bile acids increased endogenous levels of VASP phosphorylation resulting in almost non-functional platelets after liver injury.

Conclusions: These results indicate that liver diseases affect intrinsic platelet activation and impair thrombus formation that might contribute to bleeding complications in patients with acute or chronic hepatic failure and resection.

PB 075 | D-Insight - An Integrated Animal and Cell Model System to Interrogate the Expression and Secretion Dynamics of F2 Real-time *in vivo*

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Background: The coordinated dynamics of F2 gene expression and protein secretion determine the well-balanced equilibrium between coagulation and blood loss, and ensure proper execution of numerous other F2a-mediated functions beyond blood coagulation.
Aims: Here, we report on a genetic model that allows to interrogate and define regulatory principles controlling F2 gene expression and secretion in a spatiotemporal manner.
Methods: By applying a multimodal genetic tagging strategy, we generated a novel constitutive knock-in mouse model based on fluorescence and luminescence reporters separated by P2A peptides for tailored multicistronic expression. This mouse model, termed D-Insight, allows the "visualization" of regulated F2 gene expression and protein secretion in its natural context, and the coupling between these processes real-time *in vivo* by the use of non-invasive optical imaging. Complementary, we generated primary hepatic cell lines derived from this mouse model. This cell line permits the deconvolution of F2 gene expression-, protein biosynthesis- and secretion dynamics in high resolution and in a scalable high-throughput format *ex vivo* with bioluminometry and fluorescence microscopy.
Results: We confirm that this model faithfully recapitulates established (patho)physiologically relevant modifiers of F2 expression, and thereby show that the complementarity of this experimental setup may help to foster the reverse and forward translation of conditions impinging on F2-modulating circuits.
Conclusions: This experimental model thus represents a versatile tool to identify physiologically relevant rheostats involved in the cross-talk between F2 gene expression and protein biosynthesis and secretion. It will also help deciphering underlying mechanisms of disordered hemostasis e.g. in response to pathophysiological cues and other (environmental) perturbations.

PB 076 | Role of Disruptive Hydrodynamic Forces in Determining Occlusive and Non-occlusive Arterial Thrombosis Scenarios

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Background: The mechanisms responsible for difference between occlusive and non-occlusive thrombosis scenarios are not clear. Disruption of thrombus by flow (locally accelerated because of vessel lumen narrowing) was previously suggested to be important.
Aims: To investigate flow pattern changes and parameters of thrombi induced by paper patches of different length in FeCl3-induced model of murine arterial thrombosis.
Methods: Real-time videomicroscopy was used to monitor thrombus formation. Doppler flow probe was applied to validate occlusion. End-point thrombi were analyzed with scanning electron microscopy and confocal microscopy. Computational fluid dynamics was used to investigate the relationship between thrombus formation and flow parameters.
Results: Occlusion occurred in 1 case out of 6 for the damage size 1 mm long (versus 5 cases of 6 for 3 mm). Non-occlusive thrombosis was always a two-phase process, with initial formation of a large thrombus followed by its disruption and stabilization. This stabilization always occurred at a thrombus height less than 40% of the vessel diameter (90±15 microns for 1 mm, 211±50 microns for 3 mm). The structure of occlusive thrombus was non-homogeneous, with large regions composed of erythrocytes compressed into tightly packed polyhedrals. Computational flow dynamics confirmed that the vessel with thrombus functioned as a constant differential pressure system. This resulted in local acceleration of blood flow near thrombus and disruption of unstable thrombi, which could prevent occlusion. Chances of this were higher for small damage size because such thrombi did not increase overall vessel hydrodynamic resistance greatly.
Conclusions: Transition between non-occlusive arterial thrombosis and vessel occlusion is determined by disruption of thrombi by hydrodynamic forces during the phase when thrombus is not yet strong enough. Thrombus geometry determines local flow forces and is a critical factor in this process.

PB 077 | Sickle Cell Red Blood Cells Alter Properties of Mouse and Human Clot

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Background: Sickle cell disease (SCD) is associated with increased risk of venous thromboembolism. It has been proposed that clot contraction-mediated packaging of red blood cells (RBC) is significantly altered in SCD and may affect clot stability.
Aims: We investigated the cellular mechanism of clot retraction in SCD.
Methods: *Ex vivo* clot retraction was performed using blood from sickle cell (SS) Townes mice and SS patients. The number of RBC extruded from the clot into serum was expressed as a % of initial RBC in whole blood.

Results: In mice, dramatically less RBC were extruded into the serum from SS clots than control (AA) (0.8±0.8% vs 19.4±0.8%, n=3, p< 0.0001). A similar result was observed in human blood. FXIIIa inhibition with T101 increased the release of AA (by 64%, n=6, p< 0.05) but not SS RBC from mouse clots. Mixing the platelet poor plasma (PPP) and cellular fraction of AA and SS mouse blood revealed that entrapment of SS RBC is mediated by the cellular fraction of blood (AA blood 6.9±3.6%; SS blood 0.4±0.4%; AA cells/SS PPP 6.7±6.4%; SS cells/AA PPP 0.1±0.2%, n=3 per group). Mixing the platelet free plasma (PFP), platelets and RBC of AA and SS mice demonstrated that the entrapment of SS RBC within the clot is entirely mediated by yet unknown properties of SS RBC (n=3).

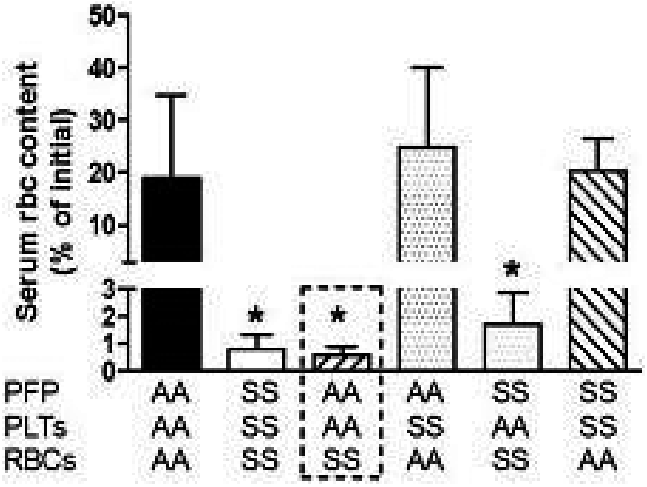

FIGURE 1 Effets of cellular compartments on the entrapment of AA and SS RBCs within the clot

Lowering hematocrit (HCT) of AA mouse blood reduced RBC extrusion from the clots. However, increasing HCT of SS mouse blood to that of AA blood did not increase the number of SS RBC extruded from the clot, suggesting that the entrapment of SS RBCs within the clot is not simply caused by lower RBC number in SCD. Electron microscopy analysis of the clots demonstrated that mouse and human SS RBC underwent sickling and formed "spicule-like" processes intertwined with fibrin fibers that prevented formation of tight polyhedral shapes observed in AA clots.

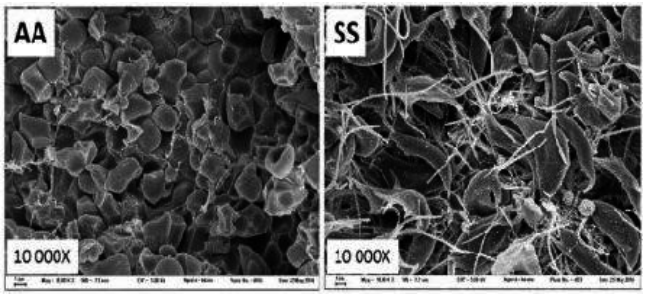

FIGURE 2 Scanning electron microscopy of AA and SS blood clots formed ex vivo

Conclusions: Sickling of SS RBC alters the process of clot retraction and changes clot structure. In vivo consequences of these observations are currently under investigation.

PB 078 | Porcine Model of Methicillin-resistant Staphylococcus Aureus (MRSA) Sepsis-induced Disseminated Intravascular Coagulation (DIC) and Multiple Organ Dysfunction Syndrome (MODS)

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Background: Sepsis-induced DIC is associated with a high mortality with no existing therapy except for supportive care. MRSA infections are increasingly becoming a significant healthcare burden. Currently there is a paucity of published MRSA sepsis models. Large animal models are now recommended prior to human clinical trials.

Aims: Our aim is to validate a published model (Soerensen et al 2013) and establish a hemodynamically relevant porcine model of MRSA sepsis-induced DIC.

Figure 1. Platelet Counts, Prothrombin Time, Activated Partial thromboplastin Time D-dimer, and Fibrinogen levels in sham and Methicillin-Resistant Staphylococcus Aureus (MRSA) septic pigs at 0, 24h, 48h, 60h, and 70h post-MRSA injection.

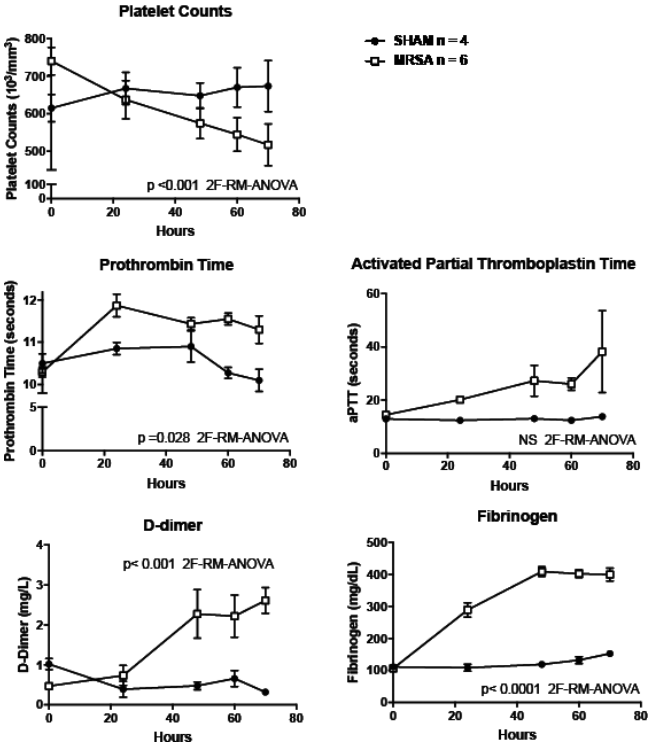

FIGURE 1 Platelet counts, PT, aPTT, D-dimer, and fibrinogen levels in sham and MRSA septic pigs.

Methods: Ten four-week old pigs (8 kg) were implanted with jugular and carotid catheters and a telemetry device in a femoral artery

for continuous hemodynamic monitoring. Four days later 6 pigs were injected intravenously with MRSA (USA300, TCH 1516 strain) dose of $1x10^9$ CFU/kg and 4 pigs (Sham) were injected with saline. Fluid resuscitation was given for heart rate > 50% or mean arterial blood pressure < 30% from baseline. Point-of-care complete blood count, prothrombin time (PT), activated thromboplastin time (aPTT), D-dimer, and fibrinogen were done at 0, 24, 48, 60 and 70h post-MRSA injection. Factors V and VII, protein C, and antithrombin activities were also measured. Pigs were euthanized and necropsies performed.

Results: Platelet counts decreased and PT, D-dimer, and fibrinogen increased significantly in septic compared to sham pigs over time ($p< 0.05$; 2FRM-ANOVA). Factors V and VII, and protein C activities significantly decreased in septic pigs by 24h post-MRSA injection. Antithrombin activity significantly decreased in septic pigs by 48h post-MRSA injection. Histopathologic examination shows microvascular thrombi, necrosis, hemorrhage, infarction and infection in the kidney, liver, and lung of septic pigs.

Conclusions: Our 70h porcine model of MRSA-sepsis induced DIC showed consumptive coagulopathy, organ injuries, and vascular thromboses. Characterization of this model is ongoing with the aim of having a pathologically and hemodynamically relevant large animal model to study DIC and evaluate potential therapeutic targets.

Figure 2. Factor V, Factor VII, Protein C and Antithrombin activites in sham pigs at 0h and 70h and in Methicillin-Resistant Staphylococcus Aureus (MRSA) septic pigs at 0, 24h, 48h, 60h, and 70h post MRSA injection.

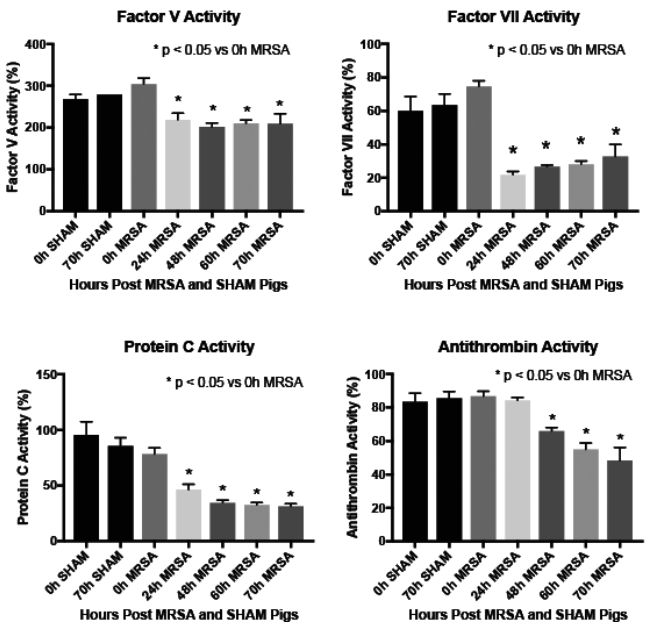

FIGURE 2 Factors V, VII, protein C and antithrombin activities in sham and MRSA septic pigs

PB 079 | Role of bone morphogenetic protein receptor type II in vascular remodeling during thrombosis

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Background: Several lines of evidence have implicated the role of inheritance and genetics in pathogenesis of pulmonary arterial hypertension (PAH). Mutations in the bone morphogenetic protein type II receptor (BMPR2), a member of the TGF-β superfamily, have been identified as a risk factor for pulmonary arterial hypertension. Fibrotic vascular occlusion is a feature of PAH and chronic thromboembolic pulmonary hypertension (CTEPH), therefore we wanted to study the role of BMPR2 in murine model of chronic venous thrombosis.

Aims: In the present study we investigated the role of BMPR2 deficiency in fibrotic vascular remodeling following thrombosis. Additionally, we aim to study the cellular composition of chronic thrombotic material excised during pulmonary endarterectomy (PEA) surgery.

Methods: We utilized a mouse model of stagnant flow venous thrombosis to study the effect of BMPR2 deficiency on thrombus formation/resolution. Wild type mice and transgenic mice, bearing a heterozygous knock-in allele of human BMPR2 mutation, R899X, were subjected to subtotal ligation of the inferior vena cava (IVC). Thrombus was harvested at different time points (days 1, 3, 7 and 14 after IVC ligation) and histological and molecular analysis were performed. Trichrome stain was performed to assess the accumulation of matrix components.

Results: We observed a significant increase of thrombus cross-sectional areas and volumes on days 1, 3 and 7 in transgenic mice. During early time points (days 1, 3 and 7) mice with BMPR2 deficiency showed more fibrin in their thrombi than wild type controls. By day 14, transgenic mouse thrombi seem to have more accumulation of extracellular matrix components like collagen.

Conclusions: These results indicate a role of BMPR2 during early thrombosis, impacting fibrotic remodeling thereafter.

PB 080 | In Vivo Neutralization of Unfractionated Heparin (UFH) and a Bioengineered Heparin Dodecasccharide (12-mer) by Protamine Sulfate in a Primate Model

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Background: Heparin, a sulfated natural glycosaminoglycan (GAG) which is widely used. Chemoenzymatic methods provide an alternate approach to synthesize heparin-related GAGs. A dodechasaccharide represents a homogeneous oligosaccharide with high affinity to antithrombin and exhibits anti-Xa activity with weak anti-IIa actions.

Aims: This study was aimed to investigate the neutralization of UFH and dodechasaccharide by protamine sulfate (PS) following intravenous administration to non-human primates.

Methods: Porcine UFH and PS were commercially obtained. The 12-mer was synthesized by a chemo-enzymatic method. In vivo studies

were carried out in individual groups of primate (n=4). Blood samples were obtained at baseline from dodechasaccharide or UFH treated groups after a 500 ug/kg IV administration to an individual group of primates (n=4). An additional sample was drawn at 5 minutes after drug administration and followed by IV PS administration at 1.0 mg/kg. Subsequent blood samples were drawn at 5 and 30 minutes post PS administration. These samples were analyzed in the APTT, anti-Xa, anti-IIa and TFPI assays.

Results: Administration of UFH increased the aPTT (>300 sec) whereas dodechasaccharide had a marginal effect. Both UFH and dodechasaccharide strongly inhibited the Xa assay (>90%) 5 mins after administration. PS neutralized the anticoagulant effect of UFH and partially neutralized the IIa and Xa activities. PS had minimal effect on the activities of dodechasacharide. While PS markedly decreased TFPI levels in UFH treated primates it had minimal effect on dodechasaccharide treated animals.

Conclusions: Unlike UFH dodechasaccharide exhibited minimal anticoagulant and anti-IIa activities, yet markedly higher anti-Xa activity. In the UFH group PS almost completely neutralized the anticoagulant effects as measured by APTTs and significantly reduced the anti-Xa effects. In contrast, PS was not effective in neutralizing the effects of dodechasaccharide in all assays.

PB 081 | Hemostatic Profile in Mouse Models of Advanced Chronic Liver Disease

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Background: Advanced chronic liver disease (ACLD), a condition including cirrhosis and fibrosis, is characterized by changes in the coagulation system that embrace both hypo- and hypercoagulability. In this rebalanced situation, hypercoagulability can contribute to fibrosis progression and cirrhosis complications.

Aims: To create ACLD murine models and evaluate hemostatic changes.

Methods: 20 adult male mice received i.p. 200mg/kg thioacetamide (TAA) 3x/week for 12 weeks or i.p. 1ml/kg carbon tetrachloride (CCl_4) and p.o. 0.3g/L phenobarbital (PB), 2x/week for 11 weeks. At the end of the treatment mice were sacrificed; blood and organs were collected.

Results: We observed a slight increase in body weight in both CCL_4/PB (28.0±2.7 vs 24.5±3.9g, P< 0.0001) and TAA (22.8±3.1 vs 21.6±3.3g, P=0.01) groups compatible with ascites development while mortality rate was higher in the CCL_4/PB than in the TAA group (32% vs 7%). In the CCL_4/PB group, liver histology displayed extensive fibrosis with both portal-to-portal and portal-to-central bridging. Albeit present, fibrosis in the TAA treated group was less extensive. Interestingly, occlusive centrilobular vein thrombosis was more prominent in the TAA than in the CCL_4/PB group, where we observed also partial periportal thrombosis. Coagulation investigation showed a slight decrease of fibrinogen in both CCL_4/PB and TAA groups compared to controls (1.4±0.1 vs 1.7±0.1g/L, P=0.04 and 1.3±0.1 vs 1.7±0.1g/L, P=0.01) and prolonged PT in both CCL_4/PB and TAA groups compared to controls (9.3±0.1 vs 8.9±0.03sec, P=0.01 and 9.8±0.2 vs 8.9±0.03sec, P=0.007). Factor VIII was highly increased in the CCL_4/PB group compared to controls (350±17 vs 194±35%, P=0.002) but not in the TAA group (278±66 vs 194±35%, P=ns).

Conclusions: These data provide the first evaluation of hemostatic profile in mice with ACLD. Evaluation of thrombin generation potential is ongoing to further characterize the bleeding and thrombotic tendency linked to murine ACLD.

PB 082 | A Dual Antiplatelet (AP) and Anticoagulant (AC) Compound - APAC - Inhibits Collagen-induced Platelet Aggregation in Mice and Protects against Arterial Thrombosis

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Background: Antithrombotic treatments are key for preventing complications in vascular disease. APAC mimics natural heparin proteoglycans and has dual antiplatelet (AP) and anticoagulant (AC) actions *in vitro* and at vascular injury sites *in vivo*. Upon local intraluminal application, APAC preserved vascular integrity in a rat femoral anastomosis model. APAC IV protected against renal ischemia/reperfusion injury in rats.

Aims: We aimed to test APACs anti-platelet effects in mice as well as its antithrombotic potential in a mouse model of arterial thrombosis (AT).

Methods: For platelet studies, we used male C57BL/6 wildtypes aged 12 w. Light transmission aggregometry was performed on citrated PRP. PRP was pretreated with either APAC or PBS at a dose of 5 µg/ml for 15 min. The agonist was collagen, concentrated 2 µg/ml.

For AT, we used a mouse model of laser-induced injury of the common carotid artery (CCA). Briefly, after IV injection of rose bengal, the CCA was targeted by a 1.5 mW green laser and the time to thrombotic occlusion measured by Doppler flow probe.

Mice were IV treated with either PBS, UFH or APAC at a dose of 0.3 mg/kg, 15 min before onset of laser injury.

Results: Collagen-induced platelet aggregation was significantly inhibited in APAC vs PBS treated PRP (Fig.1)

In line with this, *in vivo* APAC treated mice took double the time to thrombotic occlusion (97 s vs 47 s & 49 s) compared to PBS and UFH treated animals (Fig.2)

Conclusions: APAC inhibits murine collagen-induced platelet aggregation *in vitro*.

IV application of APAC but not UFH or PBS significantly delays thrombotic occlusion *in vivo*. This implies APAC's anti-thrombotic effect even after systemic application, suggesting a mechanism of self-targeting to the site of vascular injury. The compound's attractiveness, i.e. local effectiveness with less systemic side effects, is thus underlined.
Further studies will address APACs local distribution at the site of vascular injury and other mechanisms including local tissue factor expression and activity.

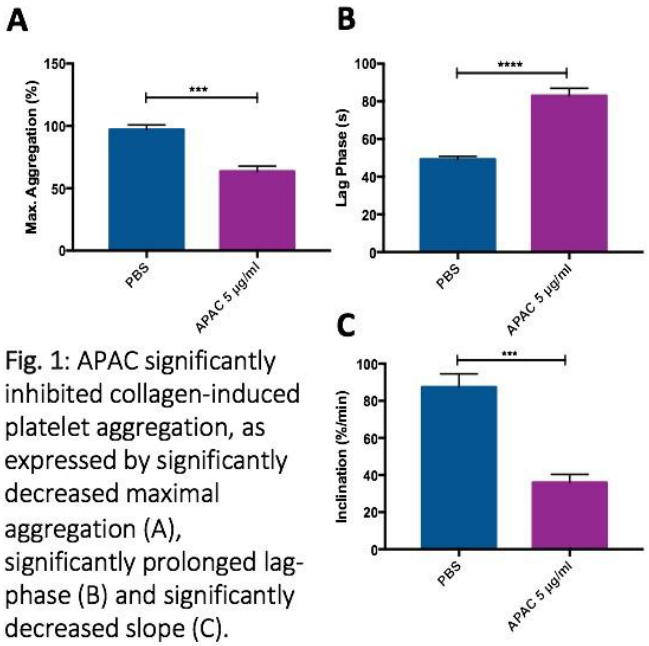

Fig. 1: APAC significantly inhibited collagen-induced platelet aggregation, as expressed by significantly decreased maximal aggregation (A), significantly prolonged lag-phase (B) and significantly decreased slope (C).

FIGURE 1 Platelet aggregation studies in mice treated with APAC

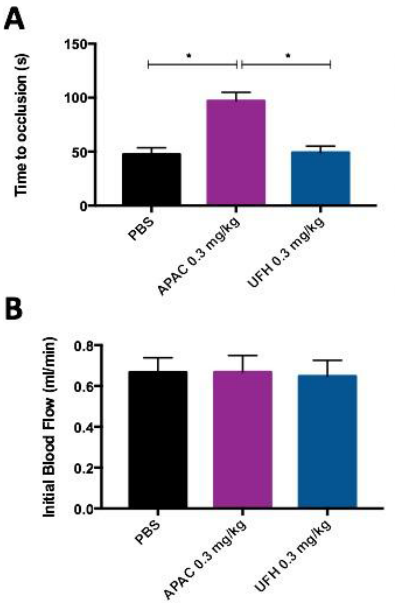

Fig. 2: Systemic IV application of APAC significantly increased time to thrombotic occlusion compared to PBS and UFH (A), whereas initial blood flow measurements did not differ between the groups (B).

FIGURE 2 The effects of APAC on thrombus formation compared to PBS and UFH

PB 083 | Blood Microsampling as a Refined Model of Platelet Thromboembolism *in vivo*

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Background: Research in the field of platelet pharmacology requires the use of mortality models of thromboembolism performed in conscious mice which inflict considerable pain and use paralysis/death as endpoints.
Aims: We have developed a refined *in vivo* mouse model of platelet thromboembolism based on blood microsampling which entails the measurement of the fall in circulating platelet counts as platelets accumulate in the pulmonary vasculature following a thromboembolism.
Methods: Three microsamples of blood were collected from the tail vein of anaesthetized Balb/c mice before and after (1 and 10 min) the injection of collagen or thrombin and platelet counts measured. The results obtained were validated by immunohistochemistry where sections of lung were stained for platelet-specific CD41 and by using our established real-time *in vivo* platelet monitoring technique. The effect of anti-thrombotic aspirin on circulating platelet counts was also assessed.
Results: The blood microsampling data showed a significant reduction in platelet counts 1 min after collagen (44.1%) or thrombin (32.1%) which returned to basal values after 10 min. These results correlated with those determined *via* real-time platelet monitoring where the maximum increase in platelet-radioactivity counts (%) in the pulmonary vasculature was found 1 min after collagen and restored after 10 min. Platelet aggregates were observed in the lung tissue of collagen-challenged mice compared to vehicle-control. Treatment with aspirin significantly inhibited the reduction in platelet counts induced by collagen, 23.4%, compared to control groups, 38.8%.
Conclusions: We developed blood microsampling as a novel and simplistic mouse model to study platelet aggregation *in vivo* which refines an animal model to a lower severity level and reduces animal use compared to mortality models. Ultimately, this method has potential value in studying the pharmacological effect of novel therapeutics on platelet function *in vivo*.

PB 084 | Thromboelastography in Mice: Impact of Blood Collection Method

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Background: Blood collection is a critical step in studying coagulation in mice. Cardiac puncture is a standard effective method for collection that provides a good quality and relatively large blood sample but the procedure requires anesthesia and is terminal thus hindering comparative studies of pre/post intervention or follow up studies. Thromboelastography (TEG) is a global sensitive haemostasis test,

which requires 340ul volume and is used in mice studies and animal models.

Aims: To investigate the feasibility of facial vein sampling for TEG analysis in mice and to compare the coagulation results to those obtained via cardiac puncture.

Methods: Citrated whole blood samples (340 uL) were obtained from 10 mice; 12 weeks via cardiac puncture and from 8 other mice via facial vein. Blood was re-calcified with 20ul CaCl2 (0.2 mol/l) and analyzed using TEG 5000, Haemoscope, according to the manufacturer's instructions. Major coagulation parameters were analyzed including R time, alpha angle, K time, MA; clot's maximum strength and CI; the overall clotting activity. Student *t* test was used to compare TEG parameters in the two different groups. P value of < 0.05 was considered a cutoff to define significance.

Results: Facial vein sampling allowed smooth easy blood withdrawal and was well tolerated by mice. There was no significant difference in any of the TEG parameters when cardiac samples were compared to facial ones. The average R time was 7.8±4.6 and 5.4±4.1; p=0.2 in cardiac and facial samples respectively. The average K time was 3.0±1.7 and 3.1±3.2 p=0.4. The average alpha angle was 55.9 ±15.6 and 61.0±18.4 p=0.5. The average MA was 64.3±14.0 and 62.0±12.9 p=0.8. The average CI was 2.4±2.6 and 2.5±1.8 p=0.9.

Conclusions: Our results demonstrate that facial vein sampling does not affect TEG parameters and can be used as an alternative to cardiac puncture in studies where follow up or post intervention is required.

PB 085 | Quantitative Proteomics of Plasma-derived Macrovesicles Identifies Early Systemic Protein Markers of a Hemophilic Bleed in Rats

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Background: Diagnosis of a hemophilic bleed is currently based on unspecific clinical symptoms. Objective systemic biomarkers of bleeding would provide a clinical tool for diagnosis, and supply valuable information in clinical trials aimed at prevention of bleeding. However, it has proven difficult to identify early (< 6h) systemic biomarkers of a hemophilic bleed in plasma. Cellular communication via macrovesicles is a rapid event, and these may carry biomarkers for diseases.

Aims: To identify early systemic signals of a hemophilic bleed by plasma macrovesicle proteomics.

Methods: Blood from hemophilic rats (n=5) was collected prior to and at different time points after an induced knee-bleed (0.5, 2, 4, 6, 24 and 48h). Membrane protein fractions from exosome (EX) and microparticles (MP) from plasma were prepared for each time point. iTRAQ labeling and off-line Hydrophilic Interaction Liquid Chromatography in combination with high accuracy mass spectrometry was used to identify and quantify individual proteins. Subsequent clustering analysis was used to identify temporal patterns of protein regulation in the two distinct vesicle fractions.

Results: In total, 225 and 703 proteins were identified in the EX and MP fraction, respectively. The difference in the number of identifications may in part be due to higher purity of the EX fraction compared to the MP fraction. Twelve protein clusters were identified in the EX fraction, of which three represented proteins with very early response to the bleed (at 0.5 and 2h). The proteins with increased concentration at 0.5h are known to be involved in platelet activation, cytoskeletal activity, coagulation activity on cell surface, and EX release. The proteins increased at 2h are known to be involved in inflammation and acute phase responses.

Conclusions: Our study suggested very early temporal regulations in the plasma macrovesicle composition post hemophilic bleeding, which may potentially guide to early systemic biomarkers of bleeding in hemophilia.

PB 086 | Improved Anticoagulant Effect of Fucosylated Chondroitin Sulfate Orally Administered as Gastro-resistant Tablets

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Background: Fucosylated chondroitin sulfate is a potent anticoagulant polysaccharide extracted from sea cucumber. Its anticoagulant activity is attributed to the presence of unique branches of sulfated fucose. Although this glycosaminoglycan exerts an antithrombotic effect following oral administration, high doses are necessary to achieve the maximum effect. The diminished activity of FucCS following oral administration is likely due to its degradation in the gastrointestinal tract. However, gastro-resistant tablet formulation may help limit the degradation of FucCS in the gastrointestinal tract.

Aims: We compared the effects of FucCS administered as an aqueous solution and as gastro-protective tablets on coagulation, thrombosis, bleeding and blood pressure. Dabigatran, Rivaroxaban and Apixaban were used in these same assays as positive control drugs.

Methods: Antithrombotic activity in rats was assessed using thromboplastin as the thrombogenic stimulus in the vena cava model. Arterial thrombosis was assessed using Rose Bengal photochemical injury model. Ex vivo anticoagulant action was assessed using thrombin time and anti IIa activity. Bleeding tendency was evaluated using bleeding time model. Statistical analysis was evaluated using ANOVA.

Results: Experiments using animal models of arterial thrombosis demonstrated that FucCS delivered as gastro-protective tablets produced a potent antithrombotic effect, whereas its aqueous solution was ineffective. However, there was no significant difference between the effects of FucCS delivered as gastro-resistant tablets or as aqueous

solution in a venous thrombosis model, likely due to the high dose of thromboplastin used. New oral anticoagulants tested in these experimental models for comparison showed significantly increased bleeding tendencies.

Conclusions: Our study provides a framework for developing effective oral anticoagulants based on sulfated polysaccharides from marine organisms. The present results suggest that FucCS is a promising oral anticoagulant.

PB 087 | Three Point Ligation of Inferior Vena Cava in Rats: Experimental Approach to Study Mechanism of Venous Thrombosis

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Background: Venous thromboembolism (VTE) is an important issue of health care concern. Conditions of reduced blood flow are associated with localized hypoxic milieu, and deep vessel injury remains an uncommon feature during venous thrombus formation. Current understanding about the pathophysiology of venous thrombosis (VT) is often limited by lack of an appropriate model system that could mimic the actual clinical scenario. Although several techniques to restrict blood flow by ligation exist under clinical experimentation, however an effective approach to initiate thrombogenesis in the absence of an overt endothelial damage still remains to be established.

Aims: To introduce certain modifications in the inferior vena cava (IVC) ligation technique and characterize a flow restriction based rat model for venous thrombosis (VT).

Methods: For the proposed model, ligation was introduced at three particular sites i.e. the IVC below the left renal vein, left iliolumbar branch and right iliolumbar branch of male SD rat. This three point ligation technique produced a thrombus which closely resembled the clinical settings.

Results: Compared to the already existing IVC models, the current procedure was relatively simple, reproducible, involved minimal surgical damage and had a high success rate. Taking the advantage that restricted blood flow is associated with a hypoxic milieu; we blocked the transcriptional activity of hypoxia inducible factors-1(HIF-1α) using pharmacological inhibitor- CAY10585, and demonstrated that HIF-1α partly regulates venous thrombus formation. The endothelial injury was identified as a response to hypoxic milieu, accompanied by infiltration of innate immune cells and platelets in case of VT.

Conclusions: The three point ligation based model produces clinically relevant thrombus and can be used to study the mechanism of localized hypoxia induced VT. The proposed model provided evidence for the sequence of events: Reduced blood flow → hypoxia→ endothelial injury→ VT.

PB 088 | Activated factor VII for Hemostasis Enhancement in Factor XI and XIa Inhibitor-treated Primates

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Background: Monoclonal antibodies against FXI or FXIa provide long-lasting antithrombotic anticoagulation without detectable hemostasis impairment in animal models of thrombosis. The development and use of such antibodies may offer safe antithrombotic therapy or prophylaxis, however, reversal might be deemed desirable in cases of clinically relevant bleeding. Since the extrinsic pathway bypasses FXI, treatment with the hemostatic enzyme, FVIIa, may effectively terminate the antihemostatic effect, if any, of FXI inhibition.

Aims: Evaluate the hemostatic and procoagulant effects of recombinant FVIIa (NovoSeven®) in baboons that were anticoagulated with recombinant human FXI(a)-inhibitor antibodies, Bay1213790 and Bay1831865.

Methods: Animals received bolus *i.v.* injections of either Bay1213790 (3-4 mg/kg) or Bay1831865 (0.25 mg/kg), followed by an *i.v.* injection of NovoSeven® (210 or 420 µg/kg) 1-2 hours later. To evaluate anticoagulation activated partial thromboplastin time (aPTT), prothrombin time (PT) were measured in plasma, and non-activated thromboelastometry (ROTEM) was performed in whole blood. Template skin bleeding time (Surgicutt®) and blood loss were measured to assess primary hemostasis impairment.

Results: Both antibodies increased aPTT to >1.7-fold baseline, and whole blood clotting time (CT) during thromboelastometry to >2-fold, but they did not alter the hemostasis markers, PT, bleeding time, and bleeding volume. NovoSeven® shortened both PT and aPTT and normalized the CT. Procoagulant effects lasted for up to 4 hours and were back to pre-reversal anticoagulation levels the next day.

Conclusions: Our data suggest that administration of NovoSeven® reduces the anticoagulant effect of FXI(a) inhibitors, and promotes the hemostatic extrinsic pathway activity. Therefore, it may be useful to support the rapid cessation of bleeding during FXI(a) inhibitor anticoagulation without complete elimination of the antithrombotic effect of FXI(a) inhibition.

PB 089 | The Electrolytic IVC Model of Venous Thrombosis (EIM): A Cost-effective, Highly Reproducible, and Easily Controlled Tool to Investigate Venous Thrombosis under Flow Conditions

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Background: Animal models are critical to study the biology of venous thrombosis (VT) *in vivo*. The electrolytic model (EIM) was developed to simulate VT in the most likely clinical scenario, i.e. under blood flow conditions.

Aims: EIM has been criticized for the high cost and lack of fibrinolysis characterization. We aimed to decrease cost and further characterize the model.

Methods: VT was induced in C57BL/6 mice aged 10-12 weeks. Electric current was delivered either by the previously described stimulator or a simple precision voltage-to-current converter (VIC) powered by a standard direct current power supply. VIC was validated by measuring thrombus weights (TW) at Day 2 post-VT. Another group of mice was harvested at Day 2, 6, and 11 to investigate the fibrinolytic system. In a separate group of animals the dynamics of thrombus volume was assessed *in vivo* by MRI.

Results: VT was achieved in all mice without complications. The use of a VIC resulted in 90% cost reduction. TWs were similar between VIC and stimulator (19.4±1.8mg vs. 21.5±0.6mg, *p=NS*). Variation in TW reproduced by two investigators (MES, ORP) was less than 10%. Positive immunostaining for plasminogen was observed, with decrement from Day 2 to Day 11, arranged from periphery to the center of the thrombus. PAI-1 was overexpressed (*p< 0.05*), with a down-regulation trend of t-PA (*p=NS*) at Day 2 (Fig. 1). The opposite PAI-1 to t-PA relationship was observed at Day 11 (Fig. 1). Modification of current or time significantly reduced thrombus size (*p< 0.0001*) as assessed by MRI (Fig. 2).

Conclusions: The EIM is highly reproducible and may be performed at minimal cost using a VIC. The fibrinolytic system is suppressed at the acute stage of VT, showing impaired ability to respond to thrombogenesis in the vein wall. The desired thrombus size can be easily manipulated by current and time adjustments. Future studies will target the dynamics of VT composition using MRI, ultrasound and histology.

Figure 1

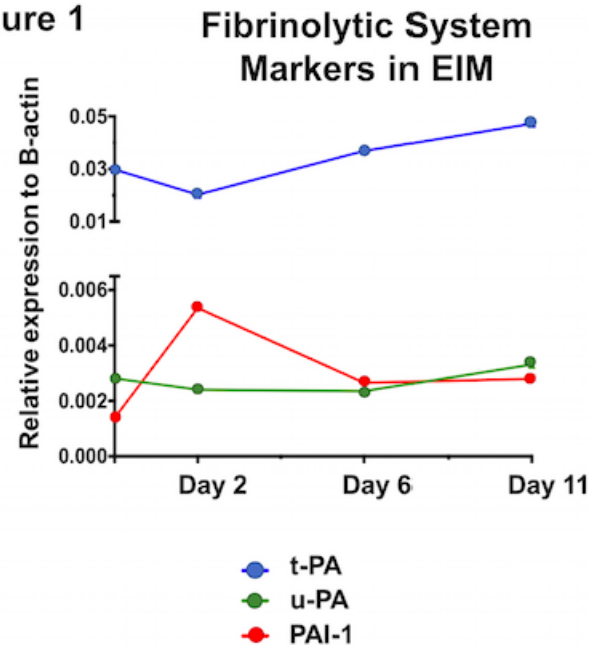

FIGURE 1 Vein Wall qRT-PCR for PAI-1, t-PA and u-PA. The PAI-1 upregulation during acute VT coincides with the downregulation of t-PA.

Figure 2

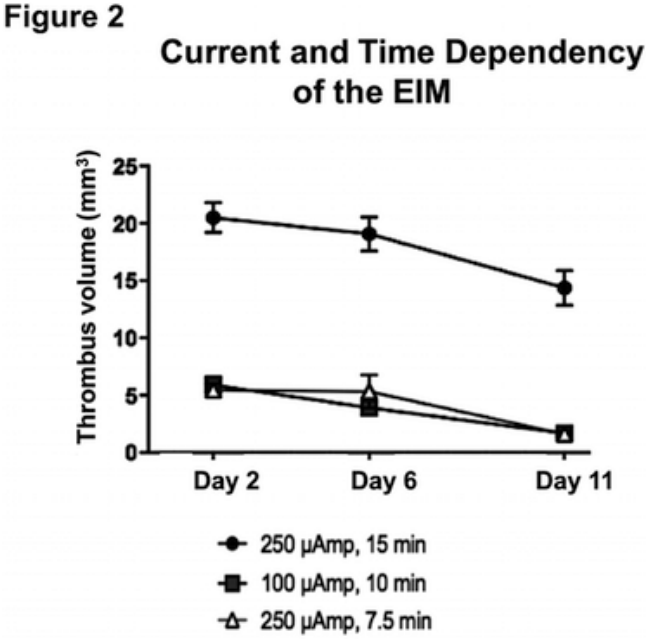

FIGURE 2 Thrombus volume over time was determined by manual tracing of contiguous axial MRI images, showing the current and time dependency of the EIM.

PB 090 | A Functional and Thromboelastometric-based Micromethod for Assessing Crotoxin Anticoagulant Activity and Antiserum Relative Potency against Crotalus Durissus Terrificus Venom

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Background: Substitution of rodent lethality assay (RLA) by means of in vitro techniques to assess antivenom potency is relevant to achieve the ‚3Rs' goals on animal experimentation. When one key lethal toxin is identified, then the ability of antivenom to neutralize some of its biological and measurable activities is likely to correlate with lethality neutralization. Crotoxin, toxin responsible for the lethality of crotalic venom, presents anticoagulant activity in vitro.

Aims: Our objective was to adapt sensitive thromboelastometry assay for assessing relative potency of anti-crotalic serum (ACS) in neutralizing anticoagulant effect.

Methods: 280 µL of citrated chicken plasma samples (CPS) were simultaneously incubated (10 min at 37°C) before recalcification with (1) ellagic acid and phospholipid-based activator (EAPA), semilogarithmic doses of (2) purified crotoxin, or (3) ACS.

Results: Clotting time (CT) values of control recalcified CPS are strongly prolonged (1698 ± 189 s, n = 8). EAPA dose sufficient to short CT to 750 s was defined as mean coagulant dose (MCD). Crotoxin

prolonged dose-dependently CT related to MCD of EAPA and 60±13 ng was considered as its mean anticoagulant dose (MAD), displacing CT to 1050 s. In our conditions, 100±18 nanoliters of one specific ACS batch neutralized incoagulability induced by 5MAD of crotoxin (300 ng), reestablishing CT to 1050 s.

Conclusions: Our results show the sensitivity of our method, being the previous potency determined by the in vivo RLA assay in which 1 mL of ACS neutralized 1.8 mg of crotalic venom. Deficiency of factor XII and slow clotting process in chicken plasma turn suitable the elaboration of a dose-response curve, in contrast with short recalcification time of mammalian plasma. Blood samples are collected without significant animal distress and this assay would be useful to substitute rodents in intermediary steps of ACS production. Moreover, it is possible to reduce the amount of toxin and ACS used.

PB 091 | Hemostasis and Fibrinolysis during Learning in Rats

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Background: Investigation of learning processis is enormous problem. In particularity it realizes in different experimental models. Our model permits to study various sides of learning in rats: psycho-emotional state, mental ability and also physiological and biochemical processes, following learning process, including state of hemostasis and fibrinolysis, that are very sensitive to intellectual and emotional loads.

Aims: The aim - to study parameters of hemostasis and fibrinolysis in rats in model of learning to solve complex food-seeking task.

Methods: 120 Wistar rats were learning to solve compound food-seeking task in open labyrinth by special method during 20 seances. Blood probes were takens before experiment and after finishing, and biochemical parameters of hemostasis and fibrinolysis were defined. Control rats (n=50) were in natural conditions of experimental room and were not subjected to intellectual loads.

Results: There were not observed essential changes of hemostasis and fibrinolysis parameters in whole group of experimental animals compared to control. AT111 and APTT level decreased by 8,5%, that demonstrated slight hypercoagulation. But there was simultaneously revealed compensation by fibrinolysis reaction: fibrinolytic activity raised by 13%, t-PA activity increased by 16,5% (p< 0,1). It was evident learning process did not cause sharp biochemical events as it represented natural and necessary action in rat's population. However only 40% of rats had learned to solve the task, 60% had not. Hemostasis and fibrinolysis were different in successful and unsuccessful rats. APTT and AT111 were correspondingly 42±5 c. and 92±10,1% in successful animals and 30,1±4,1c. and 81±7,6% in unsuccessful rats. (p< 0,05). t-PA was 45±4 C.U. in learned and 23±3 C.U. in unlearned rats (p< 0,05)

Conclusions: Unsuccessful learning led to development of moderate hypercoagulable state and possibly prethrombotic situation compared to rats solved the task. It is great favourable factor-talent to learning.

PB 092 | In the Cath Lab - A Novel Rabbit Bleeding Model

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Background: Even though cardiac catheterization is a minimal invasive intervention, a sufficient anti-coagulation and anti-platelet therapy has to be considered; and thrombosis versus bleeding risk needs to be deliberated. Patients undergoing cardiac catheterization typically receive dual anti-platelet therapy (DAPT), a combination of acetylsalicylic acid and $P2Y_{12}$-inhibition, with additional anti-coagulation treatment. This standard of care (SOC) leads to an increased bleeding risk during catheter lab examination, e.g. percutaneous coronary interventions (PCI). Increased bleeding can be monitored at the arterial puncture site and is one of the unwanted adverse events observed in the cath lab.

Aims: Deriving and adopting a novel lapine arterial bleeding model, to mimick the cath lab situation as an experimental setting in rabbits.

Methods: The lapine A. femoralis is prepared and incised via a 27 gauge needle, representing the approximate vessel-needle ratio during PCI. The puncture site is sealed with a pneumatic silicon vessel occluder for 1 or 3 minutes, respectively. The occlusion pressure is adjusted and controlled via a transonic flowprobe, to prevent stasis induction. The outcome is defined as incidence of bleeding and is controlled intrinsically via the ear bleeding time. Complementary to the animal model the procoagulant capacity of catheter material was confirmed in vitro which can be reversed by anticoagulants dose dependently.

Results: 40 percent of Bivalirudin on top of DAPT treated animals, still bleed after 3 minutes of compression. In the group of additional Enoxaparin treatment, 83% of the tested animals bleed even after 3 minutes of puncture-site occlusion. In comparison, control animals with DAPT only treatment do not bleed after 3-minutes-sealing.

Conclusions: Taken together this novel arterial bleeding model successfully simulates bleeding during PCI and demonstrated that additional anticoagulation on top of DAPT extends bleeding at the puncture site, in comparison to DAPT only.

PB 093 | Von Willebrand Factor Effects Vessel Conformation and the Expression of Integrin $\alpha V\beta_3$ and Ang-2 in the Porcine Female Reproductive Tract

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Background: Besides its role in coagulation, von Willebrand Factor (VWF) was shown to be involved in angiogenesis, and defects of this pathway might play a role in miscarriage. For this reason, VWF, Integrin $\alpha V\beta_3$ (ITG) and Angiopoietin-2 (Ang-2) were analyzed by immunohistochemistry (IHC) of reproductive tissues in a pig model of Von Willebrand Disease (VWD) type 3 and type 1.

Aims: We aimed to localize VWF and other angiogenic factors in the female reproductive tract of pigs affected by VWD.

Methods: Uterus, oviduct and ovary tissue samples were collected from five mature pigs. Of these, one was affected by VWD type 3, two were heterozygous carriers (HC, type 1) and two were wildtype (WT) individuals. Hematoxylin-eosin staining was implemented for morphological evaluation. Subsequently, IHC was performed to compare the expression of VWF, INT, and Ang-2 among the different genotypes.

Results: IHC showed almost no VWF expression in all examined tissues of the VWD type 3 pig.

For ITG the uterine epithelium and glands of the type 3 pig showed strong granular intracellular staining while the epithelial and glandular cells of the HC pigs were stained diffuse and moderately. The apical cell membrane of epithelium and glands of the WT pigs showed the strongest staining.

For Ang-2, the apical cell membrane of the uterine and oviduct epithelium of the VWD pig showed a clear immunoreaction, which was rarely seen in the HC and WT pigs.

Conclusions: The results of our study confirm effects of VWF on angiogenesis. The staining pattern of ITG can be explained by increased internalization of the protein when VWF is missing in contrast to its stabilization on the cell surface by VWF in WT pigs. The staining pattern of Ang-2 in the VWD pig might be a result of a steady release due to missing of VWF-dependent Weibel-Palade-Bodies, which serve as storage organelles for Ang-2.

PB 094 | Antithrombotic *In-vivo* Effects of Quercetin-tetra-sulphate (QTS) Isolated from *Flaveria bidentis* in an Experimental Thrombosis Model in Mice

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Background: Quercetin 3,7,3´,4´-tetrasulphate (QTS) presents important anticoagulant, antiplatelet and pro-fibrinolytic actions besides inhibitory effects over tissue factor expression in human monocytes.

Aims: In order to evaluate the antithrombotic properties of QTS, this study was designed to determine the *in-vivo* effects of sulfated flavonoids in an experimental thrombosis model in mice.

Methods: QTS was purified from an alcohol/water extract (1: 1) of *Flaveria bidentis* leaves by using a Sephadex G10 column and water as mobile phase. One hour before the thrombotic challenge (tail vein injection of 150 µg of collagen + 1.8 µg of epinephrine), five groups of 10 C57BL6 male mice weighing 28±2 gr received different intraperitoneal (i.p.) doses of QTS (10, 25, 50 or 100 mg/kg, respectively) or i.p. aspirin (20 mg/kg) (antithrombotic agent), as protected controls. Control animals (n = 25) received i.p. vehicle in the same volume as QTS in treated mice. The number of dead or paralyzed mice was evaluated until 15 minutes after injection of the thrombogenic substance and percent protection rate (PR) was calculated by the equation [1-(dead+paralyzed) /total]x100.

Results: PRs were 100, 90, 70 and 20% for 100, 50, 25 and 10 mg/Kg of QTS, respectively. In positive and negative controls, the PRs were 70 and 4%, respectively. Histological studies showed pulmonary thrombi in a dose-dependent manner.

Conclusions: Taken altogether, our results suggest that QTS could be a promising candidate as antithrombotic agent and that this effect could be associated with multiple effects of this compound over the blood coagulation system.

PB 095 | Increase of Fibrinolytic and Anticoagulant Activity by Per Os Administration of Proteinase Complex Longolytin in Rats

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Background: Longolytin is proteinase complex from imperfect fungi Arthrobotrys longa and has been successfully studied as thrombolytic substance. There are some favorable factors: simplicity of obtaining, little cost and absence of toxicity - that stimulate to search another way of it using, for example as preparation of 'enzymatic therapy'.

Aims: The aim is to use longolytin by per os administration in rats and to reveal influence on hemostasis and fibrinolysis.

Methods: 120 white rats were introduced by 0,1 ml. 3% solution of longolytin in glycerol activity 30-40 C.U. every day during 7 days. There were determined parameters hemostasis and fibrinolysis on 9, 16, 22 days of experiment. Control animals got glycerol (70 rats).

Results: There were observed increase of anticoagulant and fibrinolytic activities in experimental rats: APTT raised on 32%, fibrinolytic activity by euglobulin clot lysis time elevated on 28%, t-PA activity increased in 1,5 times after 9 and 16 days. Fibrinogen level did not exchange. Control animals had not changes in their parameters. These changes prolonged during 16 days and weakened gradually to 20 day of experiment. There were not observed reversed effect in 20 days. Intravenous thrombin infusion realized to animals after experiment finished for determine of its antithrombotic state in blood. 36% of experimental rats died and 66% of control.

Conclusions: Longolytin can create in blood prolonged elevated fibrinolytic and anticoagulant state as necessary defense against thrombosis in different prethrombotic situations.

PB 096 | The Anti-factor XIa Antibody BAY 1213790 is a Novel Anticoagulant that Shows Strong Antithrombotic Efficacy without an Increased Risk of Bleeding in Rabbit Models

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Background: Coagulation factor XI (FXI) contributes to the development of thrombosis but plays only a minor role in hemostasis and is therefore an attractive target for antithrombotic therapy. Inhibition of FXIa offers the potential to reduce the risk of thrombosis without increasing the risk of bleeding.

Aims: To evaluate the *in vitro* pharmacology of the novel anti-FXIa antibody BAY 1213790 in human plasma and to assess its *in vivo* antithrombotic properties and hemostatic risk profile in rabbits.

Methods: The *in vitro* properties of BAY 1213790 were analyzed using a biochemical FXIa assay, standard clotting assays (activated partial thromboplastin time [aPTT] and prothrombin time [PT]) and a thrombin generation assay (0.1 pM tissue factor) in human plasma. Antithrombotic efficacy of BAY 1213790 was assessed in a rabbit model of arterial thrombosis induced by ferrous chloride. The hemorrhagic effects of BAY 1213790 were evaluated in rabbit models of ear, gum and liver bleeding. BAY 1213790 was administered by intravenous bolus 15 minutes before the injury/surgery.

Results: BAY 1213790 inhibited human FXIa activity with an IC_{50} of 2 nM. At a concentration of 20 nM, BAY 1213790 was associated with a 1.5-fold prolongation of aPTT; there was no significant effect on PT. Thrombin formation was inhibited with an IC_{50} (peak) of 35 nM. In the *in vivo* rabbit models, BAY 1213790 inhibited thrombus formation with an ED_{50} of 0.2 mg/kg without increasing the ear and gum bleeding time. When administered with tissue plasminogen activator (0.1 mg/kg/h), which prolongs ear bleeding time by 1.7-fold, BAY 1213790 (3 mg/kg) did not further increase the bleeding time. In a rabbit liver incision model, BAY 1213790 (10 mg/kg) had no effect on liver bleeding time or volume.

Conclusions: BAY 1213790 is a potent inhibitor of FXIa that shows strong antithrombotic efficacy without increasing the risk of bleeding, therefore offering potential as an anticoagulant with a broad therapeutic window.

PB 097 | Concomitant Administration of Factor IX Replacement Therapy with Immunosuppression Decreases Immunogenicity in Hemophilia B Mice Expressing a Human Major Histocompatibility Complex

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Background: The development of neutralizing alloantibodies directed against factor IX following replacement therapy remains a poorly understood and potentially devastating complication of hemophilia B. Due to the clinical rarity (~2-4%) of factor IX inhibitors and their unique complications including life-threatening allergic reactions and resistance to traditional strategies for immune tolerance, novel approaches for promoting and maintaining tolerance is critical.

Aims: To examine the potential of pharmacologic approaches to promote and maintain an immunologic tolerance when the initial factor IX exposure occurs in the setting of a high risk.

Methods: Factor IX knockout (FIX-/-) mice engineered to express the common human MHC II DR B1*1501 allele instead of mouse MHC II (n=5/group) were administered FIX weekly via the subcutaneous route at a dose of 200 IU/kg for 7 doses. Immunosuppression with anakinra (an IL-1 receptor antagonist) and etanercept (a TNF-alpha inhibitor) were each administered at a dose of 10mg/kg weekly via subcutaneous injection with a control group for comparison. FIX Bethesda inhibitor antibody was assessed at weeks 2, 3, 4 and 8.

Results: 25% of mice that received immunosuppression with either anakinra or etanercept developed low-titer FIX inhibitors by week 6 (2.2 BU/ml and 1.2 BU/ml, respectively). In contrast, 75% of mice in the control group that received weekly SC FIX replacement with no immunosuppression developed FIX inhibitors by week 6 (mean 6.2 BU/ml, range 0.6-20 BU/ml).

Conclusions: Immunosuppression administered concomitantly with FIX replacement therapy during a known high risk event may blunt the immune response and reduce the risk of developing a high titer inhibitor.

PB 098 | Factor VIII Memory Immune Responses, von Willebrand Factor Makes a Difference

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Background: Immune tolerance induction (ITI) with aggressive infusion of FVIII is the current strategy to eradicate FVIII inhibitory antibodies (inhibitors) and restore normal FVIII pharmacokinetics in hemophilia A patients with inhibitors. Whether the use of FVIII products containing VWF will affect the efficacy of ITI is still controversial.

Aims: To explore the impact of VWF on FVIII memory immune responses in hemophilia A mice.

Methods: The T cell proliferation assay and cytokine profile analysis were used to study F8-primed CD4 T cells. The memory B cell-based ELISPOT assay was used to explore F8-specific memory B cells. Adoptive transfer of memory B cell pools into NSGF8KO mice were used as an *in vivo* model to further evaluate the impact of VWF on FVIII memory immune responses.

Results: When $CD4^+$ T cells from primed $F8^{null}$ mice were restimulated with rhF8 plus rhVWF *in vitro*, the percentages of daughter $CD4^+$ T cells were significantly decreased in both the 1 U/ml and 10 U/ml rhF8 treatment groups compared to the groups cultured with rhF8 only (10.4±7.1% and 15.8±8.4% vs 14.0±7.5% and 21.5±10.3%, respectively). The levels of IFNg and IL10 in the rhF8 plus rhVWF groups were significantly lower than in the rhF8 groups. When memory B cell pools from primed $F8^{null}$ mice were cultured with 0.05 U/ml or 1 U/ml rhF8 with or without rhVWF to induce memory B

cell differentiation into antibody secreting cells (ASCs), the number of ASCs in the rhF8 plus VWF groups were significantly less than in the rhF8 groups (3.9±1.7 and 2.2±0.8 vs 44.8±3.1 and 89.5±9.3 ASCs/10^6 cells, respectively). When memory B cell pools were transferred into NSGF8KO mice followed by rhF8 immunization with or without rhVWF, the titers of anti-F8 inhibitors and total IgG in the rhF8 group were significantly higher than in the rhF8 plus rhVWF group (45.9±18.9BU/ml and 5728±2039 vs 23.9±11.6 BU/ml and 2840±1022, respectively).

Conclusions: Our data demonstrate that VWF attenuates FVIII memory immune responses in hemophilia A mice.

PB 099 | New Insights into FV-short Function and Interaction with TFPIα

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Background: Factor V-short is a FV splice variant present at low levels (< 2%) in normal plasma. It lacks residues 756-1458 in the B-domain, which includes the basic region (BR). In full length FV, the BR in concert with acidic region 2 (AR2; 1493-1537) functions as an autoinhibitory unit to keep FV inactive. In FV East Texas bleeding disorder, FV-short is elevated due to enhanced splicing and this paradoxically increases TFPIα. TFPIα through its C-terminal BR forms a high affinity complex with FV-short.

Aims: To examine how TFPIα via its BR regulates FV and FV-short.

Methods: We expressed and purified FV-short and its thrombin resistant variant QQ (709/1545Q) and characterized their interaction with a TFPIα-BR fragment or a full length TFPIα variant-R107A.

Results: In a purified system, we found that FV-short, QQ, and FVa were functionally equivalent when assembled in prothrombinase. FV-short and QQ activity was blocked in the presence of TFPIα-BR while there was no effect on FVa. Fluorescence anisotropy experiments revealed that TFPIα-BR bound with high affinity (Kd< 1 nM) to FV-short with no detectable binding to FVa. In a thrombin generation assay (TGA) using FV-short, both TFPIα-BR and TFPIα-R107A reduced TG. This was not only due to decreased in FV-short procoagulant function but also to delayed cleavage at R1545 as confirmed by the higher sensitivity of the QQ variants to both TFPIα-BR and TFPIα-R107A and by data from western blotting experiments. In contrast to FV-short, there is no detectable binding of TFPIα-BR to plasma-derived FV. However the BR fragment reduced TG in normal plasma. Simultaneous fluorescence and western blotting experiments revealed that TFPIα-BR only binds partially cleaved forms of FV and binding is eliminated once AR2 is removed.

Conclusions: These data show that TFPIα through its BR downregulates TG by engaging forms of FV with an available AR2, such as FV-short and partially proteolyzed FV and AR2 removal by cleavage at 1545 eliminates its ability to impact prothrombinase assembly.

PB 100 | Structural and Functional Characterization of Anticoagulant TIX-5, the Specific Inhibitor of the Activation of FV by FXa

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Background: The prothrombinase complex consists of factors Xa (FXa) and Va (FVa) on an anionic phospholipid surface and it converts prothrombin into thrombin. Both coagulation factors require activation prior to complex assembly. The specific anticoagulant tick inhibitor of factor Xa towards factor V (TIX-5) inhibits the activation of FV by FXa.

Aims: To unravel the structure-function relationships of TIX-5 that define its mechanism of action.

Methods: The TIX-5 structure was modeled based on homology with the Der F7 allergen, and point mutations were introduced at predicted interaction sites. TIX-5 variants were expressed in *Drosophila* cells, purified by affinity and ion exchange chromatography, and tested for their anticoagulant activity in plasma using the Calibrated Automated Thrombogram (CAT). Interactions of TIX-5 variants with an anionic phospholipid layer were determined by Surface Plasmon Resonance analysis.

Results: The TIX-5 predicted structure was elongated and consisted of 2 beta sheets wrapped around the C-terminal α2 helix. At one end of the structure, two hydrophobic loops were predicted with potential phospholipid binding properties. On the other end, a negatively charged area which potentially interacts with FV and a predicted protein binding site were located. CAT analysis revealed that mutations at both, the negatively charged area and protein binding site, led to decreased anticoagulant activity of TIX-5. Mutations at the predicted phospholipid interaction site led to total loss of anticoagulant activity. Consistently, these mutations also resulted in decreased phospholipid binding.

Conclusions: Our structure-function analysis guided by a homology model of TIX-5 indicates correct prediction of its interaction sites. The results are in line with a protein that inhibits activation of FV by FXa on a phospholipid surface. We have identified the crucial phospholipid- and protein-binding sites on TIX-5 that will aid our understanding of the importance of the FXa-mediated activation of FV in coagulation.

PB 101 | Inhibition of Contact Activation by Histidine Rich Glycoprotein is Mediated by its Histidine-rich Domain

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Background: We have shown that histidine-rich glycoprotein (HRG) regulates the contact system by binding factor (f) XIIa with high affinity and inhibiting fXII autoactivation and fXI activation. In addition, HRG also binds nucleic acids and polyphosphate and attenuates their capacity to activate the contact system.

Aims: To identify the domain in HRG responsible for binding to fXIIa.

Methods: HRG was isolated from human plasma. The $(HHPHG)_4$ peptide was synthesized based on the His-His-Pro-His-Gly (HHPHG) consensus sequence in the histidine-rich region (HRR) of HRG. FXII autoactivation was performed in the presence of dextran sulphate or silica and activity was monitored by chromogenic assay. Clotting in normal or HRG-deficient human plasma was initiated with silica or activated partial thromboplastin reagent (APTT-SP).

Results: As shown previously, HRG produces a dose-dependent inhibition (IC_{50} of 200 nM) of fXII autoactivation induced by either dextran sulphate or silica, synthetic activators of the contact pathway. Like HRG, $(HHPHG)_4$ inhibits fXII autoactivation stimulated by silica (IC_{50} of 1000 nM). Neither HRG nor $(HHPHG)_4$ affects fXIIa chromogenic activity. Clotting initiated by APTT-SP or silica is accelerated in HRG-deficient plasma, but is prolonged in a dose-dependent and saturable manner by addition of either HRG or $(HHPHG)_4$. In normal or HRG-deficient human plasma, neither HRG nor $(HHPHG)_4$ influences clotting initiated via the extrinsic pathway by addition of RecombiPlasTin.

Conclusions: A synthetic peptide analogue of the HRR of HRG recapitulates the effects of HRG on the contact system. Therefore, the HRR likely mediates the interaction of HRG with fXIIa, and may be an important probe for investigating the role of HRG in thrombosis.

PB 102 | Dabigatran and Rivaroxaban Inhibit Thrombin Generation Much Stronger than the Growth of a Fibrin Clot

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Background: Low amount of tissue factor (< 5pM) produces *in-vitro* ~100 nM of thrombin, which is much greater than is required to form a clot. However, tissue factor is localized to a vascular wall, and thrombin concentration can decrease with distance. It is unknown how distribution of thrombin correlates with the rate of the fibrin cloth propagation from the damage site, particularly in the presence of anticoagulants.

Aims: To investigate *in-vitro* and *ex-vivo* the effect of different anticoagulants on spatial propagation of fibrin clot and thrombin generation.

Methods: Thrombodynamics-4D is an *in-vitro* assay for simultaneous monitoring of thrombin activity and fibrin clot propagation from the immobilized tissue factor. We used plasma from healthy individuals, spiked with unfractionated heparin, dabigatran, and rivaroxaban, and samples from patients, receiving anticoagulant therapy after an orthopedic surgery.

Results: Thrombin activity propagates from the activating surface as a moving peak. Fibrin clot follows thrombin and reaches size of 2-3 mm in 60 min. Spiking of normal plasma with 0.15 IU/ml of heparin prevented moving peak formation without affecting the initiation phase. Thrombin generation persisted in 1 mm region near the activating surface. Addition of rivaroxaban and dabigatran did not have strong effect on fibrin clot growth, but the initial formation of thrombin was delayed. 0.15 μM of dabigatran decreased amount of thrombin 2-fold, while the rate of clot growth decreased 6%.

The samples from patients after orthopedic surgery showed that 3000 IU of LMWH resulted in dramatically decreased clot growth rate and absence of moving peak of thrombin. In contrast, 2-3h after 220mg of dabigatran or 10mg of rivaroxaban thrombin generation was significantly reduced, but the clot growth still persisted. Besides, the anticoagulants had distinctly different effects on the shape of the thrombin peak.

Conclusions: Amount of thrombin generated during clot formation does not fully determine clot propagation in space.

PB 103 | Factor VII Activating Protease (FSAP) and Marburg I (G534E) Polymorphism; How One Amino Acid Determines Function

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Background: In the gene encoding FSAP (*HAPB2*) there is a single nucleotide polymorphism (SNP) called Marburg I (MI) SNP which causes the amino acid change G534E. This SNP has lower enzymatic activity and is associated with an increased risk of stroke, carotid stenosis, liver fibrosis and thyroid cancer.

Aims: To understand the mechanism behind the lower enzymatic activity of the MI-SNP of FSAP.

Methods: The catalytic domain of wild type (WT) FSAP was expressed in bacteria and characterized by an array of biochemical methods. The naturally occurring Gly residue at position 534 was mutated to Glu (MI-SNP), Ala, Asp, Leu and Gln. by site directed mutagenesis. Michaelis-Menten kinetics was performed with the different isoforms. The catalytic domain of FSAP was modelled by I-TASSER using tPA as a template.

Results: The correct expression of the catalytic domain of FSAP was confirmed by sequencing, reactivity to antibodies, activity assays against various chromogenic substrates as well as sensitivity to known FSAP inhibitors. The kinetic data indicates a very close resemblance to plasma purified FSAP. The Glu mutant (MI-SNP) showed very little activity. Asp, Leu and Gln mutants showed significantly lower activity,

whereas the Ala mutant demonstrated about 20% of the activity of WT-FSAP. The modelling data shows high overlap between the helices and sheets of FSAP and tPA as well as in the configuration of the catalytic triad. The MI mutation causes a conformational shift in two β-strands near the active site residues altering the intermolecular distances between catalytic triad residues.

Conclusions: The successful preparation of recombinant catalytic domain of FSAP opens up possibilities to perform structural and functional analysis. At the MI-SNP site only Gly or Ala were tolerated whereas other amino acid substitutions led to a loss of activity. The altered configuration of the catalytic triad in MI-SNP is responsible for the loss of its proteolytic activity.

PB 104 | Acquired Hemophilia A in Dutch Patients, a Single Center Experience

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Background: Acquired hemophilia A (AHA) is a rare autoimmune bleeding disorder that can cause life-threatening bleedings and requires immunosuppressive treatment (IST). To date, the optimal regimen for hemostatic therapy and IST is not determined.

Aims: Description of a Dutch cohort of AHA patients in order to obtain insight in the effectiveness and safety of current treatment.

Methods: A retrospective cohort study between 1999 and 2016 was performed in the Van Creveldkliniek, University Medical Center Utrecht.

Results: The cohort comprised 25 patients with AHA (60% male). The median age at diagnosis was 77 (IQR 66-80). Median FVIII level and anti-FVIII inhibitor titer at diagnosis were 5 IU/dL and 17 BU, respectively. Median follow-up was 223 days (IQR 70-500). An underlying condition was identified in 36% (*table 1*). 36 bleeding episodes occurred in 20 patients, of which 36% were severe. FVII and FVIII replacement therapies were used to treat 40% of the bleedings each. 96% of all patients were treated with IST. Outcome data after IST was available in 18 patients (*table 2*). After first line IST, 7 (39%) reached complete remission (CR). 3/3 (100%) of the patients treated with rituximab and steroids reached CR, in contrast with 3/12 (25%) of the patients treated with steroids only. Of the 11 patients with no CR after first line IST, 8 (67%) received second line IST, 2 (22%) maintenance IST and 1 (11%) died by an unknown cause. In patients with second line IST, remission data was available in 4 (50%), of whom 3 (75%) achieved CR. Remission data at final follow-up was available in 13 patients, of whom 8 (62%) reached CR. Survival data was available in 23 patients, of whom 10 (43%) were deceased. Of these, 10% died by complications of IST, no patients died by fatal bleeding.

Conclusions: This study shows first line IST fails to achieve CR in a majority of patients with AHA, although combination treatment with rituximab and steroids shows favorable results.

TABLE 1 Disease characteristics

Total, N	25
Idiopathic, N (%)	**10 (40,0)**
Any underlying condition, N (%)	**9 (36,0)**
Solid malignancy, N (%)	5 (55,5)
Postpartum, N (%)	2 (22,2)
Other (MGUS and aplastic anemia), N (%)	2 (22,2)
Unknown, N (%)	**6 (24,0)**
Patients with registered bleedings, N (%)	**20 (80,0)**
Patients with registered bleedings, n	**36**
Severe[1], n (%)	13 (36,1)

TABLE 2 Outcome of immunosuppression therapy

First line immunosuppressive therapy regimen	Patients with available outcome data on first line therapy N=18	Patients with CR after first line therapy N=7 (38,9%)	Patients with no CR after first line therapy N=11 (61,1%)
Steroids, N (%)	12 (66,7)	3 (25,0)	9 (75,0)
Steroids and rituximab, N (%)	3 (16,7)	3 (100)	0 (0)
Steroids and cyclophosphamide, N (%)	1 (5,6)	0 (0)	1 (100)
Steroids and MMF, N (%)	2 (11,1)	1 (50,0)	1 (50)

PB 105 | Salvianolic Acids Inhibit Blood Coagulation and Platelet Aggregation: A New Class of Herb Derived Dabigatran Analogs

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Background: Salvianolic acids (SAs), including SAB (also called MLB) and SAC, are major components of *Salvia miltiorrhiza* root (Danshen). Danshen extracts were used to control cardiovascular disease for centuries, and its depside salt was approved in China to treat chronic angina. Although Danshen extracts are observed to inhibit thrombosis, the mechanism has not been adequately explored.

Aims: To explore the mechanism of SAs in anticoagulation.

Methods: *In vitro* coagulation assay, isothermal titration calorimetry (ITC), and molecular docking.

Results: We observed that MLB/SAC (250 μM) significantly reduced clot size and wet/dry weight. Using thromboelastography, we found that MLB/SAC markedly decreased the mechanical strength of the clot and modestly delayed initiation of coagulation in blood plasma. We further observed that MLB significantly reduced the density of the fibrin network, suggesting that SAs targets coagulation factors. Recent network pharmacology analyses predict that MLB interacts with VWF, factor XIII (FXIII), or thrombin. We found that MLB did not

inhibit VWF-dependent platelet agglutination, or significantly alter the binding of activated FXIII (FXIIIa) to fibrinogen. However, MLB significantly reduced the generation of FXIIIa from FXIII, suggesting a direct inhibition on thrombin activity/generation. We also found that MLB markedly inhibited thrombin-induced gel-filtered human and mouse platelet aggregation. These data suggest that MLB is a direct thrombin inhibitor. Indeed, ITC showed that MLB/SAC bind thrombin, with SAC possessing a 2-fold stronger affinity. MLB/SAC contain structural similarities to dabigatran, and molecular docking models docked MLB/SAC within the thrombin active site, interacting with the same residues that dabigatran contacts within the thrombin active site.

Conclusions: These data demonstrate a novel role for SAs in the inhibition of blood coagulation and platelet aggregation, likely through direct thrombin inhibition, as a new class of herb derived dabigatran analogs.

PB 106 | Fusion to Albumin Extends the Circulatory Half-life and Antithrombotic Effects of the Kunitz Protease Inhibitor Domain of Protease Nexin 2 in Mice

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Background: Protease Nexin 2 (or Amyloid Precursor Protein) is a potent inhibitor of activated coagulation factor XI (FXIa). Its FXIa inhibitory activity is contained within a 57 amino acid (aa) Kunitz Protease Inhibitor (KPI) domain. KPI exerts antithrombotic effects in vivo but is limited by its rapid clearance.

Aims: To extend the circulatory half-life ($t_{0.5}$) of KPI via gene fusion to human serum albumin (HSA) and to characterize the pharmacokinetic and pharmacodynamic profile of KPIHSA in vivo.

Methods: KPI (63 aa), KPIHSA (598 aa), and HSA (588 aa) were expressed in *Pichia pastoris* in His-tagged form and purified from conditioned media via Ni-chelate chromatography. Clearance of ^{125}I-labelled proteins was tracked by serial blood sampling in CD1 mice following intravenous injection and $t_{0.5}$ were solved by biphasic curve fitting. ^{125}I-fibrin(ogen) deposition in the $FeCl_3$-treated vena cava and time to occlusion (TTO) of the $FeCl_3$-treated carotid artery were compared in mice treated with KPI or KPIHSA.

Results: The mean initial $t_{0.5}$ of KPI, KPIHSA, and HSA in mice were 0.048h, 0.58h, and 1.3h, and the mean terminal $t_{0.5}$ were 0.80h, 8.2h, and 17.5h, respectively. TTO was significantly lengthened in mice treated 2 min prior to $FeCl_3$ arterial injury artery with 14.3 nmoles of KPI (39 ± 17 min, mean ± SD, n=6, $p < 0.05$) or KPIHSA (49 ± 15 min, $p< 0.01$) versus saline vehicle (12.5 ± 4 min); KPI and KPIHSA did not differ. Recovery of ^{125}I-fibrin(ogen) in $FeCl_3$-induced vena cava thrombi was identically reduced from 0.267 ± 0.110% by saline vehicle to 0.114 ± 0.069% by KPI, or 0.095 ± 0.066% by KPIHSA pre-treatment 2 min pre-injury. Extending the pre-treatment interval to 60 min eliminated the KPI effect (0.282 ± 0.180%) but that of KPIHSA was unaltered (0.101 ± 0.051, $p < 0.05$ versus 60 min KPI pre-treatment).

Conclusions: Fusion to albumin slowed the clearance of KPI 10.3- to 12.3-fold without reducing its immediate antithrombotic efficacy. KPIHSA also exhibited a more durable pharmacodynamic effect than unfused KPI.

PB 107 | Immunogenicity Assessment of OBIZUR, a Recombinant B-domain Deleted Porcine- sequence FVIII, in Patients with Acquired Hemophilia A

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Background: A recombinant B-domain deleted porcine- sequence factor VIII (rpFVIII; OBIZUR) was recently approved for treatment of bleeding episodes in adults with acquired haemophilia (AHA). We previously reported results for inhibitory antibodies against rpFVIII and human FVIII (hFVIII) from a Phase 2/3 clinical trial and evaluated their impact on treatment efficacy.

Aims: The aim of this study was to extend this data by analyzing binding IgG and IgM antibodies against hFVIII and rpFVIII and evaluating their cross reactivity.

Methods: The pivotal Phase 2/3 study (NCT01178294) and the methods in principle for antibody analytics were previously reported (Whelan 2013; Kruse-Jarres 2015). 28 subjects with AHA were evaluated for inhibitory antibodies against hFVIII and rpFVIII. 26/28subjects had supplementary samples that were analyzed for total binding IgG and IgM antibodies.

Results: Consistent with AHA diagnosis all subjects evaluated (n=26) tested positive for binding IgG and inhibitory antibodies against hFVIII at screening. 20 of those subjects showed positive results for binding anti- rpFVIII IgG (10/20 had inhibitory antibodies against rpFVIII) that can be considered cross-reactive anti-hFVIII antibodies. After treatment with OBIZUR, 10 patients were tested positive for binding IgG antibodies against rpFVIII. De novo inhibitory antibodies against rpFVIII were detected in 5 subjects. Among those 2 subjects showed a concomitant decrease of anti-hFVIII IgG. This suggests that the observed anti-rpFVIII inhibitors resulted from an immunogenic response to rpFVIII.

Conclusions: An increase in cross-reactive anti-hFVIII antibodies or a specific immunogenic response to rpFVIII could contribute to the development of antibodies against rpFVIII observed during the study. All subjects had a positive response to OBIZUR treatment within 24 h after initiation. Further characterization of subclass and isotype distribution and affinities of anti-rpFVIII antibodies will provide additional insights into rpFVIII immunogenicity.

PB 108 | Affimers as a Tool to Understand Mechanistic Pathways that Control Fibrin Clot Structure and Lysis

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Background: Altered clot structure and impaired fibrinolysis are associated with increased thrombotic events. Elucidating mechanistic pathways for altered fibrin clot characteristics will help to develop new therapeutic targets to reduce thrombosis risk. We created a library of conformational random peptides (n=3x10^{10}) mounted on a scaffold protein, named Affimers, which we hypothesised can bind fibrinogen and alter fibrin network properties.

Aims: Study the effect of fibrinogen-binding Affimers on clot structure/lysis and identify potential interaction sites.

Methods: The library of Affimers was screened for fibrinogen binders using a phage display system. Turbidimetric assays and confocal microscopy assessed the effects of Affimers on clot structure/lysis. Mass spectrometry (MS) and molecular modelling were used to elucidate fibrinogen-Affimer binding sites.

Results: We identified one Affimer, termed G2, that decreased lag phase during plasma clot formation in turbidimetric experiments from 457±8.7 to 72±2.5 sec (mean±SEM) ($p< 0.001$), reduced clot final turbidity (FT) from 0.19±0.015 to 0.09±0.008 AU ($p< 0.001$) and completely prevented clot lysis at concentrations >23nM. In a purified system, G2 also prolonged clot lysis time in a dose-dependent manner. Confocal microscopy showed that G2 caused significant disruption to plasma and purified clots, explaining the change in FT. Despite screening against human fibrinogen, G2 had similar effects on mouse clots indicating that it interacted with conserved regions on fibrinogen. MS and molecular modelling identified 2 possible fibrinogen-G2 interaction sites, one located at fibrinopeptide B and a second close to the C-terminus of β-chain towards the D domain.

Conclusions: The fibrinogen-binding Affimer G2 modulates clot structure and inhibits fibrinolysis of human or mouse clots. The β-chain of fibrinogen represents one interaction site with G2, potentially offering a novel therapeutic target to modulate fibrin network structure/lysis.

PB 109 | The Most Significant Physiological Function of Protein S is Inhibition of Thrombin Generation

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Background: Protein S (PS) is a vitamin K-dependent anticoagulant for which several different functions have been ascribed: PS acts as a cofactor for both activated protein C and tissue factor pathway inhibitor (TFPI), and is an inhibitor of factor IXa (FIXa). This diversity of PS activity mandates establishing the relative physiological importance of each function in the different pathways of thrombin generation.

Aims: To quantitate the relative physiological importance of PS functions by measuring peak thrombin generation in the presence of PS alone, PS with protein C, and PS with TFPI.

Methods: Measurements of thrombin generation are performed in a defined assay using mixtures of the zymogen forms of factors VII, X, V, IX, VIII, and II, ± PS (Table 1), following the sub sampling method. The effect of PS is quantitated as a PS ratio; a ratio of 1 means little change in thrombin generation, whereas a ratio less than 1 signifies a decrease. The zymogen protein factors are incubated overnight with 15X the plasma concentration of antithrombin to ensure that no activated factors are present. The protein mix is then added to phospholipid vesicles (25%DOPS/75% DOPC) with an overall Ca^{2+} concentration of 3 mM and 10 pM tissue factor, and initiated with 0.2nM VIIa. Thrombin generation is then measured.

TABLE 1 Thrombin generation reaction pathways

1) VII, X, V, II, AT, lipid & TF ± PS → Thrombin (IIa)
2) VII, X, V, II, AT, lipid, TFPI & TF ± PS → IIa
3) VII, X, V, II, AT, lipid, TF, IX, VIII ± PS → IIa
4) VII, X, V, II, AT, lipid, TFPI, TF, IX, VIII ± PS → IIa
5) VII, X, V, II, AT, lipid, TF, PC, TM ± PS → IIa
6) VII, X, V, II, AT, lipid, TF, PC, TM, IX, VII ± PS → IIa
7) VII, X, V, II, AT, lipid, TF, PC, TM, IX, VII, TFPI ± PS → IIa

Results: When thrombin generation was measured in the reactions from pathway 3 (Table 1), the peak thrombin generated was twice the nanomolar amount without PS as it was with PS (Figure 1). The ratio of peak thrombin generation in the presence of PS to the absence of PS was 0.51, indicating that PS significantly inhibits thrombin generation when FIX and FVIII are both present.

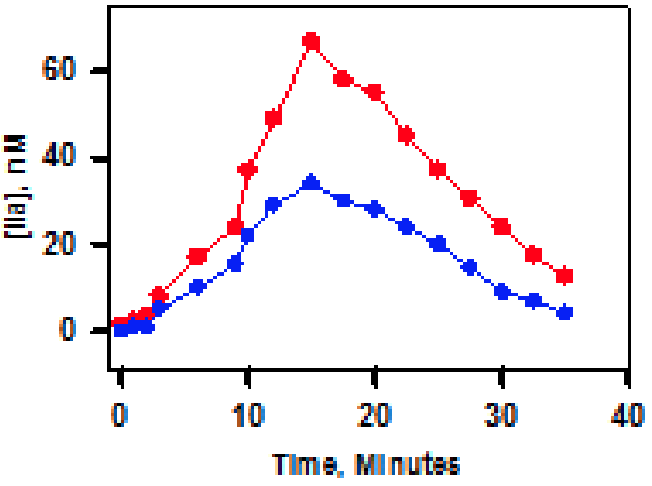

FIGURE 1 Effect of protein S on thrombin generation

Conclusions: Thrombin generation in the presence (blue curve in Figure 1) and absence (red curve in Figure 1) of PS indicates that, in a defined system, PS is a key regulator of thrombin generation. The

results of assays (pathways) 1-2 and 4-7 will further clarify the dominant role of PS with respect to its other reported functions.

PB 110 | Immunogenicity of BAX 855 in Previously Treated Patients with Congenital Severe Hemophilia A

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Background: BAX 855 is an extended half-life recombinant human coagulation factor VIII modified with polyethylene glycol (PEG) (Turecek 2012). It was recently approved in the US and Japan for on-demand treatment of bleeding events and for prophylactic treatment for patients with congenital severe hemophilia A.

The efficacy and safety of BAX 855 were extensively studied during clinical development (Konkle 2015). The assessment of BAX855 immunogenicity was of particular interest because the development of neutralizing antibodies (FVIII inhibitors) is the most serious complication following replacement therapies with FVIII products.

Aims: To fully understand the potential of BAX855 to induce antibody responses, both FVIII inhibitors and total FVIII-binding antibodies as well as antibodies against PEG-FVIII and PEG were assessed

Methods: The clinical protocols (NCT02585960, NCT02210091, NCT01736475, NCT01913405, NCT01945593, NCT01599819, NCT02615691) and the methods used for antibody analytics (Whelan 2013; Lubich 2016, Konkle 2015) were previously described. Correlation analyses were done to assess any potential consequences on PK, safety and efficacy.

Results: None of the 243 subjects included in the analysis developed FVIII inhibitors (≥ 0.6 BU/mL) A total of 44 subjects tested positive for binding antibodies at single time points. 28 of these 44 subjects showed pre-existing antibodies against FVIII, PEG-FVIII, or PEG prior to first exposure to BAX 855. 13 subjects developed transient antibodies after exposure to BAX 855 (no conclusion can be drawn for 5 subjects at data cut off). There was no confirmed causal relationship between the appearance of binding antibodies and PK parameters, adverse events and hemostatic efficacy.

Conclusions: Our data indicate that BAX855 did not show an increased risk for PTPs to develop FVIII inhibitors. The data suggest that BAX855 did not induce immune responses associated with impaired treatment efficacy or with altered PK parameters.

PB 111 | Validation of a FVIII Nijmegen Bethesda Assay for the Detection of Inhibitors to Porcine FVIII

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Background: Neutralizing factor VIII (FVIII) antibodies may develop in patients with congenital hemophilia A following FVIII replacement therapy and may also develop spontaneously in patients without hemophilia. OBIZUR [Anti-hemophilic Factor (Recombinant), Porcine Sequence] has been shown to be effective for treatment of bleeding in patients who develop spontaneous autoantibodies to FVIII, as inhibitors directed against human FVIII frequently show lower cross reactivity to porcine rFVIII. Furthermore, porcine rFVIII is less susceptible to inactivation by inhibitory antibodies.

Aims: To validate a FVIII One Stage Nijmegen Bethesda assay (NBA) to detect inhibitors against porcine VIII.

Methods: The human FVIII NBA was modified for the detection of inhibitors to porcine FVIII by substituting the test base, buffered normal pooled plasma, with buffered porcine rFVIII (OBIZUR). Human FVIII inhibitor plasma with cross reactivity to porcine rFVIII was spiked into congenital FVIII deficient plasma to obtain validation samples at 10.0, 1.0, 0.6 (LLOQ) and 0.25 BU. Validation samples were tested in the modified NBA post heat pre-treatment at 58 ± 2°C for 90 minutes. Relative accuracy, intra- and inter-assay precision, as well as specificity and sample stabilities were assessed.

Results: Relative accuracy was demonstrated for two inhibitor positive patient plasmas. Intra- and inter-assay precision ranged between 10.0 to 14.7% and 7.5 to 10.5%, respectively. Selectivity was demonstrated in six FVIII deficient donor plasmas spiked to 1.0 BU/mL and 0.25 BU. Sample stability up to 3 hours post heat treatment and incubation, as well as 3 additional freeze thaw cycles were demonstrated. Studies to assess the stability of the buffered porcine rFVIII test base when stored at -20°C and -70°C are ongoing and will be presented.

Conclusions: The porcine FVIII NBA has been validated for the quantification of porcine FVIII inhibitors in both congenital and acquired hemophilia A plasma.

PB 112 | Single-dose Safety and Toxicological Studies of Long-acting FVIIa (MOD-5014) in Rats and Monkeys Support the Initiation of Clinical Phase 1 Subcutaneous (SC) Study in Healthy Volunteers

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Background: OPKO Health is a clinical-stage public company developing biobetter long-acting versions of existing therapeutic proteins utilizing a technology termed CTP. The technology involves fusion of the C terminus peptide of hCG to the target protein.

Aims: The aim of this work was to extensively characterize the pharmacokinetic (PK), pharmacodynamic (PD), and overall safety profile of FVIIa-CTP (MOD-5014) in rats and monkeys following SC administration in order to enable the initiation of a Phase 1 clinical study in healthy volunteers.

Methods: Single-dose toxicological studies of MOD-5014 were conducted in rats and monkeys at doses of 1, 3 and 9 mg/kg. Clinical pathology was evaluated during the study and followed by 14-day recovery period to assess the reversibility of toxic effects. PK and PD parameters were also assessed to confirm proper exposure margins, which were above the initial clinical dose to be tested in the SC Phase 1 study.

Results: MOD-5014 exposures based on plasma concentrations and clotting activity increased with increasing dose and confirmed a significant margin above the expected clinical doses in the SC Phase 1 study. There were no safety concerns, unexpected test article-related mortality or adverse effects. The changes noted were related to an exaggerated pharmacological activity of the drug and were reversible after the 14-day recovery period.

Conclusions: Attachment of CTP to FVIIa led to a pronounced enhancement of PK and PD exposures and elevated half-life, supported by an excellent safety profile in toxicological setting following SC injection. Our data suggest that MOD-5014 injected SC is safe and tolerable, and has the potential to significantly improve the prophylactic treatment of hemophilic patients.

PB 113 | Low Plasma FVII:C and Activated FVII as Predictive Markers for Overt Disseminated Intravascular Coagulation

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Background: In sepsis, binding of factor VII clotting activity (FVII:C) and activated factor VII (FVIIa) with the expressed tissue factor on monocytes is the key early step of coagulation resulting in disseminated intravascular coagulation (DIC).

Aims: To measure both FVII:C and FVIIa among pediatric patients with sepsis and DIC.

Methods: Patients aged 1 month to 20 years, who had sepsis, were enrolled. Blood samples from each patient were collected at the following periods: 0, 24 and 48 hours after the onset of sepsis.

Results: Forty-seven septic patients, aged 8 months to 18.8 years, were enrolled. They were initially divided in three groups of no DIC (n=27), non-overt DIC (n=14), and overt DIC (n=6). Positive hemoculture was reported within 24 hours among 15 patients (32%). The levels of both FVII:C and FVIIa of all patients were significantly low at onset of sepsis and gradually increased at 24 and 48 hours but were still persistently low in the overt DIC group. At 24 hours, the level of FVII:C was significantly lower in the non-overt DIC [57% (41-101)] and overt DIC groups [31% (28-49)] than that in the no DIC group [83% (70-102)] with p-values of 0.032 and 0.003, respectively. Similarly, the level of FVIIa was significantly lower in the overt DIC group [2.15% (0.86-3.96)] than the no DIC group [3.83% (2.90-5.46), p=0.031]. Using FVII:C < 65% or FVIIa < 3% at 24 hours to determine overt DIC at 24 hours, the sensitivity was both 90% and the specificity was 78.4% and 70.3%, respectively. Patients with low FVII:C and low FVIIa at 24 hours after onset of sepsis had a 16.9-fold (95%CI, 1.6-179.0, p=0.019) and 23.6-fold (95%CI, 1.9-290.7, p=0.014) chance of overt DIC at 24 hours.

Conclusions: Our study demonstrated a correlation of FVII:C and FVIIa and severity of DIC among pediatric patients with sepsis. These study results showed the role of FVII in DIC in which the low levels of FVII:C and FVIIa reflected the degree of consumption of the coagulation factor among pediatric patients with DIC.

PB 114 | imabalance of Factor VIII/ Protein C System as Major Determinant of Hypercoagulability in Cushing´s Syndrome

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Background: Cushing's Syndrome (CS) is associated with an increased tendency of thromboembolic events (VTE) and hypercoagulability plays an important role in increasing cardiovascular mortality and morbidity.

Aims: To evaluate hypercoagulability by measuring thrombingeneration (TG) with and without thrombomodulin (TM) in CS patients with active disease, according to the endocrine Society Guidelines, and with disease remission.

Methods: Fifty-seven patients with CS (42 pituitary-dependent, 12 adrenal-dependent, 3 ectopic adrenocorticotropic hormone secretion) and 50 healthy controls were included. 36 patients had an active disease, 21 had the criteria for biochemical assessment of remission. Thrombophilia screening (in particular factor (F)VIII and protein C (PC) coagulometric activity) and TG endogenous thrombin potential (ETP) with and without TM were measured.

Results: CS patients showed significantly higher levels of FVIII and PC activity than healthy controls (p< 0.0001); the ratio FVIII/PC was similar between cases and controls. CS showed a significantly higher ETP in the presence of TM than controls. However, the ETP ratio (with/without TM) was not significant higher in cases compared to healthy controls. Interestingly, patients with active CS had higher levels of PC (p=0,04), a lower FVIII/PC ratio and a lower ETP ratio compared to patients with CS remission (p=0,0011). Three CS patients had VTE but their parameters did not differ compared to CS without VTE.

Conclusions: In conclusion, we confirm a prothrombotic state in CS, mainly due to a partial resistance to the anticoagulant action mediated by TM. This could be explained by the more pronounced increased of FVIII plasma levels than PC levels (similar FVIII/PC ratio). Moreover patients with disease remission showed an increased hypercoagulability tendency with higher FVIII/PC and ETP ratio, related to a more "in-balanced" ratio between FVIII and PC compare to patients with active disease.

PB 115 | Anti-Hemostatic Compounds Isolated from Coral Snake *Micrurus tener tener* VENOM

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Background: There are anti-thrombotic and anti-fibrinolytic agents among the bioactive compounds that have been identified in snake venoms and invertebrates that have a therapeutic potential.
Aims: Isolate and characterize toxins in *Micrurus tener tener* venom *(Mtt)* with anti-hemostatic activity.
Methods: *Mtt* was initially fraccionated in a Superdex 200/HPLC column; elution was performed at 0.5 mL/min with 50 mM ammonium acetate, pH 6.9. In the obtained fractions, it was evaluated the functional activity on Factor Xa (FXa) using a coagulant assay and SBTI as positive control, and the activity on plasmin using a chromogenic assay (S-2251) and aprotinin as positive control. Fraction F10 inhibited FXa (anti-FXa) and fraction F17 inhibited plasmin (anti-plasmin). The purification of anti-FXa was followed through chromatography employing C4 and XDB-C18 columns, protein elution was performed at 1 mL/min with a 0-50% acetonitrile gradient in 0.12% TFA over 30 min. Anti-plasmin was isolated in a C-18 column protein elution was performed at 1 mL/min with a 0-50% acetonitrile gradient in 0.12% TFA over 30 min; the active fraction was re-chromatographed on the same column and the elution was performed using the same acetonitrile gradient over 60 min.
Results: Anti-FXa (1 µg/mL) inhibited 85 % of FXa activity after an incubation of 30 min at 37 °C. Anti-plasmin, Tenerplasminin-1 (1 nM), showed a molecular mass of 6542 Da; the amidolytic activity of plasmin (0.5 nM) on synthetic substrate S-2251 was inhibited by 91% as well as the fibrin(geno)lytic activity. Besides, tPA-clot lysis assay showed that Tenerplasminin-1 acted like aprotinin inducing a delay in lysis time and lysis rate. Finally, this molecule did not act on thrombin, FXa nor ADP induced platelet aggregation.
Conclusions: Both inhibitors are the first anti-FXa and anti-plasmin activities reported in *Micrurus* venoms that exhibit a high potential as anti-thrombolytic and anti-fibrinolytic agents, respectively.

PB 116 | Zinc Binds and Regulates the Activity of the Natural Anticoagulant Protein Z

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Background: Protein Z (PZ) is a natural anticoagulant which acts as a cofactor of protein Z dependent protease inhibitor (ZPI) in inhibiting fXa in presence of procoagulant Ca^{2+} and phospholipid membrane. PZ, bound to membrane, positions linked ZPI in close proximity of fXa, also bound to the same membrane. Presence of Ca^{2+} is required for PZ to bind to membrane and as such cofactor activity of PZ is regulated by Ca^{2+}. Multiple studies have reported the binding of bivalent metal ions other than Ca^{2+}, such as Mn^{2+}, Mg^{2+} and Zn^{2+}, to various coagulation proteins such as fVIIa, fXIa, fXa, APC, PS etc. These ions are also observed to modulate the activity of those clotting factors and anticoagulants, however, nothing is known whether those metal ions can bind to PZ and regulate its anticoagulant activity.
Aims: To study the binding of bivalent metal ions, namely Zn^{2+} and Mg^{2+}, to PZ and their effect on the anticoagulant activity of PZ.
Methods: We employed various biophysical techniques, coagulation assays and molecular modeling for the current study.
Results: Preliminary experiments revealed that both Zn^{2+} and Mg^{2+} bind to PZ but with disparate affinity. Binding K_d for Zn^{2+} was measured to be ~117 µM whereas that for Mg^{2+} was observed to be ~210 µM. Binding of Zn^{2+} was found to be pH dependent. It was interesting to notice that the affinity of Zn towards PZ was increased ($K_{d\sim}$65 µM) in presence of Ca^{2+} indicating a synergistic effect of Ca^{2+} on Zn binding. Similar effect of $Ca^{2+}$was not observed in case of Mg^{2+} binding. Zn^{2+} was also found to increase the affinity of PZ towards phospholipid in the absence of Ca^{2+}. PZ-ZPI mediated fXa inhibition was also observed to be regulated by Zn^{2+}. Finally, molecular modeling studies using the two crystal structures available for PZ suggested two putative Zn binding sites, His 206-His208-Glu226 and Cys 97-Cys 101-Cys 110 on PZ.
Conclusions: Collectively our observation suggests that Zn^{2+} binds PZ and regulates its anticoagulant activity.

PB 117 | Effects of SERPINC1, PROC, PROS1 and EPCR Polymorphisms on the Plasma Levels of Natural Anticoagulants in Healthy Individuals

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Background: Antithrombin (AT), protein C (PC) and protein S (PS) are natural anticoagulants and EPCR plays a role in PC activation. There are several polymorphisms (SNPs) that may influence their plasma levels. So far, controversial data have been published regarding their effects that is, at least in part, caused by gene-gene, gene-environment interactions in different populations.
Aims: Our aim was to investigate the effects of 12 SNPs (PROC rs1799809, rs1799808, rs1799810, rs2069928, rs1401296, PROCR rs867186, rs6088735, rs8119351, SERPINC1 rs222758, rs121909548, PROS1 rs8178649 and rs121918472) on the plasma levels of AT, PC and PS in healthy individuals.
Methods: A multiplex PCR-primer extension assay was developed to investigate the SNPs simultaneously in 366 healthy volunteers (median age 36; IQR 24; ratio of females 58.1%). AT activity was measured in heparin-cofactor (hc-AT) and progressive (p-AT) FXa-based assays, PC activity and free PS concentration were measured by chromogenic assay and latex-immunoturbidimetry, respectively.

Results: AT Cambridge (rs121909548) was not detected in our population. Mean hc-AT and p-AT activities were 97%(±8.9) és 106%(±10.5), respectively and rs2227589 was without effect on AT levels. Mean free PS concentration was 105±20.7 and as it was expected, PS Heerlen (rs121918472) decreased it markedly (105 vs 72%, p=0.045), while rs8178649 was without effect. Mean PC activity was 115%(±24.9) and among PROC promoter SNPs rs1799809 and rs1799810 decreased, while rs1799808 increased it significantly even after adjustments. PROCR rs867186 and rs8119351 significantly increased, rs6088735 decreased PC activity. Taken the combined effects of PROC and PROCR SNPs into consideration the lowest and highest PC levels showed more than 30% difference (p< 0.001).
Conclusions: PC levels are highly influenced by genetic factors. These observations may have clinical relevance from the point of view of thrombotic risk that is worthy of further investigations. (OTKA-K 116228)

PB 118 | Identification of a Novel Factor VII Activating Protease (FSAP) Zymogen-activating Peptide by Phage Display

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Background: Factor VII activating protease (FSAP) circulates as an inactive zymogen in blood. *In vivo*, zymogen activation is mediated in an auto-catalytic manner by charged macromolecules such as histones and nucleosomes. The currently known activators are non-specific in nature and specific inhibitors for FSAP have not been designed to date.
Aims: To identify specific peptide modulators of pro-FSAP activity using phage display.
Methods: We have used a phage display approach to identify peptides that bind to pro-FSAP. The effect on peptides pro-FSAP activity was determined and the interaction was further characterized at a molecular level.
Results: Peptides that bind specifically to pro-FSAP but not to other hemostasis factors were identified. One of the, 11 amino acids long cyclic, peptides could increase the activity of pro-FSAP but none had any inhibitory potential. Neither a linear form of the peptide nor scrambled peptide or truncated forms; with up to 3 amino acids deletions at the N- and C-terminus, showed any activity. Alanine scanning suggested that the entire peptide was important for this activity. The peptide was quite specific since other zymogens such as plasminogen and pro-urokinase were not activated. Binding studies with domain deletion mutants indicated that the N-terminal region of FSAP was the key interaction site for this peptide. Upon screening of a panel of monoclonal antibodies against FSAP we identified an antibody that could block the activation of pro-FSAP by this peptide. Overlapping peptide libraries were used to map the antibody and activating-peptide binding site at the N-terminus of FSAP. However, the peptide did not activate pro-FSAP zymogen in a complex biological fluid such as plasma.
Conclusions: We have identified a pro-FSAP activating peptide. Its inability to activate pro-FSAP in plasma may be due to a low affinity, to instability or to quenching by other binding protein(s). This peptide may serve as a lead compound to develop a therapeutic concept based on pro-FSAP activation.

PB 119 | Stability of Plasma for Add-on Coagulation Tests

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Background: Until now, most international and national guidelines recommend to evaluate most coagulation parameters within a 4 hour-delay after blood.
Aims: This study was carried out to determine at what time point an add-on routine coagulation test can be safely honored on specimens that have been received in the laboratory earlier.
Methods: For that purpose, we evaluated citrated (3.2%) blood samples obtained from 208 patients: 60 had normal coagulation profile, 73 were on vitamin K-antagonist, 60 were on heparin therapy (unfractionated heparin, UFH, n=19, or low molecular weight, n=41), and 15 had abnormal coagulation tests for miscellaneous reasons. Prothrombin time (PT)/INR, activated partial thromboplastin time (aPTT), fibrinogen, factors (F) V, VIII, and IX, antithrombin (AT), and D-dimer were measured immediately after being received in the laboratory less than 2 h after sampling (T0), and in the same capped tubes that had been left at room temperature for 3 h, 6 h, 12 h, and 24 h. All tests were not performed in each sample. Comparison were performed using Wilcoxon test and the bias calculated according to Bland-Altman.
Results: Changes in PT/INR, fibrinogen, FV, AT and D-dimer were not statistically significant up to 24 h, or analytical comparison were significant but with mean bias below the recommended values of Ricos et al. (1999) or the GEHT (2014). The same applied for aPTT, except for patients on UFH in whom a significant and clinically relevant shortening effect was observed after a 3 h-delay. FIX level was stable for up to 12 h, whereas FVIII level significantly declined after a 6 h-storage.
Conclusions: These results suggest that within an 24 h-period and with plasma on spun-down cells kept at room temperature, add-on tests for PT, aPTT (except in patients on UFH), fibrinogen, FV, AT and D-dimer could be performed without yielding results with clinically significant differences from those obtained on the original unstored plasma. The maximum delay would be of 12 h for FIX, and 6 h for FVIII.

PB 120 | Microplate-based Method for Quantifying Polyphosphate in Biological Samples

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Background: Inorganic polyphosphate (polyP) is highly procoagulant, proinflammatory and regulates the complement cascade. We hypothesize that polyP released from platelets, inflammatory cells, and/or infectious organisms is important in the pathophysiology of conditions such as sepsis and trauma. Available methods for quantifying polyP in complex biological samples (plasma, serum or cell lysates) are difficult and extremely laborious, severely limiting their use in clinical studies.
Aims: High-throughput assay for quantifying polyP in complex biologic fluids.

Methods: We developed an ELISA-style microplate-based assay that allows quantification of polyP in plasma, free from interference from polyP-binding proteins. This assay leverages the extremely high polyP binding affinity of the cationic polymer, polyethylenimine (PE). Plasma samples are incubated in PE-coated microplate wells in the presence of EDTA and a high salt concentration. Wells are then washed with high concentrations of salt and urea, leaving the captured polyP bound to the PE-coated surface and stripping off plasma proteins. A recombinant bacterial polyP-binding protein complexed with streptavidin-peroxidase is then bound to the captured polyP, after which the bound peroxidase is detected using a chromogenic substrate.

Results: The assay readily quantifies medium-chain and long-chain polyP (≥ 130-mer and longer) in plasma with a linear working range of 10-100 nM (measured in terms of phosphate monomer).

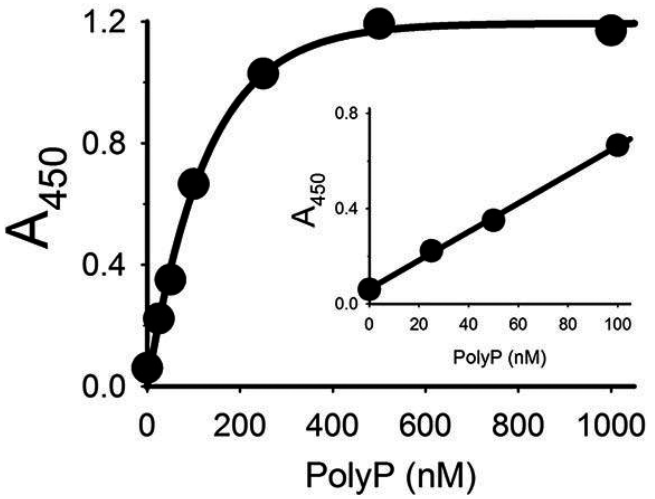

FIGURE Example of a standard curve

Conclusions: This assay may provide new insights into the roles of polyP in a variety of clinically important diseases.

PB 121 | Differential Investigation of Post-translational Modifications in Recombinant and Plasma-Derived Human Coagulation FIX

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Background: Idelvion, a long-acting albumin fusion protein linking recombinant coagulation factor IX with recombinant albumin (rIX-FP) is produced in CHO cell line and approved for the treatment and prophylaxis of patients with hemophilia B. The post-translational modifications (PTMs) of recombinant products are strongly dependent on the cell line used for manufacture as well as on the manufacturing process itself. Alterations in PTM's can influence the biological activity and the pharmacokinetic profile of the biopharmaceutical.

Aims: To fully characterize the PTM's (with focus on N- and O-glycosylations) of Idelvion in comparison to commercially available plasma derived and rFIX.

Methods: PTM's located on the activation peptide as well as the O-glycans and the Gla domain in the light chain (LC) were characterized using a novel middle-down high resolution LC-MS method after activation by hFXIa. The released and fluorescent labeled N-Glycans were identified and quantified using in-line HPLC-FLD-MS.

Results: The degree of gamma carboxylation was confirmed, and O-linked glycans on the light chain were characterized. The PTMs on the activation peptide (AP) of rIX-FP were compared to those in plasma and recombinant FIX. The AP of the rIX-FP is highly similar to that of recombinant FIX, and similar N-Glycan structures are also found in plasma-derived FIX. The N-linked glycoforms of rIX-FP are multi-antennary complex-type glycans, including sialylated and partially sulfated forms.

Conclusions: A middle-down HPLC-MS method using high resolution mass spectrometry was developed in order to characterize the intact AP, LC, the heavy chain (HC) and the albumin domain (HSALink) of Idelvion after activation with hFXIa. The middle-down LC-MS method revealed high similarity in the PTM's of Idelvion compared with plasma derived FIX (Mononine™). The accurate mass measurement of the intact fully modified AP of Idelvion confirms a very complex N-glycosylation pattern suggesting that both N-glycosylation sites are occupied.

PB 122 | Clinical Significance of Prothrombin G20210A Mutation in Homozygous Patients

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Background: Prothrombin G20210A mutation (PTM) is associated with an increased risk for venous thromboembolism (VTE); however, data regarding clinical implications of this mutation are scarce, particularly in homozygous carriers.

Aims: This study aimed to assess significance of PTM as a risk factor for VTE, cardiovascular (CV) disease, obstetric complications and neuropsychiatric symptoms in patient population.

Methods: The Rambam institutional database was retrospectively searched for patients evaluated for PTM between the years 1996-2015. Clinical data of PTM carriers and non-carriers were compared.

Results: Two hundred and thirty nine patients (30 homozygous, 95 heterozygous, 114 non-carriers) were included in this analysis. VTE events were diagnosed in 50% of homozygous [odds ratio (OR) 5 (95% CI 2.1-11.92)], 27.4% of heterozygous carriers [OR 1.88 (95% CI 0.9-3.67)], and 16.7% of non-carriers. VTE rates were significantly higher in homozygous individuals than in non-carriers without severe VTE risk factors [OR 8.17 (95% CI 2.71-24.62)]. VTE recurrence rate after discontinuing anticoagulant therapy was high in carriers (20% homozygous, 11% heterozygous, 4% non-carriers; P=0.006). Of 163 women of reproductive age, 148 had obstetric complications; their prevalence was highest among homozygous women [66.7% vs. 23.7% in heterozygous, 21.9%

in non-carriers; P=0.001; OR of 7.14 (95% CI 2.27-22.45)]. Percentage of neuropsychiatric symptoms was higher in homozygous (34%) than in heterozygous (14%) individuals or non-carriers (11%; P=0.007).

Conclusions: PTM carriers, particularly homozygous individuals, are at an increased risk for VTE and its recurrence compared to non-carriers. Obstetric complications are more frequent in homozygous women. An association between PTM and neuropsychiatric symptoms is suggested.

PB 123 | Impact of Reagents on FVIII Inhibitor Titration

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Background: Reliable measurement of FVIII inhibitor titre is critical in the follow up of haemophilia A patients, but a wide heterogeneity in reagents used for this procedure remains and is likely a source of variability in the results obtained.

Aims: In order to study the impact of buffers and source of FVIII used during the assay, we performed a multicentre study involving 27 laboratories.

Methods: One normal plasma sample without FVIII inhibitor (NP) and another sample containing low FVIII inhibitor titre (< 2 BU) (PP) were studied. 4 aliquots of each were sent to 27 laboratories and tested undiluted and diluted (1:1) using 4 different procedures: Pro A = ‚home made procedure' with variable reagents from one centre to another; Pro B, using Imidazol buffer (ImB) for samples dilution and the preparation of control mixture; Pro C, using ImB (as in Pro B) plus non-buffered normal pool plasma; Pro D, using ImB (as in Pro B) plus buffered normal pool plasma.

Results: Interestingly, no false positive or negative result was found when labs used "home-made" procedure (Table 1).

Moreover, lower FVIII residual activity in NP and higher FVIII inhibitor level in PP were measured when non-buffered normal pool plasma was used (Pro C, Figure 1), with more false positive results (Table 1).

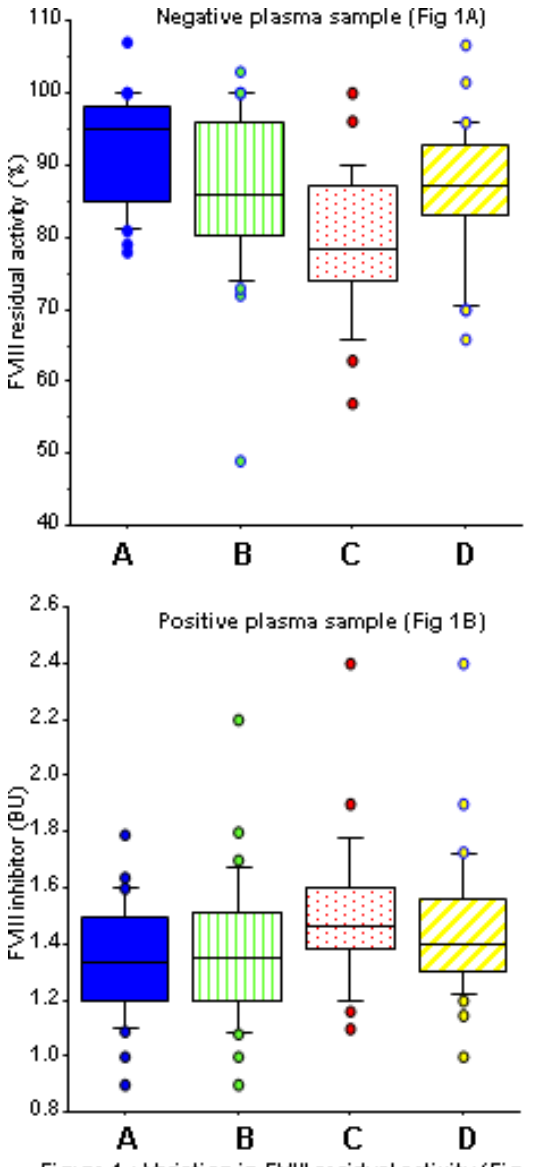

Figure 1 : Variation in FVIII residual activity (Fig 1A, negative plasma sample) and FVIII inhibitor titer (Fig 1B, positive plasma sample) according to the procedures.

FIGURE 1

TABLE 1 FVIII residual activity (%) and FVIII inhibitor (BU) in negative and positive samples

	Negative plasma sample (A)	Negative plasma sample (B)	Negative plasma sample (C)	Negative plasma sample (D)		Positive plasma sample (A)	Positive plasma sample (B)	Positive plasma sample (C)	Positive plasma sample (D)
FVIII residual activity Mean (%)	92	86	79	86	Mean FVIII inhibitor (BU)	1.34	1.37	1.50	1.46
Range (%)	[78 - 107]	[49 - 103]	[57 - 100]	[66 - 106]	Range (BU)	[0.9-1.8]	[0.9-2.2]	[1.2-2.4]	[1.0-2.4]
CV (%)	8.2	13.4	12.5	10.8	CV (%)	15.5	19.7	17.7	18.2
False positive results (> 0.6 BU)	0/27	0/27	3/27	1/27	False negative results (< 0.6 BU)	0/27	0/27	0/27	0/27
False positive results (> 0.4 BU)	0/27	2/27	8/27	4/27	False negative results (< 0.4 BU)	0/27	0/27	0/27	0/27

12 of 27 laboratories used ImB in Pro A and they measured higher FVIII residual activity in control mixture (48 %) compared to non-users of ImB (43%). Additional experiments showed that FVIII was less stable after 2 hours in control mixtures prepared with Owren Koller buffer (Stago) or Diluant factor (Werfen), than with ImB, hepes buffer or FVIII-DP (FVIII degradation < 10%). As expected, FVIII stability in test mixtures was also better with standard buffered plasma than with un-buffered or frozen-buffered plasma pools.

Conclusions: This study supports that the use of Imidazol buffer and standard buffered plasma improves the measurement of FVIII inhibitor titre.

PB 124 | Evaluation of endogenous thrombin potential among patients with antithrombin deficiency-Serbian AT deficiency study group results

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Background: Inherited antithrombin (AT) deficiency is a rare autosomal dominant disorder, caused by mutation in *SERPINC1*. It is classified into two types, where type I is a quantitative disorder characterized by both decreased antigen and activity, mostly associated with an increased risk of venous thromboembolism. While type II is a functional disorder classified into three subtypes according to the site of the causative mutation. Unlike other forms of AT deficiency Type II HBS represents a minor thrombotic risk in its heterozygous form, while homozygous type II HBS AT deficiency is characterized by early onset of arterial and venous thrombosis, as well as pregnancy related complications.

Aims: The study was conducted in order to evaluate endogenous thrombin potential (ETP) among patients with AT deficiency with regard to the type of AT deficiency.

Methods: 24 Serbian families with AT deficiency; total 65 participants were included in the study. Genotyping using Sanger fluorescent sequencing method and determination of ETP for all participants were performed. With regard to the genetic results the participants were divided in three groups: Type I deficiency (20), Type II HBS (31) and family members without mutation, as a control group (14).

Results: ETP level expressed in AUC (%) with median value of 110 (IQR 27.0) was observed in Type I; in the group with Type II HBS, the median value of 102 (IQR 18.5) was obtained; P=0.96. Among non-carriers an ETP level with median of 85 (IQ 16.5) was observed, that was significantly lower compared to the carriers of AT deficiency, P=0.001; P< 0.001.

Conclusions: The carriers of AT deficiency had significantly higher ETP in comparison with the non-carriers. With regard to the type of AT deficiency, the difference of ETP between Type I and Type II HBS was not observed.

PB 125 | Anti-FVIII IgG1 Antibody in Previously Untreated Patients with Hemophilia A Might Be Protective Against Inhibitor Development: Preliminary Results from the Hemfil Cohort Study

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Background: The development of FVIII inhibitors is the most fearful complication in patients with hemophilia A (HA). Some studies have reported that IgG1 and IgG4 are the predominant subtypes of antibodies in these patients.

Aims: To investigate the presence of anti-FVIII IgG1 antibodies in plasma samples of severe and moderately-severe (< 2%) previously untreated patients (PUPs) with HA (T0) and their relation with inhibitor development.

Methods: This cross-sectional study, derived from the HEMFIL Cohort Study, included 26 samples from PUPs with HA. An ELISA was performed to detect specific anti-FVIII IgG1. Samples were serially diluted from 1:10 to 1:640 and a numerical score was assigned based on the positivity at each sample dilution (i.e. score=1 for positive sample at 1:10; score=7 for positive sample at 1:640). A pool of five plasma samples from non-hemophilic individuals was used as negative control. Samples from HA patients were considered positive if the optical density was higher than the result of negative control plus two standard deviations. Fisher´s exact test was used for statistical analysis.

Results: A total of 13/26 (50%) and 13/26 (50%) developed inhibitor and did not after 75 exposure days (ED), respectively. At T0, 18 samples (69.2%) were considered positive for anti-FVIII IgG1 of which 5 (19.2%) were high titer (≥1:160). A total of 3/5 and 2/5 developed inhibitor and did not, respectively. A total of 7 out of 8 patients (87.5%) without anti-FVIII IgG1 at T0 developed inhibitors. Otherwise, 12/13 patients (92.3%) who reached 75 ED without inhibitors presented anti-FVIII IgG1 at T0 (p=0.01).

Conclusions: The presence of anti-FVIII IgG1 antibody in PUPs with HA before starting FVIII replacement was associated with lower incidence of inhibitor. We hypothesize that anti-FVIII IgG1 antibody might be protec for inhibitor development. Evaluation of a larger cohort with adjustment for confoundings will be required for more conclusive results. Financial support: FAPEMIG; CAPES; Fundo Nacional de Saúde.

PB 126 | Cost-effectiveness of Recombinant Porcine Factor VIII (OBIZUR) for Treatment of Hemorrhagic Events in Acquired Hemophilia A (AHA)

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Background: A clinical trial showed that recombinant porcine Factor VIII (rpFVIII) is a highly effective and safe option for treating serious hemorrhage in AHA.

Aims: To estimate the cost-effectiveness of rpFVIII relative to recombinant activated FVII (rFVIIa) and Anti-Inhibitor Coagulant Complex (AICC).

Methods: We developed a patient-level simulation model to compare clinical events, and costs of the 3 treatment strategies in AHA patients. The model included medication use , hospitalizations, thrombotic events, recurrent bleeds, and product switching. Patient characteristics and model parameters were based on data from the rpFVIII trial, the EACH2 registry (rFVIIa and AICC) and input from a hemophilia expert panel. All product costs were based on US ASP prices (rpFVIII: $4.53/U; rFVIIa: $1.90/mcg and AICC: $1.92/U). We conducted one-way and probabilistic sensitivity analyses to assess model robustness.

Results: Model simulation results showed bleeds were controlled in 90% (95% CI: 79-98%), 90% (95% CI: 83-96%), and 91% (95%CI: 83-97%) of patients who initiated treatment with rpFVIII, rFVIIa, and AICC, respectively. Estimated discounted lifetime costs were $672,000, $264,000, and $134,000 and quality adjusted life years (QALYs) were 6.56 (95%CI: 5.38 - 7.63), 6.77 (95%CI: 6.07 - 7.48), and 6.69 (95%CI: 5.91 - 7.50) for rpFVIII, rFVIIa, and AICC, respectively. Expected QALYs for rpFVIII increased to 6.92 (95%CI: 5.90 - 7.75) when we assumed deaths rates equal to the rates observed for rFVIIa.

Conclusions: Given comparable efficacy, AICC showed the lowest cost per treated bleed. rpFVIII becomes comparable to rFVIIa if drug utilization and price are both reduced by 50% and 33%, respectively. However, results should be interpreted with caution as sources from which input data were drawn may not be comparable due to differences in methodology, and possible differences in AHA severity and related co-morbidities. More robust comparisons may be achievable with the availability of observational data for rpFVIII.

PB 128 | APC-Resistance Testing Based on Genetic or Functional Assays: To Be or Not to Be ?

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Background: APC-resistance (APCR) was initially detected through functional assays and subsequent identification of the Factor V Leiden (FVL, R506Q) mutation. Several conditions for acquired APCR but also other mutations in FV leading to APCR have been identified. Today, however, many laboratories perform only DNA tests for R506Q when suspecting APCR.

Aims: To investigate the impact of functional versus genetic tests considering other polymorphisms than FVL inducing APCR.

Methods: Literature search on genetic and functional assays for APCR either based on modified aPTT or snake venom. The latter does neither require Ca^{2+} nor phospholipids, tolerates an incorrect blood to citrate ratio (sample tube under-filling), shows no interference by lupus anticoagulants (Schöni, 2007) and is quite robust against the effect of other mutations than FVL which can lead to APCR (Table 1).

Results: The data confirm the widespread presence of FV mutations leading to APCR and thromboembolic (TE) sequelae, including in

TABLE 1

Name	Mutation	APCR	Clinical Consequences	Reference
FV Bonn	A512V	+	Venous thrombosis, recurrent abortions	Pezeshkpoor et al. J Thromb Haemost. 2016
FV Nara	W1920R	+	Serious DVT, Reduced levels of FV (~10%)	Nogami et al Blood. 2014
FV Cambridge	T306R	+	Venous thrombosis	Williamson et al. Blood. 1998
FV Hong Kong	D666Q	+	Venous thromboembolism	Cai et al. Thromb Res. 2010
FV Liverpool	I359T	+	Spontaneous venous thrombosis. Low APC cofactor activity for inactivation of FVIIIa	Mumford AD, et al. Br J Haematol. 2003 Steen M, et al. Blood. 2004
Not named	E666D	+	Familial history of strong venous thrombosis	Xin-Guang C, et al. Clin Appl Thromb Hemost. 2015 Sharma A, et al. Clin Appl Thromb Haemostas 2015
Not named	R485K	(+)	Thrombosis Praeeclampsia Increased risk for CAD	Hiyoshi M, et al. Thromb Haemost. 1998 Watanabe H, et al. Thromb Haemost. 2001 Faisel F et al. Eur J Hum Genet. 2004 Le W, et al. Clin Genet. 2000
HR2 haplotype FV(R2)	H1299R and other poly-morphisms	+ (mild)	HR2 haplotype FV(R2). Reduced APC cofactor activity of FV(R2). Lower levels of FV, less efficient inactivation of FVIIIa	Luddington R, et al. Thromb Haemost. 2000 Bernardi F, et al. Blood. 1997 Faioni EM, et al. Blood. 1999

ethnic groups like in indigenous populations of Africa, America, East Asia, and Australia in which FVL is virtually absent. Also constellations such as pseudo-homozygosity or the FV Graz mutation (Prüller, 2013) that may compensate for FVL and its impact on APCR should be kept in mind.

Conclusions: Genetic tests for the R506Q polymorphism may miss other causes of APCR associated with TE episodes, or on the contrary, like in the case of FV Graz constellation, may predict APCR though functional tests are normal. DNA sequencing identifies FV mutations but cannot reliably predict its impact on APCR. Functional testing with robust assays seems to be a more suitable and cost effective (Prüller et al, 2014) first line strategy to determine APCR before using DNA based methods.

PB 129 | Alteration of the Hemostatic System in Cirrothic Patients

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Background: Cirrothic patients present a rebalance hemostatic system as a consequence of the liver synthetic capacity.

Aims: The aim of this work is to study whether procoagulant factors, physiological inhibitors and D-dimer are related to the severity of liver disease in cirrothic patients, assessed by the MELD score.

Methods: Population: 110 consecutive patients with liver cirrhosis who attend the hemostasis laboratory as part of the initial pre-liver transplant evaluation. Cirrhosis was diagnosed by a combination of clinical, laboratory and imaging findings. Severity was assessed through the MELD score.

Control group: 100 healthy individuals with equal% of blood group zero and non-zero and ages similar to the group under study.

Methodology: Factor levels by coagulable method in one stage; Antithrombin (AT), protein C (PC) and plasminogen (PLG) by chromogenic method ; free PS and DD by immunoturbidimetry. All test have been performed on ACL TOP platform with dedicated reagents (HemosiL).

Results: 110 pts were evaluated with hepatic cirrhosis (28 females, age between 19-71 years), MELD:16 (6-33),mean and rank. During the 23-month follow-up, 19 pts were transplanted and 9 presented a thromboembolic event. All parameters were significantly different from the control group (p< 0.001,see Table). Although AT and PC descend with the severity of the disease, the free PS presents a random behavior. The correlation between FVIII / PC vs MELD (r = 0.50) and FVIII / AT vs MELD (r = 0.49) is moderate but higher than the correlation between INR and MELD. Hemostatic parameters of 101 pts who didn´t have a thromboembolic events were not significantly different from the 9 patients who presented a thrombotic event during the follow up.

Conclusions: The cirrhotic patients have a hypercoagulable state, demonstrated by the elevated values of DD and the increase of the FVIII / PC and FVIII / AT ratio. These hypercoagulable parameters correlates moderately with the level of liver damage assessed through MELD score but not with DD level.

TABLE Hemostatic parameters in cirrothic patients

	Cirrothic pts median and rank	Control Group median y rank
FBG (g/L)	2.03 (6.10-5.81)	3.24 (2.01-4.15)
FV (%)	55 (14-179)	92 (86-125)
FVII (%)	44 (1-174)	98 (78-116)
FVIII (%)	193 (71-499)	89 (56-156)
AT(%); FVIII/AT ratio	42 (7-143); 4.36 (0.82-32.70)	101 (93-105); 1.02 (090-1.23)
PC (%); FVIII/PC ratio	36 (5-132); 5.35 (0.90-48.49)	105 (92-132); 0.98 (0.75-1.85)
PSfree(%); FVIII/PS ratio	78 (20-167); 2.58 (1.01-25)	84 (69-125); 1.20 (0.67-1.99)
PLG (%)	55 (38-125)	97 (85-120)
D Dimer (ngDD/ml)	1017 (42-4726)	96 (42-256)

PB 130 | In vitro Antithrombin is the Main Inhibitor of Thrombin Generation at the Surface of Endothelial Cells Acting Independently to Heparan Sulfates

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Background: We characterized the antithrombin (AT) binding *in vitro* to living endothelial cells (HUVEC) and the potency of AT on thrombin generation assay (TGA) under supraphysiological conditions (1.0 to 2.0 U/ml; Catieau et al. Abstract 4168, ASH 2015 annual meeting).

Aims: To further depict the participation of the endothelial surface in the different anticoagulation processes and, specifically its modulation of the antithrombin potency at low doses.

Methods: HUVEC were grown in a complete EGM2 medium (Lonza) for no more than 6 passages. Cells were grown to confluence, washed with non-supplemented medium and treated or not with a mix of heparanase I, II and III (2U/ml, 2U/ml and 3U/ml respectively, Sigma) on EBM2 +/- neutralizing anti-EPCR (0.1 µg/well - Millipore). TGA, following 0.5 pM of TF/2 µM of phospholipids induction, was performed at the surface of HUVEC in AT-deficient plasma spiked with various amounts of AT (0, 0.1; 0.5 and 1 U/ml).
Results: :When the concentration of AT was increased in AT-deficient plasma, the profile of the TGA changed in function of the dose. All lag-times were similar suggesting that the Tissue Factor Pathway inhibitor inhibition was not affected in the conditions assayed. However, when no AT was spiked there was no decrease of the signal following the peak but it progressively decreases in function of the dose of AT spiked. In all conditions, the combing of heparin sulfate did not modify the efficiency of the TGA confirming our previous data. To distinguish the anticoagulation coming from AT to those from the protein C pathway, an anti-EPCR antibody was added. The addition of such antibody did not change the profile of the TGA at the different AT concentrations suggesting a minor role for the protein C pathway in comparison to AT.
Conclusions: The TGA at the surface of the HUVEC *in vitro* allowed identifying the AT as the major anticoagulant pathway working independently to heparan sulfates.

PB 131 | Pregnancy and Delivery Experiences in a Patient with Severe Factor X (FX) Deficiency Treated with a High-purity Plasma-derived Factor X (pdFX) Concentrate

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Background: A female, born in 1997, was diagnosed at our center in 2001 with a basal FX:C of 3% of normal due to a homozygous missense mutation in the *F10* gene, p.Gly20Arg/c.61G>A. She had an extensive history of spontaneous and traumatic bleeds resulting in synovitis and arthrosis in her left knee and arthrosis in both ankles. She also suffered from menorrhagia (routinely treated with antifibrinolytics). The experiences of this patient with pdFX for on-demand/prophylactic treatment in a phase 3 clinical trial have been presented.
Aims: To present pregnancy and delivery experiences for this patient.
Methods: Since completing a phase 3 clinical trial in 2012, the patient has received 1500 IU pdFX (≈23 IU/kg) twice weekly under a compassionate-use program.
Results: At 17 years of age, the patient reported a spontaneous abortion at 6wk + 3d (considered by reporter as very unlikely to be pdFX related) and underwent curettage. Three subsequent spontaneous abortions were reported but unconfirmed by gynecologic examination. The patient reported another pregnancy < 2 years after the first spontaneous abortion. The frequency of pdFX treatment (1500 IU) was increased from 2 to 3 times/wk to prevent nose bleeds and protect the pregnancy. Spontaneous labor commenced at 39wk + 5d (2d following a routine pdFX dose). An additional 1500 IU pdFX dose was administered for the delivery, which required no assistance. No bleeding complications occurred during delivery of baby or placenta, placenta delivery was not delayed, and postpartum bleeding was not excessive (although lochia was increased compared with a patient without a bleeding disorder). The baby was healthy with no reported bleeding diathesis. Three weeks after delivery, the patient resumed twice-weekly routine prophylaxis.
Conclusions: These results show that pdFX was safe and effective in maintaining hemostasis during pregnancy and obstetric delivery in this patient with severe factor X deficiency.
Funding: Bio Products Laboratory

PB 132 | Impact of Thrombin (Dabigatran) and FXa Inhibitors (Rivaroxaban, Apixaban, Edoxaban) on Hemostasis Assays

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Background: Direct Thrombin (Dabigatran [DAB]) and FXa (Apixaban [APIX], Rivaroxiban [RIV]), Edoxaban [EDOX]) inhibitors are direct oral anticoagulants (DOACS) for use in clinical practice as an alternative to the vitamin K antagonists (VKA) for the prevention of thrombotic events that do not require routine laboratory monitoring.
Aims: The impact of the DOACS on IL Hemostasis primary diagnostic assays on the ACL TOP was evaluated.
Methods: RIV, APIX, EDOX, and DAB, were spiked into normal pooled plasma (NPP) in the range of 0-1000 ng/mL. PTs were acquired using HemosIL RecombiPlasTin 2G (RPT 2G), ReadiPlasTin (RdPTn), PT HS Plus, and PT Fibrinogen (PT-Fib), while APTTs were determined using HemosIL SynthASil (SSL), APTT-SP, and SynthAFax (SFX). Results are in ratio for interference (1.25), peak plasma levels (C_{max}≈200 ng/mL).
Results: RIV/APIX/EDOX induced a linear, reagent concentration-dependent prolongation of the PT/APTT. DAB exhibited a similar but curvelinear response with a lag-time for PT. RIV impacted the PT with ratio ≈1.7 and APTT ≈1.4 at the C_{max}. It was sensitive at ≈74ng/mL and ≈125 ng/mL, with 2x CT (clot time) of 300-400 ng/mL and ≥ 650 ng/mL for PT and APTT, respectively. DAB impacted the APTT with ratio ≈2 at the C_{max} with sensitivity at ≈ 25 ng/mL and 2x CT at 150-200 ng/mL. It impacted the PT at > 350 ng/mL with 2x CT at ≥ 650 ng/mL. APIX was sensitive at > 200 ng/mL for PT and > 500 ng/mL for APTT, with 2x CT at ≥ 650 ng/mL PT and ≥ 1000 ng/mL APTT. EDOX impacted the PT with ratio ≈1.6 and APTT ≈1.4 at the C_{max}. It was sensitive at ≈75 ng/mL and ≈100 ng/mL, with 2x CT of ≈300 ng/mL and ≈650 ng/mL for PT and APTT, respectively.
Conclusions: The PT is more sensitive to RIV and EDOX (≈75 ng/mL) than APIX (> 200 ng/mL), and insensitive to DAB (>350 ng/mL). The APTT is more sensitive to DAB (≈25 ng/mL) than EDOX (≈100 ng/mL)

or RIV (125 ng/mL), and insensitive to APIX (> 500 ng/mL). The greatest sensitivity was seen with SFX to the DOACS; RdPTn and RPT 2G to RIV, APIX, and DAB; and PT HS Plus to EDOX.

PB 133 | Role of Factor-Xa Inhibitor Treatment on Platelet Function and Thrombus Formation

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Background: Treatment with factor Xa inhibitor (FXAI) rivaroxaban associates with a slightly decreased number of acute coronary syndromes (ACS). This is in contrast to oral thrombin inhibitor treatment, which n associates with an increased number of myocardial infarction, likely due to a pro-thrombotic effect on platelet function and thrombus formation

Aims: Our study aims to investigate whether the clinical observed reduced frequency of ACS under rivaroxaban is due to a play of chance or linked to so far undefined underlying mechanism. We therefore asked whether rivaroxaban treatment affects platelet function under static or flow conditions.

Methods: We analyzed platelet function from patients receiving either rivaroxaban (20mg once daily) or dose adapted VKA (vitamin-K antagonist) for the prevention of stroke due to atrial fibrillation. Whole blood aggregation was determined by impedance-based aggregometry after stimulation with ADP, collagen, TRAP and ristocetin. Next we determined single platelet activation and reactivity by measuring p-selectine surface expression upon stimulation with ADP, thrombin and convulxin. Further we investigated platelet adhesion and thrombus under arterial flow conditions on vWF- and collagen-coated flow chambers. Arterial thrombus formation was analyzed in a carotid injury model in FXaI and VKA treated mice.

Results: Our data did not reveal any significant differences on platelet function under static and flow conditions in FXaI or VKA treated blood. In-vivo we found a trend towards a decreased arterial thrombus formation under rivaroxaban compared to VKA treatment.

Conclusions: Within our study we did not find any evidence for an antithrombotic effect of rivaroxaban on platelet function. However future studies are required to investigate the complex interplay between platelets and the coagulation system in more detail.

PB 134 | Prolonged Targeted Temperature Management Causes an Impaired Coagulation: A Randomised Clinical Trial

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Background: Mild hypothermia *per se* is presumed to impair coagulation. However, no studies have previously investigated whether hypothermia beyond 24 hours impair the coagulation even further.

Aims: Investigate whether prolonged duration of targeted temperature management (TTM) impair the clot formation compared with standard duration.

Methods: Resuscitated comatose out-of-hospital cardiac arrest patients (n=82) were randomized to standard (24 hours, n=42) or prolonged (48 hours, n=40) duration of TTM at 33±1°C. Blood samples were collected 22±2, 46±2, and 70±2 hours after obtaining target temperature and were analysed by ROTEM® using EXTEM®, INTEM® and FIBTEM® and for thrombin generation using the Calibrated Automated Thrombogram®Assay. Additionally, standard laboratory investigations were analysed. Informed content was obtained and the regional research ethic committee approved the study.

Results: At the 22-hour sample; ROTEM® and thrombin generation yielded no differences between standard and prolonged treatment except for a minor increase in time to maximum velocity (EXTEM® and INTEM®) in the prolonged group.

At the 46-hour sample; Using ROTEM® we found a 11% longer INTEM® clotting time (p=0.005) and 12% longer time to maximum velocity (p=0.007) in the prolonged group compared with the standard group. Thrombin generation analyses showed a 411 nM*minute decreased endogenous thrombin potential

(p< 0.001), a 30% longer lag time (p=0.04), a 106 nM decreased peak concentration (p< 0.001), a 36% longer time to peak (p=0.01) in the prolonged group compared with the standard group.

At the 70-hour sample; No differences were found in ROTEM® analyses between groups, however, the thrombin generation showed that there remained a longer lag time, a decreased peak and time to peak (all p-values ≤0.02) in the prolonged group compared with the standard group.

Conclusions: Clot formation and thrombin generation were impaired in prolonged TTM compared with standard treatment.

PB 135 | Features of Clinical and Laboratory Diagnostics of Disturbance of Hemostasis System in Patients with Retinal Vein Occlusion on the Background of FV Leiden Mutation and LA

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Background: The number of patients with retinal venous occlusions is increasing, especially among young people. Often, they have revealed a genetic predisposition to thrombosis. Risk factors for thrombosis are

genetic resistance to activated protein C (RAPC), genetic defect in factor V (FV Leiden) and the presence of lupus anticoagulant (LA).

Aims: Explore the features of laboratory and clinical disturbances in the hemostatic system in patients with OVS on the background of FV Leiden mutation and LA.

Methods: A total of 150 patients (150 eyes) with RVO (mean age — 42 ± 10 years) were examined and divided into three groups. Group 1: patients with RVO, FV Leiden and LA (n = 12); group 2: patients with RVO and FV Leiden (n = 11) without LA; group 3: patients with RVO without FV Leiden and LA, selected from remaining 107 people for a comparable number of groups (n = 30). The control group was 50 people without RVO, but with hypertension.

Results: . It was shown that RAPC index in patients with FV Leiden mutation and the LA has the less value (0,6 ± 0,01) on comparison to patients with RVO (1,50 ±0,18) ($p < 0,05$). They also have enhanced V, VIII and von Willebrand factors and intravascular platelet activity. LA exacerbates endotheliosis in the microvasculature of the retina and in combination with FV Leiden mutation increases the thrombogenesis, participating in the pathogenesis of ischemic thrombosis of central retinal vein and its branches, which clinically manifested as retinal thrombo-hemorrhagic syndrome.

Conclusions: The hemostasis regulation genes polymorphisms detection (as well as lupus anticoagulant detection) is recommended to clarify the diagnosis and selection of adequate therapy.

PB 136 | Causes of Isolated Prolonged Activated Partial Thromboplastin Time in a Haemostasis Reference Hospital

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Background: The activated partial thromboplastin time (aPTT) measures the time it takes plasma to clot when exposed to substances that activate the intrinsic and common pathways of coagulation.

Some patients have unexplained prolonged aPTT. Causes of a single prolonged aPTT included: Hemophilia A or B, inherited factor deficiencies (XII, XI, X, V), acquired factor inhibitors and lupus anticoagulant inhibitors.

Aims: To determine the causes of isolated prolonged aPTT (>1.3 RATIO) in a Haemostasis reference hospital.

Methods: We performed a retrospective study of 224 consecutive patients with isolated prolonged aPTT presenting to our hospital during 2016. All patients had normal prothrombin time and thrombin time. For all patients a standard panel of tests was performed. First, we detected lupus anticoagulant (LA) using two different sensitive tests: Dilute Russell Viper Venom Time (DRVVT) and Silica Clotting time (SCT). If the result was negative, we performed an inhibitor assay. If the sample had not an inhibitor we measured coagulant factors VIII, IX, XI and XII, which are involved in the intrinsic arm of haemostasis.

Results: The most common cause of an isolated aPTT in our study was the presence of LA (81.3%). In the 182 cases of LA positivity, 108 (59.3%) samples were positive for DRVVT and SCT tests, 34 (18.7%) for DRVVT and 40 (22%) for SCT (fig.1).

Fig. 1

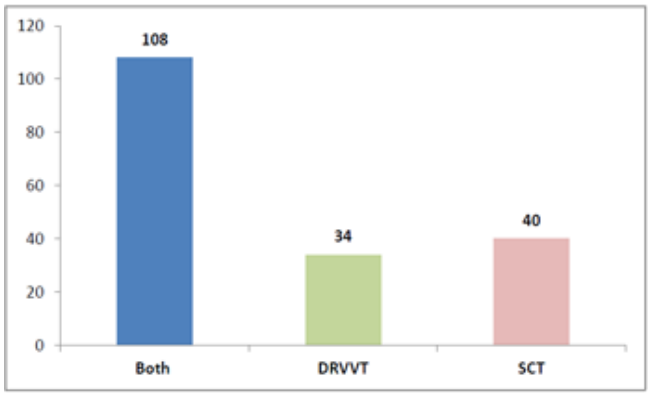

FIGURE 1

The inhibitor assay was positive in 5 cases (2.2%) due to a presence of monoclonal gammapathy in the plasma. Prolonged aPTT due to a factor deficiency was in 37 known cases (16.5%): 24 A haemophilia A, 2 haemophilia B, 6 factor XI deficiencies and 5 factor XII deficiencies (fig. 2).

Fig. 2

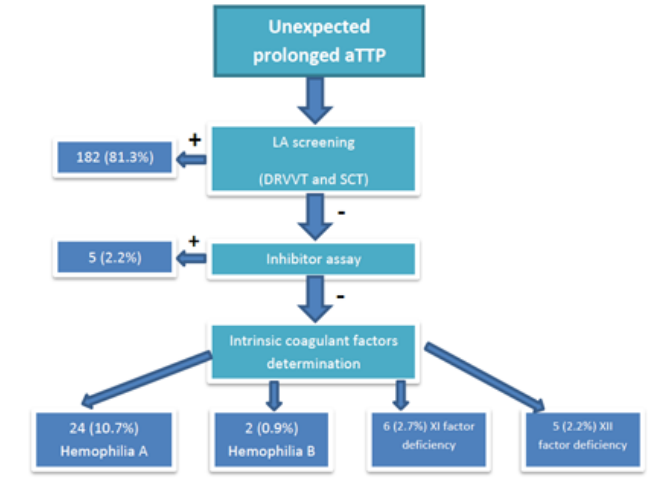

FIGURE 2

Conclusions:

- Our study suggests that most of the causes of isolated prolonged aPTT may signify an underlying thrombophilic condition.
- Our data suggest that dRVVT and SCT are sensitive tests for detecting LA.
- Using a standard test panel for screening prolonged aTTP allow for studying the patients underlying pathologic conditions and optimize the sample.

PB 137 | Evaluation of Lupus Anticoagulant and Anti Cardiolipin Antibodies in Poly Transfused Beta Thalassemia Major Patients at Hyderabad

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Background: Beta thalassemia is a congenital hemolytic disease caused by defective globin synthesis resulting in decreased quantity of globin chain. The disease is widespread in the subcontinent regions. The repeated blood transfusions received by anti-Phospholipid antibody positive patients is higher than anti Phospholipid antibody negative lupus anti-coagulant was detected in 16% of patients. There is increased need of blood transfusion in beta-thalassemia major patients those develop anti-phospholipid antibodies.

Aims: Aim of this study is to determine the frequency of anti-phospholipid antibodies in transfusion dependent Beta thalassemia major patients.

Methods: This cross-sectional study was conducted at department of pathology and transfusion services, at Liaquat University of Medical & Health Sciences, Jamshoro.It was carried out from October 2012 to July 2013 after approval of IRB and signing of written consent of patients.A total of 121 patients who fulfilling the inclusion criteria at pediatric department LUMHS and thalassemia centers in Hyderabad were selected.APTT will be done on citrated plasma on CA 500 automated coagulation analyzer by sysmex anti-phospholipid antibody which include Lupus anticoagulant and Anticardiolipin antibodies will be performed by commercially available kits by EIA.

Results: Out of 121 patients 38(31.40%) patients were found to be anti-phospholipid positive and 83(68.60%) were negative. Lupus anticoagulant were present in 20 (16.53%) and Cardiolipin Antibody were present in 11(9.09%) patients.

Conclusions: Anti-phospholipid is common in patients with beta thalassemia major.APS disorder in children. In this regard, aPLs are considered as a risk factor that can shift the balance between coagulation and anticoagulation toward represents the most common acquired hypercoagulation state of autoimmune thrombosis.

PB 138 | Reference Ranges for Hemostasis Parameters in Pregnant Women from the Krasnoyarsk Area

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Background: Pregnancy is known to be associated with significant changes in the coagulation and fibrinolytic systems. As the consequence, the reference ranges defined in healthy adult population are not optimal for ensuring proper diagnosis of both hemorrhagic and thrombotic complications in pregnant women. Moreover, no data was available, so far, for population from the Krasnoyarsk area, Siberia.

Aims: To define the reference ranges for coagulation parameters in pregnant women from Siberia.

Methods: A total 228 pregnant women, with a mean age of 29.5 years (range: 18-43), were evaluated after their informed consent was obtained. None had any complication during their pregnancy, delivery, or postpartum period. Plasma samples were obtained at different time of the pregnancy i.e. gestational weeks 1-12 (n=31), 13-21 (n=43), 22-28 (n=66), 29-34 (n=52), and 35-42 (n=36). Routine and esoteric coagulation parameters were evaluated on a fully automated analyzer ACL TOP i.e. prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen, coagulation factors (F) II, V, VII, IX, X, XI, XII, d-dimers, vWF:Ag, antithrombin, protein C, free protein S and plasminogen.

Results: Pregnancy was associated with significant shortened PT and aPTT. Moreover, significant changes were demonstrated for most specific parameters. Particularly, the plasma levels of fibrinogen, D-dimers, FVIII and vWF:Ag significantly correlated with gestational age, whereas free PS was negatively correlated.

Conclusions: The use of gestational age-specific reference ranges is critical for the accurate interpretation of haemostatic tests during pregnancy.

PB 139 | Recombinant FVIIIFc-VWF-XTEN Promotes Normal Fibrin Formation, Structure and Stability

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Background: Coagulation culminates in the formation of a network of fibrin fibers, the density of which depends on the procoagulant activity generated at the site of injury. In hemophilia A, the lack of factor VIII (FVIII) diminishes thrombin generation resulting in fibrin clots of low density and poor stability. Factor replacement therapy aims to correct circulating FVIII levels and therefore normalize fibrin formation. Recombinant FVIIIFc-VWF-XTEN (BIVV 001) is a novel FVIII fusion protein with an extended half-life due to fusion of the D'D3 domain of von Willebrand Factor and use of Fc-fusion and XTEN technologies.

Aims: To evaluate the procoagulant activity of BIVV 001 by analyzing fibrin formation, network structure, and stability in hemophilia A plasma.

Methods: Citrated human hemophilia A plasma was spiked with increasing concentrations of BIVV 001 or recombinant FVIII (rFVIII) up

to 1 IU/mL (based on one-stage clotting assay activity). Tissue factor/$CaCl_2$-triggered fibrin formation was monitored by absorbance over time. Stability assays were performed by the addition of tissue plasminogen activator (tPA) at the start of the clotting reaction. Fibrin network structure of fluorescently-labeled clots was analyzed by confocal microscopy and an innovative high content image analysis pipeline.

Results: Both BIVV 001 and rFVIII showed similar dose-dependent increases in rate of fibrin formation compared to baseline (12±6 vs 12±4 fold change, respectively at 1 IU/mL). During tPA challenge, BIVV 001 promoted clot stability in a dose-dependent manner similar to rFVIII. Microscopic evaluation of fibrin clots formed in the presence of BIVV 001 revealed a significant improvement in fibrin network structure over baseline, to densities comparable to rFVIII.

Conclusions: In conclusion, the hemostatic potential of BIVV 001 is indistinguishable from rFVIII based on fibrin formation, network structure and stability in hemophilia A plasma.

PB 140 | Dissecting the Catalytic Enhancement of Activated Factor IX via Foot-printing Mass Spectrometry

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Background: Like in other serine proteases, coagulation factor IX (FIX) proteolytic activation involves the generation of a novel N-terminus and intramolecular rearrangements in its catalytic machinery. However, the structural events that drive FIX activation have remained poorly understood.

Aims: We used innovative foot-printing and mass spectrometry techniques to investigate the activation of FIX in comparison with factor X (FX) and prothrombin.

Methods: Chemical foot-printing included hydrogen-deuterium exchange (HDX) and primary amine labeling by tandem-mass-tags (TMTs). FIX, FX and prothrombin were activated and trapped in their most active configuration by binding an irreversible inhibitor. For FIX, prominent changes were validated by mutagenesis in positions implicated in haemophilia B.

Results: TMT labeling revealed that FIXa was similar to FXa and thrombin in that its N-terminus became more protected once bound to a peptide substrate analogue. In all three proteins, N-terminus insertion coincided with differential Lys-exposure in one particular surface loop (the 220-loop in chymotrypsin numbering). In fact, upon activation, Lys-exposure in the 220-loop was decreased in FXa and thrombin, but increased in FIXa. Conformational changes in the 220-loop were confirmed by HDX and were most prominent after inhibition of FIXa by EGRck. Involvement of the 220-loop in FIXa activity was further addressed by E217A, E219A, K222A and K224A substitutions. All mutants displayed impaired activity towards FX. Substitutions at 217, 222 and 224 were associated with reduced N-terminus insertion, while the opposite was found for E219A. This suggests a relation between N-terminus insertion and the 220-loop.

Conclusions: We propose that substrate-driven rigidification of the 220-loop, in combination with more efficient N-terminus insertion provides an important allosteric mechanism in FIX. These data should provide the basis for exploring rate limiting events in FIXa catalytic activity in response to its cofactor FVIII.

PB 141 | Characterization of Interactive Sites of the Blood Coagulation Factor VIII and the Low-density Lipoprotein Receptor using Macromolecular Docking Prediction Algorithms

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Background: The low-density lipoprotein receptor (LDLR) is involved in clearance of the blood coagulation factor VIII (FVIII) from the circulation. The binding sites of the LDLR family receptors are formed by complement-type repeats (CRs), grouped in clusters. Upon ligand binding, a CR domain interacts with a surface-exposed lysine of a ligand (Fisher et al, 2006, Mol. Cell 22:277-83). Previously, we demonstrated that four adjacent CRs (2-5) of LDLR form an extended site for binding FVIII, and the interactive FVIII site is in proximity to or involves the C1-domain (Kurasawa et al, 2013, J. Biol. Chem. 288:22033-41). In the present work, we used computer modelling to predict details of this interaction.

Aims: To determine surface-exposed lysines of FVIII potentially involved in its binding to LDLR and assess mutual orientation of both molecules during the interaction.

Methods: Using atomic coordinates from the crystal structures of FVIII and LDLR exodomain (PDB 2R7E and 1N7D, respectively), each of the CRs 2-5 of LDLR was docked individually to FVIII using the molecular docking program DOT2. Promising poses, most similar to the canonical CR domain interaction, were filtered based on:

i) the distance of a FVIII lysine to the CR-coordinated Ca^{2+} ion and

ii) the ability of the lysine to interact with a conserved aromatic residue of the CR.

Finally, by modeling the linker regions between the CR domains orientations, the individual docking results were combined into a model of the FVIII-LDLR interaction.

Results: A number of surface-exposed lysines of FVIII, located on its A3, C1 and C2 domains, were determined as candidates for the interaction and aligned with particular CR domains of LDLR.

Conclusions: A model of the FVIII-LDLR interaction was built up. The data suggest that the site of FVIII for binding to LDLR is extended as involves all domains of the FVIII light chain. *Disclaimer: these contributions are an informal communication and do not bind or obligate the U.S. FDA.*

PB 142 | Comparison of Bleeding Efficacy of FIX-F_C and FIX_{WT} 7 Days Post-infusion

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Background: Because hemophilia B prophylaxis is based on plasma levels of factor IX (FIX), several companies have produced FIX molecules with longer plasma residence times. But, we have previously shown that FIX with its c-terminus fused to the Fc region of IgG (FIXFc) provides no more protection from saphenous vein bleeding in hemophilia B mice than does $FIX_{WT.}$ Furthermore, FIX binds tightly to type IV collagen, which underlies the vasculature; this binding appears to explain our observation that there is ~3 times more extravascular factor IX than plasma factor IX. Here we show that FIX fused at its c-terminus with albumin (FIX_A) provides no additional benefit over FIX_{WT} in the saphenous vein bleeding model.

Aims: To compare Clotting Efficacy of FIX_{WT} and FIX_A.

Methods: Factor IX was infused into either hemophilia B mice lacking the FIX antigen or mice producing normal amounts of defective FIX. Seven days post-infusion, the saphenous vein was injured, and the number of times that clotting occurred was recorded. All mouse experiments were approved by the Institutional Animal Care and Use Committee (UNC-CH, #13-144) and followed U.S. Public Health Service guidelines for animal care and use.

Results: Fig. 1 shows no statistical difference between FIX_{WT} and FIX_A seven days post-infusion at the two different concentrations.

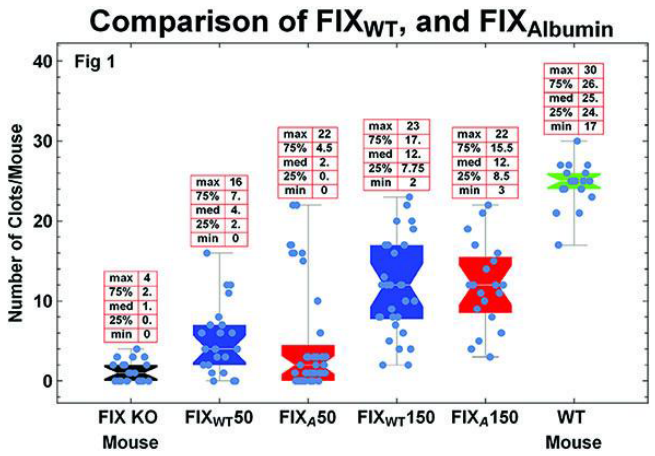

FIGURE 1 Hem B mice infused with FIXA were evaluated 7 days post infusion by saphenous vein bleeding. At neither dose was FIXA superior to FIXWT

If FIX's extracellular distribution is important for clotting efficacy, one would expect that a CRM^+ patient (a patient with defective FIX that can still bind type IV collagen) would require a higher dose of FIX for efficient prophylaxis. To test this, we utilized a knock-in mouse that expresses defective FIX_{R333Q} at normal levels. Fig 2 shows that a dose of FIX (100 IU/kg), that provides a median value of 8 clots at 7 days post-infusion in a hemophilia B mouse, provides only 5 clots in the FIX_{R333Q} mouse.

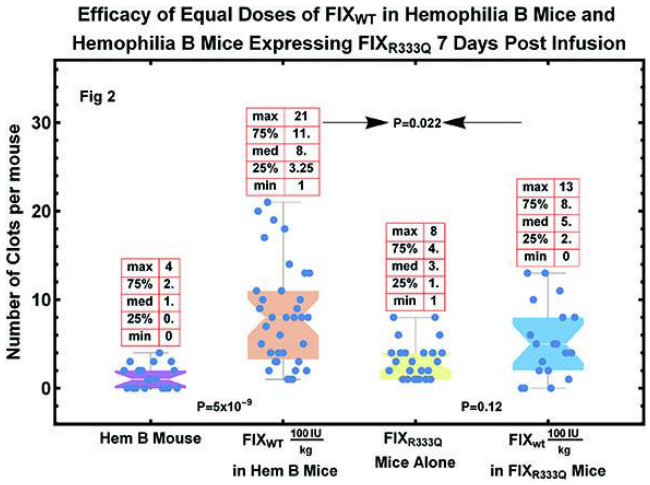

FIGURE 2 Hem B mice or mice expressing defective factor IX (FIXR333Q) showed significant differences in clotting efficacy

Conclusions: These data suggest that plasma FIX levels are not the best criterion for dosing hemophilia B. Furthermore, dosing may need to be adjusted depending upon the level of defective FIX.

PB 143 | rFVIIIFc-VWF-XTEN Demonstrates Comparable Efficacy to Recombinant Human FVIII in Mice by Acute Bleeding and Intravital Microscopy Models

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Background: Following vascular injury, the coagulation cascade activates FVIII, which leads to platelet activation and development of a stable clot. rFVIIIFc-VWF-XTEN (BIVV 001), is a novel long lasting recombinant FVIII designed to treat hemophilia A patients. BIVV 001 consists of a single chain rFVIIIFc, two XTEN linkers and the FVIII binding D'D3 domains of VWF, which prevents its association to VWF further increasing its half-life.

Aims: Compare the in vivo efficacy of BIVV 001 versus recombinant full length human FVIII (rhFVIII) in a dose-response study by clot formation in hemophilia A (HemA) mice.

Methods: HemA mice treated with BIVV 001 or rhFVIII were subjected to either tail clip or laser-induced saphenous vein injuries by intravital microscopy. For the tail clip assay, loss in blood volume was quantified for 30 minutes post injury. For the laser-injury model, the kinetics of platelet accumulation was analyzed at the site of injury for 3 minutes. The initial site was then reinjured after 5 and 10 minutes, for a total of 3 injuries induced at the same site.

Results: In the tail clip bleeding model, BIVV 001 showed comparable efficacy to rhFVIII at all tested dose levels. In the laser-injury model, the initial platelet adhesion was similar between HemA and wild-type mice but platelet deposition was decreased in HemA mice after the second and third injuries. Similarly, treatment with both FVIII molecules increased platelet deposition after the second and third injuries. Analysis of the third injuries showed similar platelet deposition profiles for BIVV 001 and rhFVIII as determined by maximal platelet accumulation, time to maximal platelet deposition, and rate of platelet accumulation.

Conclusions: BIVV 001 and rhFVIII display similar acute in vivo efficacy in HemA mice based on intravital microscopy and tail clip models when dosed based on one-stage assay activity. These results demonstrate equivalent in vivo hemostatic potential for BIVV 001 and rhFVIII.

PB 144 | How Full-length FVIII Benefits from its Heterogeneity - Insights into the Role of the B Domain

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Background: Extensive post-translational modification of natural coagulation factor VIII (FVIII) generates various sized hetero-dimeric molecular species. This unique heterogeneity results from processing of the molecule's B domain.

Aims: We explored natural FVIII heterogeneity and its effect and that of the B domain on protein stability.

Methods: FVIII molecular species containing 70% (B70), 20% (B20) or 0% (BDDrFVIII) B domain were isolated from full-length recombinant FVIII (FL-rFVIII) produced in CHO cells; FVIII from pooled human plasma (pdFVIII) was purified. Heterogeneity and thermally induced aggregation of FVIII molecular species, FL-rFVIII and pdFVIII were analyzed by SDS-PAGE, HPLC-SEC, and DLS.

Results: FL-rFVIII and highly purified pdFVIII with similar von Willebrand factor content as rFVIII showed analogous heterogenic protein profiles. The aggregation behavior of FL-rFVIII and pdFVIII was comparable; however, clear differences to and within purified monogenic molecular species were observed. Upon exposure to elevated temperatures, aggregate size increased as B domain decreased (aggregates of BDD-rFVIII>B20-rFVIII>B70-rFVIII>FLrFVIII). Time-dependent aggregation kinetics under physical stress provided deeper insights into the different aggregation pathways of FVIII molecular species. With decreasing B domain content, oligomers formed more rapidly and triggered excessive formation of large aggregates. Furthermore, FL-rFVIII and pdFVIII showed a lower propensity for aggregation than any molecular species. The absence of a B domain was shown to facilitate formation of cross-beta sheets, a structural feature not observed in B domain containing FVIII aggregates.

Conclusions: These results demonstrate similar heterogeneity of highly purified pdFVIII and FL-rFVIII produced in CHO cells, and suggest a new role of the B domain in ensuring stability of the FVIII molecule by modulating the protein aggregation pathway.

PB 145 | Monoclonal Antibodies to FcγRIIB (CD32) Modulate FVIII-specific Recall Response *in vitro*

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Background: Fc gamma receptors (FcγRs) for IgG regulate the adaptive immune response by triggering activating and inhibitory signaling pathways within immune cells. Data from a hemophilia A mouse model demonstrate that genetic deletion or blockade of inhibitory FcγRIIB (CD32) prevent the differentiation of FVIII-specific memory B cells (MBCs) into antibody secreting cells (ASCs) *in vitro*.

Aims: The mechanisms preventing the FVIII-specific recall response, however, remained unclear. Here, the potential role of CD32 inhibition was studied by modulating receptor activity with different agonistic and antagonistic anti-CD32 monoclonal antibodies (mAbs).

Methods: Splenocytes from immunized $FVIII^{-/-}$ mice were re-stimulated with FVIII in the absence or presence of anti-CD32 mAbs over 6 days. At day 6, FVIII-specific release of IFN-γ and IL-10 was quantified from cell culture supernatant and the formation of FVIII-specific ASCs was assessed. Binding of FVIII-containing immune complexes (F8-ICs) to bone marrow derived dendritic cells (BMdDCs) was also investigated.

Results: The antagonistic CD32 mAb AT128 suppressed the formation of FVIII-specific ASCs in a dose-dependent manner and reduced secretion of IFN-γ and IL-10. In contrast, the agonistic mAbs AT130-2 and AT130-5, and their $F(ab')_2$ fragments, fully supported the formation of FVIII-specific ASCs, even though the full IgG of AT130-2 reduced binding of F8-ICs to CD32.

Conclusions: These results suggest that an inhibitory signal is transmitted when F8-ICs bind to CD32 and that this is required during MBC activation to prevent apoptosis and support formation of FVIII-specific ASCs. If the inhibitory signal is lacking due to CD32 deletion or blocking with antagonistic anti-CD32 mAbs (like AT128), FVIII-specific T cell stimulation and ASC formation are suppressed whereas agonistic stimulation of CD32 with AT130-2 or AT130-5 restore T cell stimulation and ASC formation. These studies underline the central role of CD32 in regulating adaptive immune responses.

PB 146 | Factor VIII A1 Residues 346-349 is a Novel Thrombin-binding Site Responsible for cleavage at Arg372 in the Heavy Chain

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Background: Factor (F)VIII is activated by cleavage at Arg^{372}, Arg^{740}, and Arg^{1689} by thrombin. We demonstrated that FVIII interacts with thrombin through the A2 domain (residues 392-394 and 484-509) and C2 domain, and these interactions governed the cleavages at Arg^{740}, Arg^{372}, and Arg^{1689}, respectively (JBC 2000, 2005, BJH 2008). However, the residues 484-509 partially participated in the cleavage at Arg^{372}, supporting the presence of other thrombin-binding site responsible for this cleavage. We have recently reported that the residues 340-350 with sulfated Tyr^{346} in the acidic region in A1 contained the binding site responsible for cleavage at Arg^{372} by experiments using synthetic peptides.

Aims: To identify the crucial residues, we performed the N-terminal sequence analysis of the cross-linking product between the 340-350 peptide and thrombin bridged by the EDC reagent, and indicated that residues 344-349(EDYDDD) participated in cross-link formation. Therefore, the mutant forms of these residues converted to Ala (E344A/D345A, Y346A, D347A/D348A/D349A) were expressed in the BHK system and purified. We examined the interaction between thrombin and these mutants to identify the specific binding site.

Methods: The thrombin activation of these mutants was evaluated in a one-stage clotting assay, and the thrombin-catalyzed cleavage at Arg^{372} was by SDS-PAGE/western blotting.

Results: Initial velocity of the activation was 36.2±5.8 min^{-1} for WT, 22.1±1.4 min^{-1} for E344A/D345A, 15.0±3.2 min^{-1} for Y346A and 6.4±5.8 min^{-1} for D347A/D348A/D349A, respectively, indicative of restricted thrombin activation of Y346A and D347A/D348A/D349A compared to WT. The thrombin cleavage at Arg^{372} in Y346A and D347A/D348A/D349A were significantly delayed compared to WT. Band densitometry showed the cleavages velocity in Y346A and D347A/D348A/D349A were 56% and 24% of WT, but, that in E344A/D345A was similar to WT.

Conclusions: We demonstrated that A1 residues 346-349(YDDD) was a thrombin-binding site responsible for the cleavage at Arg^{372}.

PB 147 | Recombinant FVIIIFc-VWF-XTEN (BIVV 001) Demonstrates Equivalent Thrombin Generation and Whole Blood Clotting Profiles to Advate®

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Background: Recombinant FVIIIFc-VWF-XTEN (BIVV 001) is a novel fusion protein consisting of the FVIII binding D'D3 domains of VWF, a single chain rFVIIIFc and two XTEN linkers. Appending the D'D3 domain of VWF to FVIII provides the protection and stability of endogenous VWF, while avoiding the limitation in FVIII half-life extension imposed by VWF clearance. In hemophilia A mouse models, BIVV 001 showed equivalent efficacy and >3-fold half-life extension relative to Advate.

Aims: To evaluate the hemostatic potential of BIVV 001 by thrombin generation and thromboelastometry methods.

Methods: BIVV 001 was generated, purified and characterized at Bioverativ. BIVV 001 or Advate was added to naïve whole blood from severe hemophilia A donors (FVIII chromogenic activity < 0.5%) based on one-stage clotting assay with Actin FSL or labeled potency, respectively, followed by EXTEM (1:15000 dilution of Innovin®) and INTEM (1:300 dilution of INTEM reagent) analysis on ROTEM®. For thrombin generation assay (TGA), platelet-poor plasma obtained from the same visits was spiked with BIVV 001 or Advate and the assay was performed using modified CAT® method (PPP low reagent from Stago as trigger).

Results: BIVV 001 and Advate were compared side-by-side for each visit. In EXTEM and INTEM assays, increasing FVIII levels (0-30%) resulted in shortened clot time for both FVIII molecules. Despite the inter- and intra- subject differences, at similar FVIII activity, the two FVIII molecules tested showed comparable whole blood clotting profiles. In TGA, BIVV 001 and Advate showed clear dose responses in the measured range (0-100%) and comparable thrombin generation parameters.

Conclusions: BIVV 001 demonstrated equivalent whole blood clotting and thrombin generation profiles to Advate at similar FVIII activity levels. These results, together with in vivo pre-clinical efficacy data, suggest that Actin FSL based one-stage assays can predict the hemostatic potential of BIVV 001.

PB 148 | Altering FIX Zymogenicity Extends Procoagulant Function and Improves Hemostatic Function in Murine Hemophilia B

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Background: Strategies to improve hemophilia B (HB) treatment include extending zymogen factor IX (FIX) circulating half-life (e.g. long-acting FIX) and biologic function (e.g. FIX-Padua).

Aims: We investigated whether evading plasma-based inhibitors (e.g. antithrombin, AT) once FIX is converted to the protease FIXa would be a safe and effective strategy to mitigate the HB phenotype.

Methods: We altered the FIX zymogen to protease transition through mutagenesis at position 16 or 17 (chymotrypsin numbering) and generated a panel of variants that were characterized *in vitro* assays.

Results: In the absence of FVIIIa, all variants have reduced active site function relative to wild-type (wt)-FIXa and were zymogen-like. Importantly, the variants had reduced reactivity with AT as assessed by ELISA and prolonged biologic or "activity" half-life in plasma (6-20-fold > wt-FIXa). Consistent with work examining the FX zymogen to protease transition, the procoagulant activity of the FIXa variants could be partially rescued when incorporated into the intrinsic FXase complex. Following characterization in plasma-based systems, FIX-V16L appeared most promising and was evaluated *in vivo*. In a tail-clip model in which blood is collected for 10 min, intravenous administration of either FIX-V16L or wt-FIX zymogens into HB mice (n=10;

250 μg/kg; p< 0.05) reduced blood loss equally *vs*. PBS control (n=10). However, in a tail vein transection model where blood loss is assessed over 60 min, FIX-V16L (n=6-7; 75 or 125 μg/kg) was significantly more efficacious compared to wt-FIX (n=7-8; 75 or 125μg/kg) especially when comparing blood loss at >40 min. FIX-V16L appears to be safe, as thrombin-AT and D-dimer levels were not elevated following protein injection.

Conclusions: These data show that extending the biologic half-life of FIXa has a therapeutic benefit in vivo. We conclude that tuning of the FIX(a) zymogen-protease state is an effective way to evade physiologic inhibitors and yield variants with attractive biotherapeutic properties.

PB 149 | Evaluation of Recombinant FVIIIFc-VWF-XTEN (BIVV 001) Activity in One-stage Clotting and Chromogenic Assays and its Correlation with in vivo Efficacy in Hemophilia A Mice

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Background: Recombinant FVIIIFc-VWF-XTEN (BIVV 001) is a novel fusion protein consisting of the FVIII binding D'D3 domains of VWF fused to a single chain rFVIIIFc and two XTEN linkers. BIVV 001 was engineered such that thrombin activation removes both the VWF and XTEN moieties, resulting in a similar activated form as rFVIIIFc. In hemophilia A (HemA) mice preclinical studies, BIVV 001 showed >3-fold half-life extension relative to Advate®.

Aims: To evaluate the activity of BIVV 001 in one-stage clotting assays (OS) using different APTT reagents and chromogenic assays (CS) and assess activity correlation with in vivo efficacy in HemA mice

Methods: BIVV 001 was generated, purified and characterized at Bioverativ. OS and Siemens CS were performed on Siemens Sysmex CA1500 instruments. All other CS assays were done manually. The WHO 8th FVIII concentrate standard was used as calibrator for all assays. In vivo acute efficacy by tail clip bleeding model was determined after dosing HemA mice at select doses with either BIVV 001 or Advate.

Results: For OS, we observed 27.6% CV among the 3 major APTT reagents that collectively account for 94% of FVIII assays performed in US clinical hemostasis laboratories. The CS activities from 4 FVIII chromogenic kits were similar with 8.5% CV. The molar specific activity for BIVV001 by CS is similar to Advate and CS:OS ratio is ~2.2. BIVV 001 demonstrated equivalent in vivo efficacy to Advate when activity for dosing was determined by Actin FSL based OS.

Conclusions: BIVV 001 retains the molar specific activity equivalent to conventional FVIII molecules when assessed by CS, a two-stage assay utilizing an initial thrombin activation step, demonstrating that the FVIII portion is fully functional. The addition of the VWF and XTEN moieties appear to reduce the specific activity as assessed by OS, however OS is shown to be more physiologically relevant as it correlates with in vivo efficacy. Therefore the potency of BIVV 001 is assigned using OS, which will be used for all future studies.

PB 150 | Evaluation of Impact of Codon-Optimization on the Functional Activity of the Blood Coagulation Factor VIII by Clotting, Chromogenic and Thrombin Generation Assays

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Background: Codon-optimization is a useful approach to increase the production of recombinant proteins expressed at low levels. However, the change of codon usage may affect protein folding, post-translation modifications and functional properties. We evaluated the impact of codon-optimization on the activity of a B-domain deleted (BDD) coagulation factor VIII (FVIII) as a global measure of correct protein structure.

Aims: To compare the activities of the BDD-FVIII variants expressed from a codon-optimized (CO) or the wild-type cDNA (WT).

Methods: Each of WT and CO was produced in three preparations from the respective independent CHO clonal cell lines; the protein concentrations were determined by ELISA and confirmed by absorbance at 280 nm. The preparations were tested using chromogenic, one stage clotting and thrombin generation assays using the 8th International Standard for FVIII Concentrate as standard; in parallel, we tested two commercial BDD-FVIII variants as controls.

Results: By the chromogenic assay, the average specific activity of the CO preparations was about 1.5-fold higher than WT, though this difference was not statistically significant. Because the deletion of the B domain can be associated with assay-dependent discrepancy in the FVIII activity, the proteins were tested by other assays. All samples of WT, CO, and the control BDD-FVIII variants, generated similar amounts of thrombin and had similar clotting time-derived activities. Compared to the control BDD-FVIII variants, thrombin generation by the WT and CO started earlier, suggesting differences in purity between the control and in-house samples.

Conclusions: The codon-optimization of the BDD-FVIII did not affect negatively the functional activity, supporting structural similarity of the CO and WT. We hypothesize that the apparently higher specific activity of the CO reflects its better structural preservation due to significantly higher concentrations during the production. *Disclaimer: our contributions do not bind or obligate FDA.*

PB 151 | An *In silico* and *In vitro* Approach to Elucidate the Impact of Residues Flanking the Cleavage Scissile Bonds of FVIII

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Background: Coagulation Factor VIII is activated by an ordered limited thrombin proteolysis with different catalytic efficiency at three P1 Arginine residues: Arg^{759}> Arg^{1708}>Arg^{391}, indicating the flanking residues of the latter to be less optimal.
Aims: This study aimed to investigate, *in silico* and *in vitro*, the impact of possessing hypothetically optimized residues at these three catalytic cleavage sites.
Methods: The structural impact of the residues flanking Arginine cleavage sites was studied by *in silico* analysis through comparing the cleavage cleft of the native site with the optimized sequence at each site. Moreover, recombinant FVIII proteins were prepared by replacing the sequences flanking native thrombin cleavage sites with a proposed cleavage-optimized sequence. FVIII specific activity was determined by assessing the FVIII activity levels in relation to FVIII antigen levels.
Results: Our *in silico* results show the impact of the residues directly in the cleavage bond, and their neighboring residues on the insertion efficiency of the loop into the thrombin cleavage cleft. Moreover, the *in vitro* analysis shows that the sequences flanking the Arg^{1708} cleavage site seem to be the most close to optimal residues for achieving the maximal proteolytic activation and profactor activity of FVIII. The residues flanking the scissile bonds of FVIIII affect the cleavage rates and modulate the profactor activation.
Conclusions: We were able to provide insights into the mechanisms of the specificity of thrombin for the P1 cleavage sites of FVIII. Thus, the P3-P3´ residues surrounding Arg^{1708} of FVIII have the highest impact on rates of thrombin proteolysis which contributes to thrombin activation of the profactor and eventually to the thrombin generation potential.

PB 152 | *In vitro* Characterization of the *Factor 8* Gene Mutations Associated with Assay Discrepancy

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Background: Haemophilia A is caused by lack or reduced amount of Factor VIII (FVIII). The severity of disease is closely correlated to the FVIII activity which can be measured with a one-stage or a two-stage assay. In 30% of patients with non-severe phenotype, a discrepancy between the results of the two assays is observed.
Aims: In vitro characterization of missense mutations in the *F8* associated with assay discrepancy.
Methods: *F8* variants were transiently expressed in COS-1 cells. FVIII specific activity of the variants was determined based on FVIII activity levels (FVIII:C) using two one-stage assays (FVIII:C_{1st} and FVIII:C_{Bonn}) or a chromogenic assay (FVIII:C_{chr}). Furthermore thrombin generation test (TGT) was used to investigate whether it could reflect the haemostatic potential of the variants.
Results: Thirteen missense mutations were analysed. Six mutations belong to the group 1, associated with higher one-stage assay values. Discrepant results were observed for p.T294I, p.R550H and p.P1844S. Group 2 mutations associated with higher chromogenic assay values consist of seven mutations. Discrepancy was confirmed for p.E739K, p.R1708H, p.S2138Y and p.V697L. Except for the p.R1768H mutation all variants of group 1 showed a significant reduction of thrombin peak in TGT. For variants of group 2, TGT was able to show differences in lag-time for p.E739K, p.R1708H, p.Y1669F and p.V697L. The thrombin generation potential was only reduced for the p.R546W. The variants p.S2138Y and p.D2150 showed similar results to FVIII wild-type.
Conclusions: In the majority of variants the *in vitro* results confirmed the assay discrepancy phenomenon observed *in vivo*. The inability to confirm the discrepancy in the rest of the mutation might be due to modifications in the artificial in vitro system leading to loss the discrepancy detected in patients' plasma.

PB 153 | Corn Trypsin Inhibitor Mediates Differential Effects on Thrombin Generation Parameters in Hemophilic Patients Compared with Healthy Volunteers

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Background: Thrombin generation assay (TGA) is an important tool for evaluating the hemostatic potential of hemophilic patients. The addition of corn trypsin inhibitor (CTI) to collection tubes for blood samples intended for TGA was recommended by ISTH to abrogate the contact activation pathway when low tissue factor (TF) concentration is used. However, it has already been suggested that the impact of CTI on thrombin generation (TG) might differ between healthy volunteers and hemophilic patients.
Aims: To evaluate the impact of CTI on TG in hemophilic patients and healthy subjects.
Methods: This study was approved by a medical ethics committee. Forty healthy male volunteers (age 18-45), and 21 hemophilic patients (8 with severe and 13 with mild or moderate disease) were included after informed consent was obtained. Blood was collected into 3.2% citrate Monovette tubes with or without 1.45 µmol.L^{-1} CTI. TGA was performed in fresh platelet-rich plasma (PRP) and frozen platelet-poor plasma (PPP) with 1 pmol.L^{-1} TF. TG parameters were recorded using

the calibrated automated thrombogram method. Endogenous thrombin potential (ETP) and thrombin peak (P) measured in blood collected in tubes with or without CTI were compared by Student's paired t-test.

Results: In PPP, ETP and P values remained unchanged irrespective of CTI use in severely hemophilic patients, but were significantly reduced by CTI use in healthy volunteers ($p < 0.001$). In patients with mild or moderate hemophilia, CTI also had a statistically significant effect on ETP and P ($p < 0.01$) (Figures 1 and 2). The same results were obtained in PRP. The effect of CTI on TG varied from one volunteer to another in both PPP and PRP.

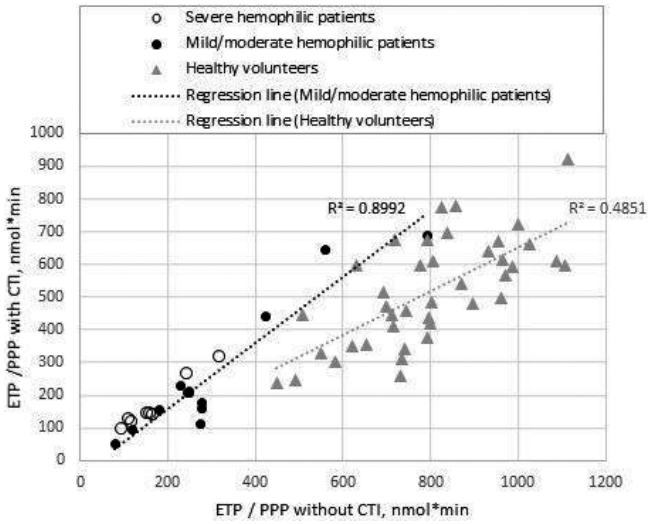

FIGURE 1 Influence of CTI addition to blood samples of healthy volunteers and hemophilic patients; ETP measured in PPP without CTI (x) and with CTI (y)

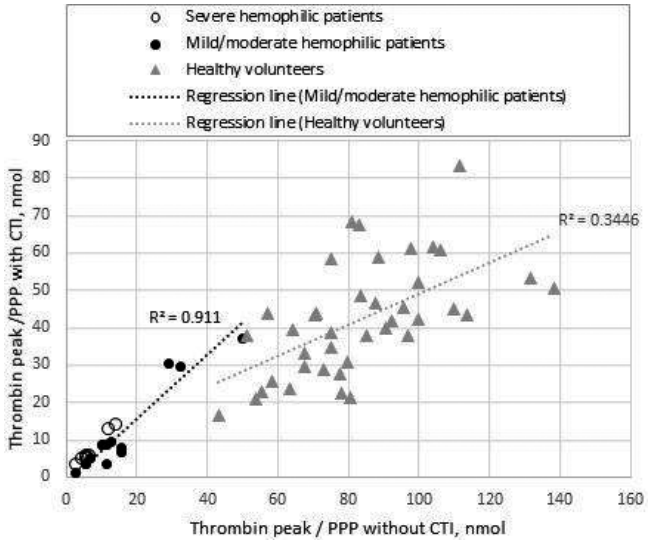

FIGURE 2 Influence of CTI addition to blood samples of healthy volunteers and hemophilic patients; Peak measured in PPP without CTI (x) and with CTI (y)

Conclusions:

- Values of TG in healthy volunteers cannot be determined in blood collected with CTI.
- TG in hemophilic patients can be evaluated in blood collected in Monovette tubes without CTI.

PB 154 | Influence of Pharmacodynamics of Factor VIII on Bleeding Phenotype of Children under Long-term Prophylactic Regimen

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Background: Plasma factor VIII activity (FVIII:C) has limited value as a predictor of bleeding risk in patients with severe haemophilia A (SHA). In contrast, procoagulant capacity evaluated by global tests is emerging as a valuable parameter to predict bleeding phenotype in this population. This may suggest a superior significance of global procoagulant capacity over FVIII:C itself as guidance to personalization of prophylactic treatment. So far, this field has been poorly evaluated in the paediatric population.

Aims: To assess the influence of pharmacodynamics (PD) of FVIII evaluated by thrombelastography (TEG) on the bleeding phenotype of children with SHA on long-term prophylaxis (LTP).

Methods: Children with SHA on LTP were recruited. PD and pharmacokinetics (PK) of FVIII were evaluated by kaolin-activated-TEG in whole blood and by determining plasma levels of FVIII:C respectively before and 30 min, 4, 8, 24 and 48 h after one prophylactic infusion. EC50 (FVIII:C at which 50% of the maximal response is observed) was calculated for each patient. Annualised bleeding rate (ABR) was obtained retrospectively from patient diaries. The study was conducted in accordance with the Declaration of Helsinki and was approved by the hospital ethics committee. Written informed consent was obtained prior to any trial-related activity.

Results: Twenty children under LTP were included of which only 4 (bleeders) registered bleeding events during the previous 12 months. Overall, these patients displayed no significant difference in the FVIII half-life, but a lower FVIII recovery than the average patient. Most markedly K-time and alpha angle of bleeders displayed nine times higher EC50 values than the non-bleeders suggesting a lower response to FVIII:C.

Conclusions: TEG evaluations may be useful to detect SHA patients with lower response to FVIII which may contribute to a higher ABR in children on LTP. Further studies are needed to confirm if TEG evaluations may be useful to detect patient on prophylaxis at higher risk of bleeding.

PB 155 | Influence of Neighbourhood Socioeconomic Status on Mortality Is Mediated by Factor VIII: Results from a Cohort Study

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Background: Socioeconomic status (SES) is a major determinant of mortality

Aims: To investigate if the association between neighbourhood SES and mortality can be explained by the presence of cardiovascular risk factors, morbidity or factor VIII levels

Methods: A large cohort study followed 4225 patients with venous thrombosis and 5991 controls from a Dutch population-based case-control study for a median of 5.6 years. Neighbourhood SES was measured on postcode level by using a status score based on mean income level, percentage of households with low income, unemployment rate, and education level. Participants were categorized in 4 percentile groups (0-15, 15-30, 30-70, >70). Cox proportional hazard models were used to evaluate risk of death between groups. All analyses were adjusted for age and sex, and additionally for the possible mediators BMI, chronic disease, smoking, alcohol consumption, and factor VIII levels

Results: The relative risk of mortality increased with decreasing status score. The hazard ratio of death for each 1 unit decrease in status score was 1.11 (95% CI, 1.00-1.22) in patients, 1.24 (95% CI, 1.06-1.44) in controls, and 1.16 (95% CI, 1.07-1.26) in all participants. The risk of death was 1.59-fold increased (95% CI, 1.20-2.11) in all included participants with status score below ≤15th percentile in comparison with the reference group of participants with status score above 70th percentile. Adjustment for possible mediators showed strongest effect for factor VIII levels. Per one unit decrease in status score the HR of death was 1.16 (95% CI, 1.07-1.26) without correction and 1.07 (95% CI, 0.93-1.23) after correction for factor VIII. Smoking, and chronic diseases also led to attenuation, but to a lesser extent.

Conclusions: A lower neighbourhood SES was associated with a higher mortality risk in healthy individuals as well as in patients with prior venous thrombosis. Factor VIII and chronic diseases might serve as an explanation behind this association.

PB 156 | Novel F9 Gene Variants in Hemophilia B Patients from Turkey

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Background: Hemophilia B (HB) is an X'linked bleeding disorder affecting one in 30000 males. Carrier females with reduced FIX activity may also suffer from bleeding. including menorrhagia. It is associated with the mutations in the F9 gene. mutations are identified in 97% of the cases. 2% of the HB patients develop inhibitor associated with mainly large deletions or missense, nonsense, small deletions/insertions, splice site mutations. Molecular genetic analysis of F9 gene in HB patients is important to predict the inhibitor risk, for the carrier identification, prenatal diagnosis for the proper management with hemophilia.

Aims: There are 1050 registered HB patients in Turkey. 412 of them are clinically diagnosed as severe, 358 as moderate and 280 as mild HB patients but the genetic profile is not reported. The aim of the present study is to screen the F9 gene of 22 HB patients in İstanbul, Turkey to establish molecular genetics of HB in Turkey.

Methods: F9 gene of the HB patients was PCR amplified and analyzed by Sanger sequencing of exons, 5' and 3' untranslated regions and exon/intron junctions. Initially four patients clinically diagnosed as severe HB, with FIX:C is less than 0,01 IU/ml were screened.

Results: Sanger sequencing revealed 10 different variations in the patients. 8 patients had single variations including novel exon 6 delAAGCACCCAAT/insACAGCACTCAAAG, novel p.Arg43Thr, p.Arg191Cys, p.Thr194Ala, p.Arg75Ter, p.Gln237Ter, novel IVS4+7A>G, novel p.His415Pro. 2 of the patients had double variations p.Arg226Gln/ p.Thr194Ala, p.Arg162Ter/p.Thr194Ala.

Conclusions: F9 gene variations were identified in all of the patients. 60% of the variations are missense mutations and 30% are stop mutations.7 of the variations were known mutations except p.Thr194Ala which is an SNP associated with deep vein thrombosis. It is observed in 30% of the patients. The p.Arg43Thr was predicted to be highly damaging according to Polyphen analysis. In silico analysis of IVS4+7A>G and p.His415Pro will reveal their pathogenic effect.

PB 157 | Evaluations of the Activated Partial Thromboplastin Time-based One-stage Clot Assays for Monitoring of Half-life Extended Factors VIII and IX Formulations

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Background: Coagulation factor activities of patients with hemophilia are commonly measured by the aPTT-based one-stage clot (OS) assays. Discrepancies have been reported among OS assays, and the modified factor VIII (FVIII) and factor IX (FIX) products for half-life extension may add to variabilities.

Aims: The objective of this study is to assess the characteristics of PEGylated, Fc-IgG and albumin (Alb) fusion constructs of FVIII and FIX formulations in various OS assays comparing with a chromogenic substrate (CS) assay.

Methods: FVIII deficient plasma was spiked *in vitro* with either octocog alfa, turoctocog alfa (B-domain deleted [BDD]), rurioctocog alfa pegol (PEGylated) or efraloctocog alfa (Fc-IgG fused BDD). A plasma lacking FIX was added with either nonacog alfa, eftrenonacog alfa (Fc-IgG fused), or albutrepenonacog alfa (Alb fused) with 0.1, 0.3 and 1.0 IU/mL based on labelled potencies. Samples were tested in OS assays

with each calibration on an automated blood coagulation analyzer (CS-2400) using APTT-SLA (SLA), ACTIN FS (FS), Coagpia APTT-N (APTT-N), SynthASil APTT (SynthASil), APTT-SP (SP), and PTT-LA. Recoveries were also assessed by CS assay using new reagents for FVIII and FIX activities.

Results: All of the FVIII products were recovered within ±25% in the assays of SLA, FS, and APTT-N, although recoveries for SynthASil, SP and PTT-LA were highly variable. Regarding FIX products, acceptable eftrenonacog alfa recovery was demonstrated in APTT-N, SynthASil, SP and PTT-LA, however, some overestimation was observed in SLA and FS. The target recoveries of 0.3 IU/mL albutrepenonacog alfa demonstrated 48.5±1.7% for OS assay with FS and 124.9±1.5% for CS assay.

Conclusions: FVIII can be measured in plasma samples containing modified products in OS assays using ellagic acid as an activator. Recovery of individual FIX products considerably depended on assay system. The choice of reagents for OS assays can affect the accurate measurement of activities for half-life extended factor products.

PB 158 | Population Pharmacokinetic Analysis of Perioperative Factor IX Dosing in Hemophilia B

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Background: To maintain hemostasis in the peri-operative setting, hemophilia B patients receive replacement therapy with factor concentrates to normalize FIX activity levels (FIX:C). FIX exhibits high inter-patient pharmacokinetic (PK) variability, which may produce over- and underexposure.

Aims: In this study, we aimed to construct a population PK model for FIX, which allows the assessment of average PK parameters and their associated inter-patient variability (IIV) in the perioperative period.

Methods: Retrospective data of 118 hemophilia B patients (median (range) age 40.1 years (0.15-90.45), body weight 81.5 kg (5.3-132)), undergoing 255 surgical procedures was available from ten hemophilia treatment centers in the Netherlands and the United Kingdom. Average PK parameters were estimated using nonlinear mixed-effect modeling.

Results: FIX:C versus time profiles were adequately described using a three-compartment model. Population PK parameter estimates and IIV (%) were: CL: 293 mL/h/70kg (21.1%), V1: 5010 mL/70kg (16.8%), Q2:102 mL/h/70kg, V2: 4780 mL/70kg, Q3: 1310 mL/h/70kg, V3: 2080 mL/70kg. With rising age, CL and V1 decreased 0.8% and 1.2% per year, respectively, until the age of 32 years. Compared to the United Kingdom, V1 was 14.5% higher for patients treated in the Netherlands. Furthermore, an increase of 16.4% and 22.1% for CL and V1, respectively, was associated to the use a recombinant product. A complication, such as an infection or bleeding during the surgical procedure, resulted in 16.4% increase of CL.

Conclusions: Measured perioperative FIX:C were described adequately by the established population PK model. The estimated population PK parameters were different from those reported for prophylactic treatment. This model will be used in a prospective clinical trial to perform PK-guided dosing of hemophilia B patients undergoing a surgical procedure.

PB 159 | Which Tests Can Better Indicate Clinical Phenotype of Pediatric Patients with Hemophilia on Prophylaxis?

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Background: Even if pharmacokinetic guided prophylaxis dosing of FVIII can opportunity to maintain a specific trough level, the variability of bleeding at different factor levels remain open issue.

Aims: The aim of this study is to assess whether global hemostasis tests, such as thrombin generation assay (TGA) and thromboelastography (TEG), can indicate bleeding pattern of severe hemophilia better than trough level and pharmacokinetic profile, particularly in the prophylactic setting.

Methods: The study group and healthy control group were consisted of 39 patients with hemophilia A (28 severe, 3 moderate, 8 mild) and 75 respectively. All patients for FVIII-inhibitor were negative. Basal or after washout (72 hours) factor (F) VIII, inhibitor level, TEG, TGA (platelet poor and rich plasma) of the participants were performed. Recombinant factor VIII product was administered to the patients at 40-50 IU/kg dosing for patients on prophylaxis. After factor replacement, the above tests were repeated at 30th minute, 6th, 24th and 48th hours.

Results: TGA and TEG parameters of the patients with mild and moderate hemophilia A were better than severe hemophilics. After administered factor concentrates, the patients with 48th hour FVIII < 3 % (n=10) and ≥ 3 % (n=12) were assessed with regard to annual bleeding

rate (ABR) and hemophilia joint health score (HJHS). ABR and HJHS were similar in these groups (P=0,368, and P=0,631 respectively). TGA and TEG parameters of these groups were also similar (ETP P=0,773, peak thrombin P=0,340). When the patients with 48th hour FVIII < 5 % (n=14) and ≥ 5 % (n=8) were evaluated in respect to ABR, HJHS, TEG and TGA parameters. We did not find a significant difference between two groups regarding above tests.

Conclusions: Neither pharmacokinetic profile with reduced number of samples nor trough levels may be correlate with clinical bleeding phenotype in patients with hemophilia A on prophylaxis. TGA and TEG promise for using clinical practice in these patients.

PB 160 | Acquired Haemophilia and Eosinophilic Fasciitis

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Background: Acquired haemophilia A (AHA) is an uncommon bleeding disorder, affecting predominately elderly patients. It results from developing autoantibodies to factor VIII. It is associated to autoimmune disorders, cancer, drugs, infections and often no underlying disease is found.

Aims: To present an uncommon association.

Methods: We present the case of AHA complicating eosinophilic fasciitis (EF).

Results: A 24-year-old patient diagnosed with EF as she complained from erythematous swelling of the extremities with thickened skin. Laboratory findings showed eosinophilia and a full-thickness wedge biopsy revealed infiltration of the deep fascia and the subcutis with lymphocytes, histiocytes and eosinophils.

The patient was treated with corticosteroids for 6 months with resolution of cutaneous symptoms; however, she developed cholestasis and cytolysis considered as a hepatic flare-up of the disease. Corticosteroids where increased to 1mg/kg/d of prednisone.

Two months later, while she was still taking 1mg/kg/d of prednisone, she presented diffuse ecchymosis.

Blood count cells and prothrombin time were normal, however, activated partial thromboplastin time (aPTT) was prolonged. Anti phospholipid antibodies were negative and Von Willebrand factor was normal. Factor VIII level was 1% and the screening of anti factor VIII was positive allowing the diagnosis of acquired haemophilia A.

Osteo-medullary biopsy performed to screen for malignant haemopathy associated with EF or AHA was normal.

Regarding the persistence of prolonged aPTT with a haemorrhagic syndrome despite high doses of corticosteroids, immunosuppressive therapy was proposed. The patient received 3 pulses of Cyclophosphamide relayed by azathioprine with resolution of the ecchymosis and normalisation of the aPTT.

Conclusions: Both EF and AHA are uncommon conditions that may be the first manifestation of hematologic malignancies. To our Knowledge, no such association were described. A prolonged follow up is necessary to screen malignant associated condition.

PB 818 | Efficacy, Safety, and Pharmacokinetics of a High-purity Plasma-derived Factor X (pdFX) Concentrate in the Prophylaxis of Bleeding Episodes in Children < 12 Years with Moderate to Severe Congenital Factor X Deficiency (FXD)

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Background: Congenital FXD is a rare bleeding disorder characterized by spontaneous joint and mucocutaneous bleeding and gastrointestinal or intracranial hemorrhage. pdFX is a US- and EU-approved treatment for congenital FXD, but data in children < 12 y have been unavailable.

Aims: To investigate pdFX efficacy, safety, and pharmacokinetics in children < 12 y with moderate to severe congenital FXD.

Methods: In this 6-month, open-label, multicenter, phase 3, prospective study in children < 12 y, all subjects had a confirmed diagnosis of moderate to severe congenital FXD (basal FX:C < 5%), severe bleeding history, or an *F10* gene mutation causing a documented severe bleeding type. Subjects received routine prophylaxis at recommended 40-50 IU/kg twice weekly to maintain trough FX:C levels ≥5%. Each investigator assessed efficacy based on standardized criteria and presence of breakthrough bleeding. All subjects provided informed consent and the protocol was approved by appropriate independent ethics committees.

Results: Mean age of the 9 trial completers was 6.8 y. Eight subjects had severe and 1 had moderate FXD. Overall, 537 prophylactic infusions were administered; mean dose/child was 38.6 IU/kg. Ten bleeds in 3 of 9 children were reported: 6 minor, 3 major, 1 unassessed. Investigators rated overall pdFX efficacy as excellent in all subjects. Overall mean incremental recovery was 1.74 IU/dL per IU/kg. FX trough levels were maintained >5% after visit 4 (days 29-42) in all subjects.

A total of 28 treatment-emergent adverse events (TEAEs) were reported in 8 children; none were considered pdFX related. No significant changes were noted in vital signs, physical exams, or laboratory measurements. No evidence of inhibitor development was seen.

Conclusions: pdFX is efficacious in the prophylaxis of bleeding episodes in subjects < 12 y with moderate to severe FXD. Safety profile in this population is consistent with previous results in subjects ≥12 y.

Funding: Bio Products Laboratory

PB 819 | Characterization of an Aptamer Inhibiting Coagulation Factor XIa Identified through in vitro Screening of a Single-stranded DAN Library

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Background: Either factor XIIa or thrombin activate coagulation factor XI (FXI) to FXIa. FXIa then activates Factor IX (FIX). Animal model and epidemiological data support FXI/FXIa as a therapeutic target, because it appears to influence thrombosis more than hemostasis. Only protein and small molecule inhibitors of FXIa, but not nucleic acid-based agents, have been described to date.

Aims: To select and characterize DNA-based inhibitors of FXIa activity.

Methods: A 78 base, single-stranded DNA aptamer library containing a 40 base randomized core was iteratively screened. Candidates were selected for binding to FXIa and against active site-blocked FXIa in each round. The selected aptamer pool was then amplified using asymmetric PCR. After ten positive/negative rounds, individual sequences from the selected pool were synthesized and characterized.

Results: Six of 89 different selected aptamers inhibited chromogenic substrate S2366 cleavage by FXIa. Five of the six were highly homologous. The most active anti-FXIa aptamer had a 36 base central core of 5'- AACCTATCGGACTATTGTTAGTGATTTTTATAGTGT - 3' and was designated **F**actor **EL**even **I**nhibitory **AP**tamer (FELIAP). FELIAP, but not a scrambled aptamer control (SCRAPT), competitively inhibited FXIa-mediated S2366 cleavage, FIX activation, and complex formation with antithrombin. No effect of FELIAP on FXI activation was observed. FELIAP inhibited modified clotting and thrombin generation assays to a significantly greater extent than SCRAPT. Immobilized FELIAP bound FXIa with an equilibrium binding constant (K_D) of 1.9 ± 0.2 nM (mean ± SD, n= 3) as judged by surface plasmon resonance. Deletion of all 38 bases flanking FELIAP's central core abrogated anti-FXIa activity, but activity was retained in FELIAP derivatives with as few as 5 flanking nucleotides.

Conclusions: FELIAP binds FXIa with high affinity, at or near its active site. The first FXIa-inhibitory aptamer to be described, FELIAP will be optimized in length and composition to develop related aptamers for in vivo use.

PB 820 | Three Dimensional Thrombus Architecture Formed under Flow Conditions

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Background: Various commercial and home-made microfluidic devices use extensional and steady state shearing forces to assess *in vivo* thrombus formation. Sometimes within these systems the thrombus has been imaged but is largely limited to two dimensions. To characterise the role of anti-platelet agents and anticoagulants on the three dimensional structure of thrombus formation under flow conditions, alternative methodologies need developing.

Aims: We aimed to therefore develop a flow model of thrombosis using confocal microscopy to determine the composition of fibrin and platelets in the three dimensional forming thrombus.

Methods: Channel slides were coated with 50 $\mu g.mL^{-1}$ fibrillar type I collagen in 500mM acetic acid pH 2.3 for 1 hour at 37°C. Slides were then blocked with a 1% w/v BSA solution in HBS buffer for 20 minutes at 37°C, and washed with HBS buffer. Hirudin or citrate blood samples were spiked with DiOC18(3) and $10\mu g.mL^{-1}$ Alexa Fluor 594 conjugated human fibrinogen and incubated for 30 minutes. Following addition of 7.5mM $CaCl_2$ and 3.75mM $MgCl_2$, blood was perfused through the coated chamber at varying shear rates. Flow was terminated at a maximum time of 10 minutes, and the thrombus was washed with HBS buffer supplemented with 1 $U.mL^{-1}$ heparin. Slides were mounted on a Zeiss LSM880 inverted microscope. Confocal z-stacks were recorded for both labelled platelets and conjugated human fibrinogen.

Results: We have generated confocal images that validate this approach to assessing the role of anticoagulants and anti-platelet agents on the composition of the thrombus under flow conditions. The images show that under normal circumstances, platelets are coated with fibrin layers on the interface of where the blood is flowing.

Conclusions: This study shows that for the first time it will be possible to determine the role of antithrombotic agents employing a more physiological approach to monitoring thrombus formation under flow conditions and aid the development of next generation antithrombotic agents.

PB 821 | Administration of Dexamethasone during Initial Exposure to Factor VIII Reduces Inhibitor Development in Hemophilia A Mice Via Thymic Tolerance Induction Mechanisms

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Background: The most severe complication of hemophilia A (HA) treatment is the development of inhibitory antibodies against factor VIII (FVIII). In a humanized HA model with a 30% likelihood of inhibitor incidence, administration of dexamethasone (Dex) during initial FVIII exposure results in long-term, antigen-specific tolerance.

Aims:

1. Determine if Dex administration during initial FVIII exposure reduces the FVIII immune response in a HA mouse model with a 100% likelihood of inhibitor incidence.

2. Determine short- and long-term treatment effects on lymphocytes.
3. Investigate treatment effects on thymic and splenic mRNA expression.

Methods: All experiments were performed in E16KO C57Bl6 HA mice. Mice received FVIII or FVIII+Dex (FVIII 6 IU/dose IV, Dex 75 µg/dose IP) for 5 consecutive days.

1. Blood was collected 5 weeks later. Plasma anti-FVIII antibody titers and inhibitory activity were measured.
2. Thymus, spleen and blood were collected either 3 days or 3 weeks later. Lymphocyte populations were assessed via flow cytometry.
3. Thymus and spleen were collected 3 days later. mRNA expression was assessed via NanoString.

Results:

1. 77% of FVIII+Dex mice v 100% of FVIII mice developed anti-FVIII IgG. FVIII+Dex mice had lower anti-FVIII IgG titers and inhibitory activity (p=0.08) than FVIII mice.
2. FVIII+Dex mice had a lower percentage of splenic $CD19^+$ cells than FVIII mice 3 days after treatment and an increased percentage of thymic $CD4^+CD8^-CD25^+FoxP3^+$, PD-$L1^+$ and PD-1^+ cells. No differences detected among blood lymphocytes at 3 days, or among lymphocytes from any source at 3 weeks.
3. FVIII+Dex mice had decreased mRNA expression of T cell development mediators in the thymus compared to FVIII mice. No differences in splenic mRNA expression were detected between the 2 groups. $p < 0.05$ unless stated otherwise.

Conclusions: Dex given during initial FVIII exposure reduces the anti-FVIII immune response in a second HA mouse model. This is associated with effects of Dex on lymphocyte populations and thymic gene expression.

PB 822 | Dual Mode of Interaction of Fibrinogen with the Staphylocoagulase•Prothrombin* Complex

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Background: Fibrin deposition is a hallmark of staphylocoagulase (SC)-positive *S. aureus* endocarditis. The N-terminal SC(1-325) fragment activates prothrombin (ProT) non-proteolytically. The SC(1-325)•ProT* complex cleaves fibrinogen in the E-domain to form fibrin. Full-length SC(1-660) contains one pseudorepeat (PR) and 7 tandem repeat sequences (R1 to R7). It also binds fibrinogen, mediated by SC C-terminal repeat sequences.

Aims: The Aim was to identify the SC binding sequence(s) on fibrin(ogen).

Methods: We used size exclusion chromatography; native PAGE; fluorescence labeling; CD, NMR and mass spec analysis; and Ala scanning to study the interactions.

Results: SC(1-325) did not bind fibrinogen Fragment D (FragD), whereas SC(1-660) bound multiple Frag D molecules. SC(1-660) bound to fluorescein-labeled ProT ([5F]-FPRProT) and FragD in a ternary complex. Native-PAGE and fluorescence titrations showed no interaction of [5F]PR and [5F]R1 with FragD, whereas [5F]PR-R1, -R2, -R3, -R6, -R7, bound FragD with K_D 50 - 130 nM, and a 1:1 stoichiometry. PR-R1 bound to FragD in the presence of GPRP with K_D 17 nM, suggesting no binding to Frag D β- and γ- holes. The full length repeat [5]PR-(R1-R7) bound to Frag D with K_D 7 - 32 nM and a stoichiometry of 5. Competitive titrations of [5]PR-(R1-R7) and FragD with PR-R1R2R3, PR-R1R6R7, PR-R3R4R7 and PR-R3R6R7 showed that the constructs bound to FragD with K_D 7 - 42 nM and a stoichiometry of 3. CD analysis of PR-R7 and PR-(R1-R7) yielded spectra without a definite structure. Mass spec analysis of [pBpa]PR-R7 cross-linked to FragD yielded putative cross-linked sites VELEDWNGR and MFK on γ- and α- chains of FragD. NMR studies identified a minimal region within PR-R7, required for FragD binding. This peptide bound FragD with a K_D of ~5 µM. Ala scanning showed that substitution of VKYRD, AGTGIR, EYNDG, ARPT, YKKP and HADG in the minimal peptide abolished FragD binding.

Conclusions: The combined data identify two different modes of fibrinogen binding to the SC•ProT* complex.

PB 823 | Sensitive, Functional Assay for Detecting Polyphosphate in Plasma

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Background: Inorganic polyphosphate (polyP) is an important procoagulant and proinflammatory molecule released from platelets and other cells. Circulating polyP in disease or following trauma is of particular interest. However, facile methods are lacking for quantifying plasma polyP, particularly of the size secreted by platelets. We here demonstrate a sensitive assay for polyP in plasma.

Aims: A method for detecting and quantifying polyP in plasma.

Methods: Modified PT clotting assays were performed with dilute tissue factor (TF), with and without added tissue factor pathway inhibitor (TFPI). PolyP in plasma was found to abrogate the anticoagulant activity of TFPI in a dose-dependent manner. That this "TFPI resistance" was due to polyP was confirmed by clotting assays in the presence of polyP inhibitor (UHRA-2 or polybrene).

Results: Adding 135 ng/ml TFPI to normal plasma profoundly prolonged the clotting time, but adding increasing polyP concentrations rendered it increasingly resistant to TFPI anticoagulant effect (Figure, panel A). This functional "TFPI resistance" assay is sensitive to nanomolar concentrations of platelet-sized polyP. Clotting times of

unknown plasma samples containing polyP were quantified with and without TFPI, making sure their clot times fell within the range of the standard curve. To confirm that polyP is responsible for any observed TFPI resistance, the polyP inhibitors UHRA-2 or polybrene were used to reverse the ability of polyP to render plasma TFPI resistant (Figure, panel B).

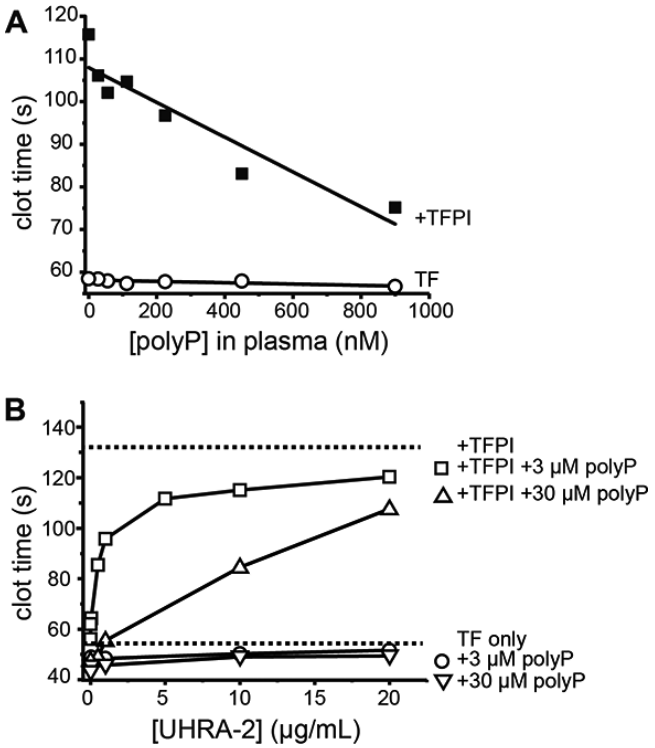

FIGURE 1 TFPI resistance (A) as a function of polyP concentration (B) reversed by polyP inhibitor

Conclusions: The effective concentration of polyP in plasma samples can be determined through this functional assay, using readily available equipment. We hope to gain further understanding of polyP's role in physiological events and, in turn, improve clotting-related diagnoses.

PB 824 | Combined IV and SC administration of Long-acting FVIIa (MOD-5014) Significantly Improves the Pharmacokinetics (PK), Pharmacodynamics (PD) and Pharmacological Long-term Outcome in Preclinical and Toxicological Models, Supporting the Initiation of a Clinical Study Combining Prophylactic and On-demand Treatments

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Background: OPKO Health is a clinical-stage public company developing bio-better long-acting versions of existing therapeutic proteins, utilizing a technology termed CTP. The technology involves fusion of the C-terminal peptide of hCG to a target protein.

Aims: The aim of this work was to extensively characterize intravenous (IV) and subcutaneous (SC) administrations of FVIIa-CTP (MOD-5014) for a potential combined prophylactic and on-demand treatment.

Methods: PK and PD were assessed following SC or IV administration in rats and monkeys using ELISA and clotting methods, respectively. MOD-5014´s long-term hemostatic effect following SC and IV injection utilizing tail-clip and survival model in hemophilic mice, as well as bioavailability, were also evaluated.

Results: MOD-5014 demonstrated significantly improved PK and PD profiles when injected IV and SC; following SC administration, half-life values were longer. The calculated bioavailability MOD-5014 injected SC was found to be approximately 40% in monkeys. The long-term hemostatic effect following SC administration, as observed in tail-clip studies as survival rates, bleeding intensity and bleeding duration, were prolonged in comparison to IV administration of MOD-5014 in rodent models.

Conclusions: Attachments of CTP to FVIIa led to enhanced PK and PD profiles when administered both SC and IV. A slower onset and prolonged half-life following SC administration indicate relatively slow absorption of MOD-5014 in comparison to IV administration, which is translated to a marked *in vivo* hemostatic effect. This suggests that SC administration could be used for prophylactic treatments, while IV administration could serve as an on-demand treatment during a bleeding episode due to the obtained quick onset, higher C_{max} and AUC. This potential combined treatment may be evaluated in an advanced clinical study.

PB 825 | Factors Influencing the Recanalization of Deep and Superficial Venous Thrombosis of Lower Limbs

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Background: Superficial (SVT) and deep venous thrombosis (DVT) represents one of the most frequent vascular diseases of lower limbs. Recanalization is a long-lasting process which depends on different factors and is important for chronic complications, such as post-thrombotic syndrome (PTS) and chronic venous insufficiency (CVI), therefore we investigated it further.

Aims: Aim of the study was to investigate the recanalization rate of SVT and DVT and the factors which are responsible for recanalization of thrombotic occlusions of veins, as well as the factors which inhibit lysis of thrombus.

Methods: Two groups of patients were included, one with idiopathic DVT, and one with SVT, with at least 10 cm thrombotic occlusion of affected superficial vein. We evaluated the recanalization rate and measured levels of systemic inflammatory markers and systemic fibrinolytic parameters.

Results: We showed that recanalization rate depends on thrombus load and is faster in distal thrombotic occlusions. We also showed that recanalization is faster and more frequent in females than in males. Recanalization of deep as well as superficial venous thrombosis is also influenced by endogenic fibrinolytic potential on the level of systemic inflammatory markers. Multivariate analysis of recanalization rate in patients with DVT showed that it is significantly influenced by level of interleukin 6 (IL-6) ($p< 0.001$) and P-selectin ($p=0.007$). Recanalization of SVT is also significantly related to levels of inflammatory markers: reactive protein (CRP) ($r=0, 39$, $p< 0.01$), IL-6 ($r=0,38$, $p< 0.01$) and tumor necrosis factor α (TNF- α) ($r=0,26$, $p=0.04$).

Conclusions: After acute thrombotic occlusion of superficial or deep veins, a process of recanalization starts which is long-lasting and depends on thrombus load, location of thrombosis and is influenced by gender. Recanalization is also significantly influenced by systemic levels of inflammatory markers, particularly levels of interleukins, which inhibits thrombolysis and diminishes recanalization rate.

PB 826 | Effect of Platelet Depletion and Thrombin Inhibition on Tail Bleeding in Wild Type and FXI-deficient Mice

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Background: Factor XI (FXI) deficiency is a rare disease with only a mild bleeding phenotype. Moreover, deficiency of FXI is related to a reduced risk of thrombotic events. Therefore FXI inhibition might represent an effective treatment option for thrombosis prevention in comparison to current therapeutic options. However, the bleeding risk of a targeted inhibition of FXI is unknown.

Aims: Target of the study was to evaluate the effect of platelet depletion alone or in combination with thrombin inhibition on bleeding tendencies in a tail bleeding model in wild type (WT) and FXI deficient ($FXI^{-/-}$) mice.

Methods: C57BL/6J (WT) and $FXI^{-/-}$ mice were treated with a platelet-depleting GPIbα antibody (R300 emfret) or control IgG and/or the direct thrombin inhibitor dabigatran. Agents were used at low doses to unravel additive effects on top of FXI-deficiency. Bleeding in mice was evaluated by using a standardized tail bleeding model. Tail was cut at 5 mm from the tip, bleeding intensity was measured over 30 min.

Results: Platelet depletion (> 95 %) resulted in an increased bleeding phenotype in WT mice (from 100 ± 96.6 % to 300.8 ± 105.8 %). The bleeding could be further increased by concomitant use of the direct thrombin inhibitor dabigatran (0.2 - 0.3 mg/kg, bleeding increase ≥ 50%). $FXI^{-/-}$ mice did not show an additional increase in tail bleeding compared to WT mice, neither after administration of the platelet-depleting antibody nor by additional administration of dabigatran.

Conclusions: A strongly reduced platelet count led to increased bleeding in WT mice. Interaction with the coagulation system by using a thrombin inhibitor further increased bleeding tendencies in platelet-depleted mice. The fact that no further bleeding increase in $FXI^{-/-}$ mice could be observed, indicates that FXI and its amplification by thrombin do not play a key role in primary hemostasis. Therefore, modulating FXI seems to be a unique and safe treatment option for the prevention of thrombosis.

PB 827 | Interrelation between Levels of Hemostatic Factors, Lipids and C-reactive Protein in Population Controls

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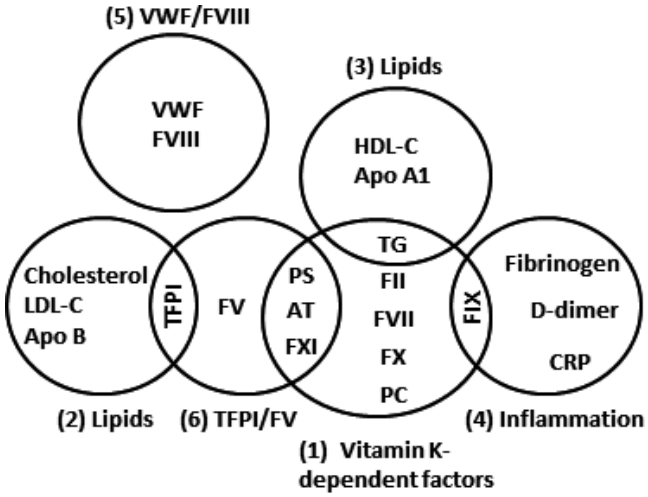
Background: Previous studies have shown that hemostatic factors cluster together. However, results were not consistent, probably due to differences in sample sizes and variables studied. Furthermore, since arterial and venous thrombosis share some traditional risk factors, we questioned if and how hemostatic factors cluster with lipids and C-reactive protein (CRP).

Aims: To assess the clustering of hemostatic factor levels and how these clusters relate to lipid and CRP levels.

Methods: We investigated 2,874 individuals (47% men) who had participated as population controls in a previous study. We imputed missing data on fibrinolytic factors which were measured only in a random subset of controls (n=733). Clusters of interrelated factors were identified by principal component analysis. A factor loading >0.40 was used as the marginal value to include factors in a cluster. Consent and ethical approval were obtained.

Results: We identified 3 clusters among the hemostatic factors: a vitamin K-dependent factor (VKDF) cluster (factors [F]II, VII, IX, X, protein C and protein S [PS]) that also included FXI and antithrombin (AT); another comprising fibrinogen, FVIII, von Willebrand factor (VWF) and D-dimer, and a third one including FV, tissue factor pathway inhibitor, PS and AT. The addition of lipid fractions and CRP (Fig. 1), led to two extra lipid clusters, with triglyceride (TG) also clustering with VKDFs. VWF and FVIII now formed a separate cluster, and CRP clustered with fibrinogen, D-dimer and FIX. When individuals with chronic diseases were excluded (n=570), the clustering pattern remained virtually the same. Upon addition of imputed fibrinolytic factors, plasminogen and α2-antiplasmin clustered with VKDFs, and tissue plasminogen activator and plasminogen activator inhibitor-1 formed a new cluster with FVII, TG and PS, with minor changes in the other clusters.

Conclusions: In this comprehensive study, we confirmed and extended cluster patterns of previous reports.



The six clusters were ranked by their eigenvalues from (1) to (6). Factor loadings within each cluster were ranked descendingly: **(1)** FX 0.79, PC 0.75, FII 0.68, FVII 0.66, FIX 0.65, TG 0.55, FXI 0.47, AT 0.45, PS 0.42; **(2)** LDL-C 0.93, Cholesterol 0.91, Apo B 0.89, TFPI 0.40; **(3)** HDL-C 0.97, Apo A1 0.91, TG -0.46; **(4)** CRP 0.79, Fibrinogen 0.73, D-dimer 0.65, FIX 0.42; **(5)** VWF 0.91, FVIII 0.91; **(6)** FV 0.63, TFPI 0.61, AT 0.54, PS 0.45, FXI 0.41.

FIGURE 1 Factor loading pattern of hemostatic factors, lipids and c-reactive protein (CRP)

PB 828 | Selective Inhibition of Protein S Anticoagulant Functions Restores in vitro Thrombin Generation in Haemophilia A and B Plasma

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Background: Protein S is an important inhibitor of blood coagulation serving as a co-factor for activated protein C (APC) in the inactivation of Factor Va and VIIIa as well as for Tissue Factor Pathway Inhibitor-a (TFPI-a) in the inhibition of Factor Xa [Walker, JBC (1980), 255:5521-5524; Hackeng, PNAS (2006), 103:3106-3111]. Recently, Bologna et al. demonstrated that knock-out of the protein S encoding gene can rescue the haemophilic phenotype in haemophilia A and B mice [Bologna, Blood (2016), 128:79].

Aims: To further elucidate the possibility to ameliorate haemophilia by blocking protein S, we developed a monoclonal antibody (mAb) targeting the EGF1-domain of protein S.

Methods:

Results: This mAb essentially normalized thrombin generation *in vitro* in haemophilia A and B plasma and clot formation in haemophilia A-like whole blood as measured by thromboelastography. Interestingly, the anti-protein S mAb appeared to function both in an APC-dependent and

-independent manner in plasma. Further, the mAb dose-dependently increased the thrombin generation in plasma triggered with Factor Xa in the absence of functional Tissue Factor and Factor VIIa, conditions previously shown to be especially sensitive toward the TFPIa/Protein S-interactions [Thomassen, JTH (2015), 13:92-100]. However, while the APC-dependent mechanism was readily confirmed using purified coagulation factors, the mAb did not appear to perturb protein S interactions with TFPIa or Factor Xa under these conditions. Thus the effect observed in plasma may require interactions of an additional coagulation protein, potentially Factor V. In addition, the anti-protein S mAb did not affect protein S interactions with C4b-binding protein or negatively charged phospholipid surface.

Conclusions: In conclusion, our data underlines the importance of protein S for the down-regulation of blood coagulation making it a potential target for the treatment of haemophilia A and B. However, the high plasma concentration makes protein S a challenging target to saturate.

PB 829 | Treatment of a Subdural Hematoma and Long-term Secondary Prophylaxis in a Patient with Severe Factor X (FX) Deficiency Treated with a High-purity Plasma-derived Factor X (pdFX) Concentrate

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Background: A male patient, born in 1991, with a basal FX activity of < 1% of normal due to a homozygous missense mutation in the *F10* gene, p.Gly262Asp, had an extensive history of spontaneous and traumatic bleeds. From 2011-2013, this patient participated in a phase 3 clinical trial of pdFX for on-demand treatment of moderate/severe FX deficiency.

Aims: To present pdFX treatment of a subdural hematoma and consecutive secondary prophylaxis.

Methods: After completing the phase 3 clinical trial, the patient received on-demand pdFX in an extension trial and under a compassionate-use program.

Results: On 10-Feb-2014, the patient presented with a severe headache for the past 2d that was unresponsive to analgesics (no recent history of head trauma). The patient immediately received 1000 IU (15 IU/kg) pdFX and was sent for a CT scan, which identified a 1.2-cm subdural hematoma in the left parietal-temporal-occipital region, with associated hemorrhagic lesions in the tentorium cerebelli. The patient

was admitted to the neurosurgical intensive care unit (ICU) and received a further 3000 IU (46 IU/kg) pdFX.

The patient remained in the neurosurgical ICU for 1wk and received daily doses of 2000-4000 IU (31-62 IU/kg) pdFX (no other administered hemostatic agents). The headache resolved 1d after admission. A second CT scan performed on 17-Feb indicated that bleeding had stopped and the hematoma had significantly regressed.

The patient was discharged on 18-Feb and began once-weekly routine prophylaxis with 2000 IU (25 IU/kg) pdFX for 5mos, on-demand therapy for 10mos, and now receives routine prophylactic treatment of 1800-1900 IU (≈25 IU/kg) pdFX every 2wks, with no bleeding episodes or long-term neurologic sequelae.

Conclusions: These results show that pdFX was safe and effective in treating a life-threatening bleed and as routine prophylaxis to reduce the frequency of spontaneous and traumatic bleeds in this patient with severe FX deficiency.

Funding: Bio Products Laboratory

PB 830 | Differential Coagulotoxic Effects of Asian Pitviper Snake Venoms: Evolutionary, Pathophysiology and Biodiscovery Implications

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Background: Snakebite is a massive global burden and is the most neglected of all tropical diseases. Snake venom neurotoxins have been the subject of intense research efforts, but comparably much less is known about coagulotoxins. Limited knowledge into the mechanisms of action of snake venom hinders our understanding of the clinical pathologies, restricting our use of these powerful natural products as lead compounds in drug design and development. One success story has been Ancrod, a kallikrein-type serine protease from the venom of the Malayan pitviper *Calloselasma rhodostoma*. Through its selective fibrinogen-degrading activity it has been commercially developed into 'Arvin' used for treatment of stroke.

Aims: Investigate unique procoagulant properties from the wide ranging Asian pitviper clades for the use in novel biodiscovery and possible drug design.

Methods: 30 Asian pitviper venoms were screened for actions upon fibrinogen and clotting. Fibrinogen 1D SDS PAGE was performed to identify possible degradation of fibrinogen chains. A novel procoagulant assay was also developed utilising a Stago STA-R Max to accurately detect real time clotting in plasma.

Results: Gels showed effects ranging from rapid degradation of the α-chain only (Fig A1), full cleavage of α, β and γ chains to form a fibrin clot (disappearance of fibrinogen) (Fig A2-A4), abnormal degradation of all three chains simultaneously (Fig A5) or rapid degradation of the α-chain followed by slower degradation of β and γ chains, without the formation of a fibrin clot (Fig A6). Clotting times revealed that not all species which degraded all 3 chains to form a fibrin clot were able to rapidly clot plasma (Fig B, C).

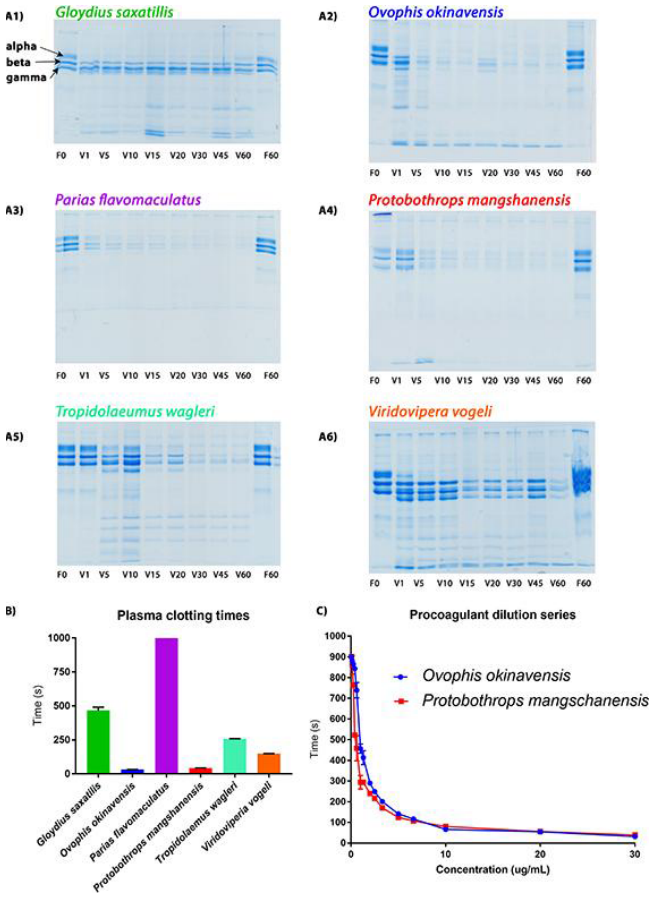

FIGURE A) Differential venom effects upon fibrinogen chains at 37°C, B) Time for venom (20 µg/ml) to induce plasma to clot at 37°C; C) clotting curves

Conclusions: Over 30 MY of evolution has subjected Asian pitvipers to rapid radiation and extensive venom diversification, leading to such properties as defibrinogenation and clotting. These results not only inform in regards to potential clinical effects by these neglected, medically important species but also provide a biodiscovery roadmap.

PB 831 | The Effect of Histone H4 on Coagulation Enzymes and Complexes in Purified Systems

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Background: It has been reported that histones interact with proteins of blood coagulation and fibrinolysis giving an overall procoagulant effect in whole blood.

Aims: To evaluate the effect of histone H4 on the amidolytic and proteolytic activity of coagulation enzymes and their complexes.

Methods: The effect of H4 on the amidolytic activity of purified enzymes FIIa, FVIIa and FXa was evaluated via chromogenic and fluorogenic assays. Similarly, the effect of H4 on the proteolytic activity of the enzymatic complexes intrinsic FXase, extrinsic FXase and prothrombinase was also evaluated. H4 titrations were performed for each of the enzymatic complexes to obtain their respective IC_{50} values.

Results: For purified enzymes tested, almost complete inhibition (95%) of amidolytic activity was observed for FVIIa while 29% inhibition was observed for FXa at saturating H4 concentrations. In contrast, a 28% increase in amidolytic activity was observed for FIIa. For the enzymatic complexes, complete inhibition was observed for both intrinsic and extrinsic FXase while only 56% inhibition was observed for prothrombinase. IC_{50} values were 0.13 μM, 0.45 μM and 0.40 μM, respectively.

Conclusions: In contrast to the effect observed in whole blood, H4 substantially inhibited the activity of 2 out of 3 coagulation enzymes and of all 3 enzymatic complexes tested. The observed contrast between purified systems and whole blood could potentially be related to the effects of cellular components and platelets that are present in blood.

PB 832 | CP-5-001: A Phase 1/2a, Open-Label, Multicenter, Dose Escalation Study to Assess the Safety, Pharmacokinetics and Pharmacodynamics Profile of a Long-acting Recombinant Factor VIIa (MOD-5014) in Adult Men with Hemophilia A or B

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Background: OPKO is developing long-acting versions of therapeutic proteins using CTP. This technology involves fusion of the C-terminal peptide of hCG to proteins and was clinically validated for several drugs while maintaining biological activity. MOD-5014 is a long-acting form of recombinant FVIIa (rFVIIa-CTP) tested for the 1st time in hemophilic patients.

Aims: Assessment of acute safety, tolerability, PK and PD profiles of single intravenous (IV) administration of escalating MOD-5014 doses in hemophilic subjects with and without inhibitors.

Methods: Single-dose, open-label, dose-escalating study performed in 2 stages. Stage 1 included 3 doses (25, 50 and 100 μg/kg), with 4 subjects per group. Dosing of all subjects was followed by a 7-, 14- and 30-day safety observation period. Safety assessments included monitoring of adverse events (AEs), injection site reactions, vital signs, physical condition and laboratory assessments (e.g. hematology, biochemistry, coagulation panel, immunogenicity). Stage 2 includes 3 doses (200, 300 and 400 μg/kg).

Results: Study analysis includes safety, PK and PD data for patients that completed the 2 stages. In Stage 1, 75% of patients reported AEs, all considered unrelated or unlikely related to study medication; none led to premature study discontinuation. One severe AE was reported during MOD-5014 treatment and was considered unlikely related to study medication. Laboratory assessments supported of MOD-5014 tolerability; no significant overall changes were observed in hematology or chemistry. There was no consistent pattern for changes in any coagulation parameter during the study attributable to study intervention. MOD-5014 presents superior long PK and PD profile in comparison to for commercial FVIIa historical data.

Conclusions: Currently MOD-5014 demonstrates promising safety and tolerability profile following single IV administration of escalating MOD-5014 doses (25, 50, 100 and 200 μg/kg) in hemophilic subjects with and without inhibitors.

PB 833 | Age-related Variation in the Glycosylation of Alpha-2-Macroglobluin

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Background: Alpha-2-macroglobulin (a2m) is a plasma glycoprotein serine protease inhibitor shown to exhibit higher levels in newborns than adults. Although our previous studies did not reveal differences in glycan number per a2m molecule (macroheterogeneity) for newborns compared to adults, we discovered that newborn a2m had significantly more sialic acid (SA), indicating microheterogeneity between the age groups.

Aims: To examine differences in the glycan moieties between newborn and adult a2m.

Methods: To determine the heterogeneity in a2m charge, 2-dimensional (2D) gel electrophoresis was carried out on both newborn and adult plasma samples, followed by western blotting. A2m was then purified from plasmas by immunoprecipitation and subjected to fluorescence-assisted carbohydrate electrophoresis (FACE) to assess differences in glycan types for newborn vs. adult a2m molecules.

Results: 2D gel results revealed a change in net charge for the two molecules, with newborn a2m species migrating to a lower isoelectric point than adult. This result was consistent with the increased SA in newborn a2m observed previously. Additionally, FACE revealed a prominent band of sialylated biantennary glycans and a secondary population of larger glycans with increased branching. Analyses

showed that there was a significantly higher proportion of the more branched glycans in the newborn sample than adult.

Conclusions: This is the first study to identify specific carbohydrate characteristics between newborn and adult a2m. The results suggest that newborn a2m tends to have higher negative charge from increased SA capping more highly branched glycans. Such differences may partly account for the increased a2m plasma levels in newborns due to slower clearance resulting from reduced uptake by receptors that bind glycans missing SA or mannose in less branched structures.

PB 834 | First Evaluation of the Safety, Pharmacokinetics and Pharmacodynamics of BAY 1213790, a Full Human IgG1 Antibody Targeting Coagulation Factor XIa, in Healthy Young Men

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Background: Anticoagulants are the cornerstone of prevention and treatment of thrombotic disorders, but those in clinical use are associated with a dose-dependent risk of bleeding, limiting their antithrombotic potential. Coagulation factor XI (FXI) contributes to the development of thrombosis, but plays only a minor role in hemostasis and is therefore an attractive target for antithrombotic therapy. Inhibition of FXIa offers the potential to reduce the risk of thrombosis while minimizing the risk of associated bleeding.

Aims: To evaluate the safety, pharmacokinetic (PK) and pharmacodynamic (PD) properties of BAY 1213790, a full human IgG1 antibody targeting FXIa, in healthy male volunteers.

Methods: In this randomized, single-blind, parallel-group, placebo-controlled dose-escalation study, BAY 1213790 (0.015-10 mg/kg) was administered as a 60-minute infusion to healthy male volunteers. Adverse events, in particular, spontaneous bleeding, infusion reactions and hypersensitivity were monitored; volunteers were followed up for 5 months. Key PK parameters included exposure (area under the plasma concentration-time curve), maximum plasma concentration and terminal half-life. The PD properties of BAY 1213790 were assessed using activated partial thromboplastin time, rotational thromboelastometry, FXI activity (clotting assay) and bleeding time. The study was approved by the local ethics committee and all volunteers provided written informed consent.

Results: BAY 1213790 demonstrated a favorable safety and tolerability profile, with a dose-dependent increase in exposure and a terminal half-life of approximately 30 days. All relevant coagulation biomarkers were influenced in a dose- and time-dependent manner. No cases of bleeding and only one case of mild infusion reaction were reported.

Conclusions: BAY 1213790 is a promising candidate for long-term and stable anticoagulation, showing no increased risk of bleeding in this first study in humans.

PB 835 | Case Study of Successful Transition to Human Cell Line Fc Fusion Factor IX Replacement without Premedication in an Adolescent Male with Severe Hemophilia B, Anaphylactoid Reaction to FIX Replacement, and Previous History of Inhibitor and Nephrotic Syndrome

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Background: The development of inhibitors to factor replacement in hemophilia continues to present significant challenges. While inhibitors develop with less frequency in Hemophilia B, this occurrence is often seen with the concurrent development of severe allergies to replacement, further compounding treatment challenges.

Aims: To describe an option for treatment of FIX deficient individuals with anaphylactoid reaction to FIX replacement and history of inhibitors.

Methods: A 12-year-old male with severe hemophilia B (missense mutation) presented with the following history: Initiation of prophylaxis with recombinant FIX at 8 months. At 10 months, he developed an intracranial hemorrhage with an inhibitor level of 5.5 Bethesda units. Daily immune tolerance induction with recombinant FIX and recombinant FVII was started. At 11 months, he developed severe allergies to FIX replacement. At 18 months, he developed nephrotic syndrome. At 3 years old, his regimen was changed to rituximab (off label), IVIG (off label), and plasma derived FIX. The inhibitor level decreased to undetectable; however, his allergy to infused FIX remained as did his risk and development of multiple line infections. At presentation, he was infusing recombinant FIX every other day with premedication before every dose. A trial of human cell line Fc Fusion FIX replacement was pursued.

Results: Fc Fusion FIX was dosed at 100units/kg without premedication. A pharmacokinetic study was completed and he was started on weekly infusions with a day 7 FIX activity of 4%. His port was removed under Fc Fusion FIX coverage and he continues weekly infusions without need for premedication.

Conclusions: This case highlights the need for further exploration of the use of human cell line therapies in hemophilia B patients with inhibitors and allergic phenotype. The decreased immunogenicity reported with human cell line derivative products may offer an opportunity for the development of improved treatment guidelines in this challenging subgroup.

PB 836 | Estimates of within-subject Biological Variation of Factor VIII, von Willebrand Factor Antigen, Fibrinogen, Prothrombin Time, INR and APTT in Pregnant Women

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Background: In pregnancy and post-partum, interpretation of results from coagulation parameters can be difficult because of the physiological changes towards a hypercoagulable state.

Aims: The aim of this study was to describe the change in concentration of several coagulation parameters during healthy pregnancies, and to estimate and compare the within-subject biological variation (CV_I) of these parameters in healthy pregnant and non-pregnant women.

Methods: Blood samples were obtained every 4th week during pregnancy and 3 samples post-partum in 20 healthy women and every 4th week during 40 weeks in 19 healthy non-pregnant women. Activated partial thromboplastin time (APTT), prothrombin time (PT), PT-INR, Fibrinogen, Factor VIII clot (FVIII) and von Willebrand factor antigen (vWF) were analysed. Analysis of variance (ANOVA), with the statistical model for repeated subsampling, was used to calculate biological variation. Data was transformed into multiples of the median (MoM) to create a steady-state situation by adjusting for the physiological changes during pregnancy. Within-subject variations (CV_I) were estimated from the transformed lnMoM data after outlier exclusion. APTT, PT and INR were analysed by two different reagents, therefore two CV_I results are presented.

Results: During pregnancy, APTT, PT and PT-INR decreased or remained largely unchanged (dependent upon reagent). Fibrinogen, FVIII and vWF increased gradually during pregnancy and decreased abruptly after delivery. The CV_I (lnMoM) in pregnancy (post-partum period excluded) and in non-pregnant women were similar, except for Fibrinogen (Table 1).

TABLE 1 CVI (back transformed from SDs for lnMoM) in pregnant and non-pregnant women

	Pregnancy CVI (95%CI)	Non-pregnant CVI (95%CI)APTT2
APTT1	3.0 (2.7-3.4)	2.7 (2.4-3.0)
APTT2	2.2 (2.0-2.5)	2.1 (1.8-2.3)
PT1 (Owren Method)	2.9 (2.5-3.2)	3.3 (2.9-3.7)
PT2 (Quick Method)	2.5 (2.2-2.8)*	2.5 (2.3-2.8)
PT-INR1 (Owren Method)	3.2 (2.9-3.5)	3.0 (2.7-3.4)
PT-INR2 (Quick Method)	2.6 (2.3-2.9)*	2.5 (2.3-2.9)
Fibrinogen (Clauss)	7.2 (6.4-8.1)	9.3 (8.3-10.5)
Factor VIII clot	12.7 (11.6-14.2)**	11.7 (10.4-13.2)
von Willebrand factor antigen	11.3 (10.1-12.8)	11.1 (9.9-12.5)

*Not Gaussian distribution of the residuals, **Not variance homogeneity

Conclusions: Transformation of coagulation parameters in healthy pregnancies to MoM is a tool to establish a kind of steady-state. Although there is a physiological change in APTT, PT/INR, Fibrinogen, FVIII and vWF:Ag during pregnancy, the CV_I (lnMoM) was comparable with the CV_I (lnMoM) of non-pregnant women.

PB 837 | A Snake-venom Enzyme (Noscarin)-based Assay for APC-resistance Is Not Influenced by Non-vitamin K Antagonist Oral Anticoagulants (NOAC)

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Background: Various coagulation assays are influenced by the presence of *non-vitamin K antagonist oral anticoagulants* (NOAC). In the case of APC-resistance (APCR) assays, the literature is not conclusive. Studies with either a small number of patient samples on either rivaroxaban (Ri) or dabigatran (Da) (Gessoni 2015) or samples spiked ex vivo with Ri (Hillarp 2011) using either aPTT based or noscarin-based APCR assays gave slightly discrepant findings. Noscarin-based APCR assay was shown not to be influenced by the presence of lupus anticoagulant (Wilmer 2004 and Schöni 2005).

Aims: To determine if NOACs could influence the results of noscarin-based APCR assay leading to misclassification of patients with known FV Leiden mutation receiving treatment with various NOACs (dabigatran, rivaroxaban, apixaban or edoxaban).

Methods: Plasma samples of patients (n=50) under NOAC treatment are collected at the Graz University Hospital and genotyped for FV Leiden mutation using a 5'-nuclease assay using specific primers and probes. The noscarin-based APCR assay (Pefakit APC-R Factor V Leiden) is performed in the absence of Ca^{2+} and phospholipid. It activates factor V via the factor V activating enzyme from *Vipera russelli* (RVV-V) by bypassing FXa, and prothrombin via noscarin from *Notechis scutatus*. These specific activation pathways reduce potential interferences in plasma. Plasma levels of NOACs are determined using Biophen DiXal assay for anti-Xa NOACs and Biophen DTI for anti-IIa NOACs with specific calibrators and controls. Agreement between phenotype results of noscarin-based APCR assay and FV Leiden genotyping has been analyzed.

Results: So far in the presence of NOACs 100% of samples (n=24) were correctly identified (wild-type, heterozygous, homozygous) by noscarin-based APCR assay compared to PCR genotyping.

Conclusions: Preliminary data suggest there is no interference of the NOACs tested in this study on the phenotyping for APCR with the noscarin-based clotting assay.

PB 838 | APC and TFPI Cofactor Activities of Thrombin-cleaved Protein S and C4BP-protein S Complex

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Background: Protein S is a non-enzymatic cofactor for APC in the inactivation of FVa and FVIIIa and for TFPI in the inhibition of FXa and TF-FVIIa. In plasma 60% of protein S (~ 200nM) is found in a high affinity complex with C4BP and 40% of protein S (~ 150 nM) circulates in a free form.

Aims: It has been reported that only free protein S expresses anticoagulant activity as a cofactor for APC and that this activity is inhibited by cleavage of thrombin-sensitive region of protein S. Alternatively, binding of protein S to C4BP results in specific inhibition of cleavage by APC at R506 of factor Va. However, regulation of protein S cofactor activity for TFPI is less well understood. In the current study we evaluated and compared the APC and TFPI cofactor activities of protein S, thrombin-cleaved protein S and C4BP-protein S complex in plasma and in model systems.

Methods: To quantify the APC cofactor activity, thrombin generation was measured in protein S-deficient plasma containing APC in the presence of different concentrations of the various forms of protein S both in the absence and presence of neutralizing antibodies against TFPI. The TFPI cofactor activity of native protein S, thrombin-cleaved protein S, and C4BP-protein S complex was quantified in model system by measuring the inhibition of Xa by TFPI. In addition, the ability of the different forms of protein S to act as TFPI cofactor was quantified in plasma by measuring thrombin generation in protein S-deficient plasma reconstituted with varying amounts of protein S.

Results: Thrombin-cleaved protein S showed full TFPI cofactor activity in the model system but C4BP-protein S was only partly active. In plasma, thrombin-cleaved protein S lacked APC cofactor activity but was fully active as TFPI cofactor. C4BP-protein S did not exhibit APC cofactor activity in plasma but was active as a TFPI cofactor.

Conclusions: In conclusion, this research gives more insight into the APC and TFPI cofactor activities of the various forms of protein S.

PB 839 | Anticoagulants Differentially Affect Procoagulant Platelet Formation

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Background: Procoagulant platelets provide a surface for assembly of coagulation complexes at the platelet thrombus. Inability to form procoagulant platelets is associated with bleeding while excess is linked with pathological thrombosis. Heparins are used in acute management of myocardial infarction and venous thrombosis, while direct anticoagulants (DOACS) are used for acute venous but not arterial thrombosis. Both bleeding and efficacy rates of heparins are higher than DOACs and we hypothesised this related to procoagulant platelet function.

Aims: To explore the effect of anticoagulants on procoagulant platelet formation.

Methods: We adapted a procoagulant platelet flow cytometry assay based on novel cell death agent, GSAO (Hua et al., Blood, 2015), to measure procoagulant platelet formation in ex vivo whole blood. Whole blood from healthy volunteers was pre-incubated with anticoagulants or vehicle control at varying doses prior to stimulation with thrombin (2U/mL) and subjected to FACS analysis. Procoagulant platelets were defined as GSAO+/CD62P+.

Results: Compared with vehicle control, unfractionated and low molecular weight heparin both markedly reduced thrombin induced procoagulant platelet formation in a dose dependent manner (fold change 0.32±0.07, n=3, p=0.0098 and 0.46±0.04, n=3, p=0.0039). Dabigatran, a direct thrombin inhibitor also inhibited thrombin induced procoagulant platelet formation (0.26±0.13, n=6, p< 0.005). In contrast, the direct FXa inhibitor, rivaroxaban, did not significantly affect procoagulant platelet formation (0.95±0.14, n=9, p=0.0835). Apixaban demonstrated minor inhibition in procoagulant platelet formation (0.78±0.07, n=10, p=0.0054) at a "subtherapeutic" level of 49ng/mL.

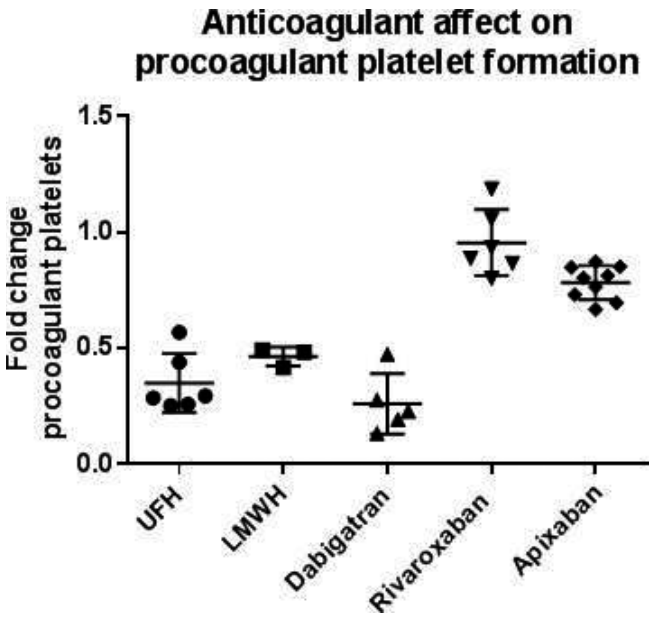

FIGURE 1: Heparins and dabigatran inhibit procoagulant platelet formation, while rivaroxaban and apixaban have minimal effect.

Conclusions: Anticoagulants used currently during acute myocardial infarction inhibit procoagulant platelet formation in addition to the known anticoagulant effects. These findings may help explain bleeding and efficacy profiles of heparins compared with oral anti-FXa agents rivaroxaban and apixaban.

PB 840 | A Novel Hypoxia Response Element Regulates Oxygen-related Repression of Tissue Factor Pathway Inhibitor Expression

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Background: The expression of tissue factor pathway inhibitor (TFPI) is repressed under hypoxic conditions. In a previous study we identified the hypoxia response element (HRE) 5'-GACATG-3' located -1065 to -1060 within the TFPI promoter and that HIF-1α transcriptionally regulates TFPI gene expression under hypoxia through binding to this HRE. HIF-2α, also known as endothelial PAS domain-containing protein 1 (EPAS1), is another key transcription factor in hypoxia. Its role in the hypoxia mediated regulation of TFPI is unclear.

Aims: To explore the possible correlation of HIF-2α/EPAS1 and hypoxia-mediated repression of TFPI expression.

Methods: Quantitative RT-PCR, Western blot, luciferase reporter gene assay, chromatin immunoprecipitation (ChIP) and mutagenesis were used to study the role of HIF-2α in MCF7 breast cancer cells.

Results: Quantitative RT-PCR revealed that the TFPI mRNA level was decreased by overexpression of HIF-2α/EPAS1. Luciferase reporter gene assay and ChIP demonstrated a HIF-2α/EPAS1 responsive region located in the TFPI promoter region at -170 to +21 relative to the transcriptional start site. Using mutagenesis a functional hypoxia response element (HRE) 5'-AAACAGGA-3' for HIF-2α/EPAS1 was identified within the TFPI promoter at -45 to -38.

Conclusions: This study provides evidence that HIF-2α/EPAS1 transcriptionally downregulates TFPI gene expression through a novel HRE. The hypoxic microenvironment may induce aberrant activation of coagulation through repression of TFPI expression.

PB 841 | Impact of Thrombomodulin on Spatial Clot Growth and Fibrin Polymerization in Cirrhotic Patients

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Background: Thrombin generation assays (TGA) with thrombomodulin (TM) demonstrated that cirrhotic patients have a hypercoagulable profile. The thrombodynamics assay (TD) gives a global assessment of coagulation by evaluating the growth of a fibrin clot after activation of tissue factor immobilized on a thin layer of a plastic surface. A recent study found that spatial clot growth was preserved in patients with cirrhosis without use of TM.

Aims: To evaluate spatial clot growth and fibrin polymerization in plasma of cirrhotic patients in presence of TM.

Methods: Nine healthy controls and 26 cirrhotic patients (9 Child-Pugh (CP) A, 10 CP-B and 7 CP-C) were enrolled. TGA were performed with calibrated automated thrombography using 5 pM of tissue factor and 4 mM of phospholipids, in presence and absence of 1 nM of TM. TD were performed without and with addition of 25 nM of TM. Fibrin polymerisation was evaluated by turbidimetry after thrombin activation
(0.5 U/ml).

Results: We confirmed a hypercoagulable state in cirrhotic patients gradually increasing from healthy controls to CP-C patients in TGA with TM. In TD without TM, the clot growth rate, the clot density and the clot size were preserved in cirrhotic patients. In TD with TM, the rate and the lag time of clot growth were decreased. The clot size was not modified by addition of TM in TD. A significant decrease in clot density was found in TD without and with TM for CP-C patients when compared to healthy controls ($p < 0.01$). No correlation with TG parameters was found. The fibrin polymerisation study revealed a significant decrease of maximal absorption without and with TM between healthy controls and CP-C patients ($p < 0.01$), strongly correlated with fibrinogen activity. TM significantly increased the lag time observed in polymerisation.

Conclusions: TM decreases several parameters of TD and turbidimetry analyses, although without differences between healthy controls and cirrhotic patients. There were no correlations with variables measured by TGA.

PB 842 | Biomechanisms of a Severe Hypersensitivity Reaction Induced by Factor IX in Hemophilia B Complicated by Factor IX Inhibitor

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Background: Patients with hemophilia B who develop factor IX (FIX) neutralizing antibodies (inhibitors) after FIX infusion are at high risk of hypersensitivity reactions upon FIX re-exposure, but the underlying mechanisms are incompletely understood.

Aims: The study was aimed at identifying potential biomechanisms of hypersensititvity reaction induced by factor IX in a patient with severe hemophilia B complicated by newly developed inhibitor.

Methods: We employed an *in vitro* cellular antigen stimulation test (CAST) to evaluate leukotriene C4 (LTC4) release from basophils stimulated by FIX in three treated children with hemophilia B, one

of whom developed FIX inhibitor and experienced anaphylaxis following FIX re-exposure. Anti-FIX IgE and IgG antibodies and markers of complement activation (C5b9, C3d and iC3b) were measured in plasma, the last also after FIX infusion. Ten healthy children served as controls.

Results: The hemophilia B patient who developed anti-FIX inhibitors and anaphylaxis had a nonsense mutation in the FIX gene (p.Arg298Stop) and, compared to normal controls, had higher plasma levels of specific anti-FIX IgE (2.285 *vs* 0.084 $OD^{492\ nm}$), with a marked LTC4 release from his FIX-stimulated basophils (519.8 *vs* 39.9 pg/ml). Further, he had higher plasma levels of anti-FIX IgG of all the four subclasses (total IgG 1.180 *vs* 0.120 $OD^{492\ nm}$) with FIX neutralizing activity (1.5 BU); mild complement activation occurred during FIX-induced anaphylaxis (C5b9 increased from 258.5 to 351.1 ng/ml). The same parameters were normal in two hemophilia B patients who tolerated FIX infusion.

Conclusions: In the hemophilia B patient who experienced anaphylaxis after FIX, but not in the hemophilia B patients who tolerated FIX, the CAST assay showed FIX-induced LTC4 release, which was associated with high plasma levels of specific anti-FIX IgE and IgG antibodies.

PB 843 | The Thrombomodulin Resistance in Prothrombin Belgrade Mutation Carriers

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Background: The prothrombin Belgrade (FIIc.1787G>A) mutation leads to Arg596Gln substitution, which impairs binding of antithrombin to thrombin and results in antitrombin resistance in mutation carries. Besides antithrombin resistance, increased endogenous thrombin potential and decreased prothrombin clotting activity with normal prothrombin antigen was reported in carriers. However, the thrombin/thrombomodulin interactions in Belgrade mutation carriers have not been elucidated so far.

Aims: We aimed to analyze the effects of the prothrombin Belgrade mutation on thrombin/ thrombomodulin interaction in a large Serbian family with this mutation.

Methods: Plasma samples from 19 family members (10 mutation carriers and 9 non carriers) were tested. We tested thrombin/thrombomodulin interaction using previously described method based on fibrinogen clotting assay. The *Oxyuranus scutellatus* venom with phospholipid and $CaCl_2$ was used for prothrombin activation. The activity was determined by fibrinogen clotting assay performed with 20µg/mL recombinant soluble thrombomodulin (+TM) or without (-TM). The results were normalized with standard human plasma (100%) and residual activity ratio (+TM/-TM) was calculated.

Results: The residual activity ratio was significantly increased ($p < 0.001$) in mutation carriers (18.94±2.30) in comparison to non-carriers (14.5±2.91), indicating the presence of thrombomodulin resistance in prothrombin Belgrade mutation carries. The increased residual activity ratio was associated with prothrombin Belgrade mutation, regardless carriers' age and gender.

Conclusions: Our results indicate that both antithrombin and thrombomodulin resistance are involved in complex mechanism by which prothrombin Belgrade influences haemostatic balance and leads to thrombophilia.

PB 844 | Hereditary Factor X (FX) Deficiency in Females: Treatment with a High Purity Plasma-derived Factor X Concentrate

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Background: A high-purity plasma-derived FX concentrate (pdFX) has been developed for treatment of hereditary FX deficiency, an autosomal recessive disorder.

Aims: This post hoc analysis describes the pharmacokinetics, safety, and efficacy of pdFX in 10 female subjects with hereditary FX deficiency.

Methods: In this open-label study, subjects (10 female, 6 male) aged ≥12 years with moderate or severe FX deficiency (basal plasma FX activity ≤5 IU/dL) were enrolled and received 25 IU/kg pdFX for on-demand treatment of bleeding episodes or preventative use for up to 2 years. All subjects provided informed consent and the protocol was approved by appropriate independent ethics committees.

Results: Nine female subjects had severe and 1 had moderate FX deficiency, were aged 25.5 (median; range 14-58) y, and received a total of 267 pdFX infusions (178 for on-demand and 89 for preventative treatment). Male subjects (5 severe and 1 moderate FX deficiency) received a total of 159 pdFX infusions (64 on-demand; 95 preventative). The mean number of infusions per subject per month was higher among females (2.48) than males (1.62). The mean pdFX incremental recovery was similar between females and males (2.05 vs 1.91 IU/dL per IU/kg, respectively), as was mean half-life (29.3 and 29.5 h, respectively). Among females, 132 assessable bleeding episodes (61 heavy menstrual bleeding, 47 joint, 15 muscle, and 9 other) were treated with pdFX. Females reported a treatment success rate (ie, subject rating of "excellent" or "good" response to pdFX) of 98%, comparable to the 100% treatment success rate among males. After study completion, 2 subjects received pdFX for hemostatic cover during obstetric delivery. Additional infusion, bleed, and safety data will be presented.

Conclusions: These results show that in female subjects with moderate or severe hereditary FX deficiency, pdFX was safe and effective and had a pharmacokinetic profile similar to that of males.

Funding: Bio Products Laboratory

PB 845 | Factor VII Deficiency: A Center Experience

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Background: Factor VII deficiency is the most frequent among rare inheritable bleeding disorders affecting 1/500000 people. Its prevalence is probably higher owing to the presence of asymptomatic patients. Mostly, surgical bleeding can be their first symptom, when spontaneous bleeding is negative. The diagnosis is determinate by FVII:C level; the clinical manifestations are heterogeneous. The reliable classification of bleeding severity activity levels is not still clear-cut, and there is an absence of a consistent correlation between symptoms and FVII clotting levels(FVII:C), that becomes so important, to achieve a safe and standardized substitution treatment for this congenital disorders.
Aims: Evaluat the clinical behavior of FVII deficiency patients,followed in Congenital Coagulopaties Center of HospitalSãoJosé
Methods: Review of the patients with FVII deficiency,followed in our Hospital,from January2007 until January2017,according FVII:C levels,bleeding complaints and the implemented replacement therapy.
Results: We observed 41 patients: 2 patients with FVII:C level< 1%:one male with severe bleeding disorder, mostly haemarthrosis that is receiving prophylactic therapy with rFVIIa (30µg/2xweek) with good results and one women with abundant menorrhagia before menopause, but without spontaneous bleeding, since then. All the others patients were mildly affected or asymptomatic, and no correlation was seen among the laboratory and clinical manifestations. We managed 28 high bleeding surgeries in 26 patients: In all of them was administered rFVIIa (15-30µg/kg) previously to the surgery. 1 or 2 administrations were sufficient in most patients. No adverse events were observed.
Conclusions: Despite de FVII:C level, the bleeding symptoms of our patients confirm what is read in the literature. All the surgeries occurred without complications. It is not well established the minimal level of FVII:C to guarantee the haemostasis in these patients, being the clinical bleeding history very important mostly if the FVII:C is > 20%.

PB 846 | ADP and ATP Inhibit Factor X Activation by Factor VIIa/Tissue Factor

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Background: ADP and ATP are released by activated platelets and make important contributions to platelet aggregation. ADP and ATP have both been shown to reduce the ability of certain membrane-interactive proteins such as synaptotagmin-1 to bind to negatively charged phospholipids like phosphatidylserine (PS). Factor X activation by the factor VIIa/tissue factor (VIIa/TF) complex is the initiation point of the blood coagulation cascade, and this reaction relies on membrane PS for function. We hypothesized that ADP and ATP may inhibit factor X activation by VIIa/TF by disrupting PS-binding by factor X and/or VIIa.
Aims: Investigate the effect of ADP and ATP on factor X activation by VIIa/TF.
Methods: We measured initial rates of factor X activation using various combinations of factor X, factor VIIa, TF and phospholipids in the presence of AMP, cAMP, ADP or ATP.
Results: Rates of factor X activation by VIIa/TF were significantly reduced, in a dose-dependent manner, by micromolar to low millimolar concentrations of ADP or ATP (Fig. 1), while equivalent concentrations of AMP, cAMP or soluble phosphoserine had no effect. Phospholipids were not required for this inhibitory effect, as assays using soluble TF without lipids yielded similar results. ADP and ATP had little to no impact on the amidolytic activities of Xa or VIIa/TF, so the effect is likely focused on recognition of factor X as a substrate by VIIa/TF. Interestingly, activation of factor X by factor VIIa without TF was only slightly reduced by ADP and was actually enhanced by ATP.
Conclusions: ADP and ATP inhibit factor X activation by VIIa/TF in a lipid-independent and TF-dependent manner. They may serve as allosteric regulators of VIIa/TF under certain conditions.

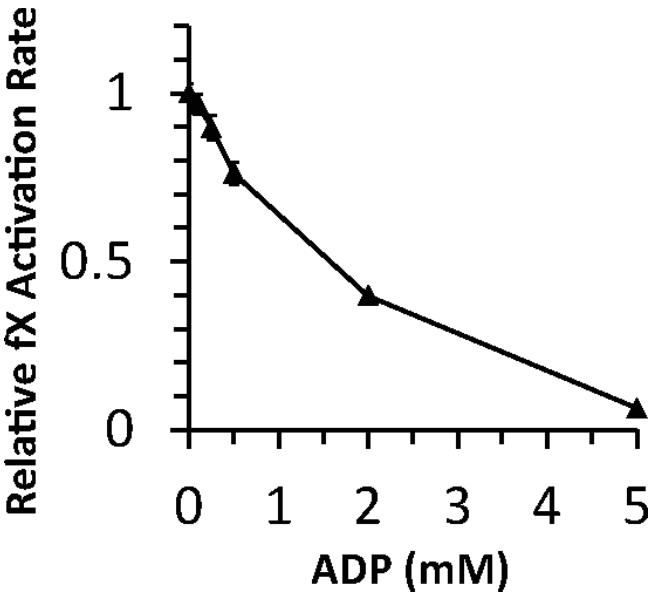

FIGURE 1 Factor X activation by factor VIIa + TF (relipidated in 85:15 PC:PS liposome) in the presence of ADP

PB 847 | Intracranial Hemorrhage in Neonates and Infants with Congenital Bleeding Disorders: The Experience of Çukurova University Hemophilia Center

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Background: Intracranial hemorrhage (ICH) is a life threatening complication of hereditary bleeding disorders in childhood resulting in high rates of mortality and disabling sequelae.

Aims: In this study, we evaluated retrospectively of our patients with intracranial hemorrhage and compared with literature.

Methods: From 1995 to 2016, 16 patients with intracranial hemorrhage were diagnosed in Çukurova University Hemophilia Center. ICH episodes, the findings of physical examination, CT scan or MRI and treatment strategies including surgical interventions were evaluated retrospectively.

Results: We reported 17 episodes of ICH from 16 patients with hereditary congenital factor deficiencies (CFD). Age range was from 1 day to 24 months. Three patients were in the neonatal period. There were 12 patients with severe hemophilia A, 1 patients with severe hemophilia B, 1 patient with factor VII deficiency, two patients with factor X deficiency. Except one patient with hemophilia A, all patients had one ICH bleeding episode. One patient had a high titer inhibitor against factor VIII. The most important factor of ICH was trauma. A history of recent trauma was documented in 11 patients. Intracerebral and subdural hematoma were more frequently seen. The most frequent symptoms were seizure and loss of consciousness. The diagnosis of congenital bleeding disorders were established in 6 patients after intracranial hemorrhage who referred to our center with ICH. In 5 patients, hematoma was evacuated by surgery. The only one hemophilia patient died due to ICH and one patient presented late sequelae.

Conclusions: Intracranial hemorrhage is the most serious complication for hereditary bleeding disorders in childhood. Urgent establishing diagnosis and quick starting treatment with prompt doses of factors to initially maintain a normal level of circulating factor is mandatory. But, despite the prompt treatment, death and late sequelae can be seen in this patients group.

PB 848 | A Novel Mutation in the F7 Gene in a Tunisian Patient with Factor VII Deficiency

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Background: Factor VII deficiency (FVIID) is a rare genetic bleeding disorder caused by decreased or absence of FVII. Its clinical expression is highly variable and the severity of the hemorrhagic syndrome is not correlated with the residual levels of FVII activity. Mutation of the factor 7 (F7) gene is the primary cause of FVIID.

Were ported the case of A 50 years old woman, who was first diagnosed as having FVIID in the context of a preoperative assessment. She suffered from menorrhagia and hematoma. Her FVII activity level is of 6%.

Aims: Our aim is to provide the mutation analysis for this woman affected with FVIID.

Methods: DNA was extracted from peripheral blood samples from the proband using standard protocol. All exons and flanking sequence of the FVII gene were amplified with PCR and then subjected to direct sequencing.

Results: The diagnosis of FVIID was confirmed by genetic testing, which revealed a faux-sens mutation in exon 9 of F7 (c.1382T>C, p.P461L). To the best of our knowledge, this is a novel mutation that has not been reported previously. Our proband carries out the novel mutation in the homozygous state, additionally she was a heterozygous for the reported p. R413G mutation localized also in the same exon of *F7* gene.

Conclusions: More investigations of the novel mutation p.P461L are necessary in order to explain its impact on the Factor VII protein and also to identify the effect of its interactions with the reported mutation p. R413G on the protein structure which make us able to correlate with the phenotype data of our proband.

PB 849 | A Rat-human Cross-species Compatibility Study: Catalytic Efficiency for Activation of Rat FX Is Decreased for Human FVIIa Compared to Rat FVIIa

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Background: In vitro studies of cross species molecular interactions constitute an important tool for understanding the limits in pharmacological animal models used to test human proteins. Under normal conditions, activated coagulation factor VII (FVIIa) requires the binding of Tissue Factor (TF) to attain full catalytic activity towards activation of factor X (FX). Pharmacological doses of recombinant human FVIIa (hFVIIa) activate FX on the surface of activated platelets, independently of TF. Consequently, the use of animal models to test hFVIIa is dependent on the binding to and ability to activate endogenous FX.

Aims: Enzyme kinetic analyses of TF-dependent and TF-independent FX-activation by human and rat FVIIa using purified proteins and rat whole blood.

Methods: Recombinant rat FVIIa (raFVIIa) and soluble TF (sTF) were expressed and purified. Binding affinity between rat and human FVIIa-TF were assessed by surface plasmon resonance. Kinetic parameters for FVIIa proteolytic activity were evaluated for both the autologous (hFVIIa:hsTF, raFVIIa:rasTF) and heterologous (hFVIIa:rasTF, raFVIIa:hsTF) complexes, as well as for FVIIa alone, in the presence of phospholipids. Finally, pro-haemostatic effect of hFVIIa and raFVIIa were compared in rat whole blood thrombelastometry (RoTEM).

Results: hFVIIa was capable of activating rat FX both in the absence and presence of TF. However, the catalytic efficiency (k_{cat}/K_M) of FX-activation was 4-fold reduced for hFVIIa relative to the rat ortholog.

Moreover, hFVIIa demonstrated lower potency than raFVIIa in rat whole blood RoTEM. Binding kinetics of heterologous FVIIa:sTF complexes revealed considerably lower binding constants relative to autologous complexes.

Conclusions: hFVIIa is able of activating rat FX, although with a decreased catalytic efficiency compared to raFVIIa alone or in the presence of sTF. These kinetic parameters may explain the decreased potency of hFVIIa in the rat whole blood TEG analysis.

PB 851 | Investigations of Plasma-soluble Fibrin: Implications of Enhanced Functionally-assayed Fibrinogen Level, Erythrocyte Sedimentation, Fibrin Self-assembly, and Clot Lysis

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Background: Fibrin(ogen) complexes, increased in various disorders, are poorly understood. We investigated complex-enriched (FgR) and depleted (FgD) fibrinogen (Fg) isolates each previously defined by its molecular imprint, solubility (4°), and fiber generation capacity.

Aims: Investigations in afibrinogenemic plasma (AP) of FgR and FgD effects on fibrin self-assembly, Fg assays, clot lysis, and erythrocyte sedimentation rate (ESR).

Methods: Isolates were ≥ 96% clottable, plasminogen-free, phenylmethylsulfonyl fluoride-treated, and their thrombin- or Reptilase-induced self-assembly was monitored by turbidity. Plasminogen activator-induced lysis of clots prepared in 20% AP containing 8 mM $CaCl_2$, and gel-sieved platelets $2x10^5$/μL, pH 7.4 was monitored by thromboelastography. For plasma Fg levels thrombin time (TT)- and prothrombin time (PT)-based assays were used. ESR of mixtures of 30% AP, pH 7.4, washed erythrocytes (Hematocrit 33%), Fg 1-4 mg/ml were monitored by a gravity-dependent procedure.

Results: FgR clots displayed shorter onset times and ~2x higher turbidity maxima, than those of FgD. By TT assay, FgR yielded Fg levels 2-2.5x higher (n=5) than predicted or those of FgD. By PT assay Fg levels were ~1.5x higher (n=2). Lysis was faster by FgR clots, n=3. For example, FgR and FgD clot lysis times were 12.67 and 37.75 minutes, respectively. FgR increased ESR, in contrast to FgD, n=4. For example, at Fg 3 mg/ml, ESRs were FgR 29, FgD 11, des-αC FgR 6, AP control 4 mm/hour, and at 0.5 and 4 FgR mg/ml, they were 5 and 47 mm/hour, respectively.

Conclusions: Faster FgR (than FgD) clot lysis is attributable to coarser fiber networks. TT-assayed Fg significantly above its actual level reflects rapid FgR self-assembly enhanced by plasma proteins. RBC-Fg links are αC-dependent, and accelerated ESR likely reflects the higher density of FgR- than of FgD-bearing RBCs. The plasma Fg level and ESR effects may also occur in clinical disorders with creased soluble fibrin and affect interpretations of corresponding test results.

PB 852 | Correlation Analysis of Standard Bethesda Method versus Modified Nijmegen Assay in Detecting Factor VIII Inhibitors, and Impact of Marginal Titer Inhibitors on FVIII Pharmacodynamics and Pharmacokinetics in Patients with Hemophilia A

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Background: Inhibitors of FVIII represent major complication of hemophilia replacement therapy.

Aims: To perform correlation analysis of inhibitors titres investigated by classical Bethesda and modified Nijmegen methods and to evaluate an impact of marginal inhibitor titres on pharmacodynamics and pharmacokinetics of administered FVIII.

Methods: We compared the results of 265 parallel inhibitor measurements by Bethesda and Nijmegen assay in 77 severe hemophilia A patients; out of them 60 patients with negative history of inhibitors (Group 1) and 17 patients with a history of inhibitors after inhibitor eradication by immune tolerance induction (ITI) (Group 2).

Results: Nijmegen method excluded a false positivity of low titer inhibitors between 0.51 and 0.9 BU/mL detected by Bethesda method and a good correlation between cut-off 0.7 Bethesda units/mL (BU/mL) and 0.5 Nijmegen BU/mL (NBU/mL) was confirmed. Comparison of 120 and 72 investigations of FVIII pharmacodynamics in Group 1 (inhibitor titre of 0.2±0.1 NBU/mL) and in Group 2 (inhibitor titre 0.5±0.15NBU/mL), respectively, showed in vivo recovery 109±19.4 (range 60-160)% and 86±32 (44-136)%, and incremental response 2.2±0,7 (1.4-3.5)%/1IU/kg and 1.6±0.6 (0.72-2.8)%/1IU/kg, respectively. Twenty pharmacokinetics studies in 20 patients from Group 1 and 30 studies in 14 patients from Group 2 showed a half life of 11,5±1,8 (8,2-16.2) h and 8.14±3.61 (2.9-16.4) h, respectively, and a clearance of 3.7±1.2 (2,6-6,5) mL/kg/h v.s. 6.48±2.24 (2.9-11.9) mL/kg/h, respectively ($p < 0.05$).

Conclusions: The Nijmegen assay is now a reference method for inhibitor testing with negativity cut-off 0.5 NBU/mL. Despite inhibitor eradication by the ITI in several patients inhibitor negativity does not correlate with pharmacodynamics and pharmacokinetics of FVIII suggesting the persitence of inhibitors undetectable by classical coagulation methods. For reliable ITI success evaluation more sensitive and specific methods are required.

PB 853 | Differences in Levels of Markers of Inflammation and Endotelial Damage between the Blood from Varicose Veins and Systemic Blood

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Background: Varicose veins (VV) represent one of the most frequent vascular diseases and are in most cases benign. However advanced disease is frequently associated with complications including thrombosis of the superficial veins. The pathogenic mechanisms responsible for development of thrombotic complication remain unclear.

Aims: The aim of our study was to investigate changes of blood constituents in VV and compare them to the systemic markers of inflammation and endothelial damage.

Methods: The study included 50 patients with primary VV. Local blood samples were taken from the leg, obtained from the dilated varicose great saphenous vein and systemic blood samples from the cubital vein.

Results: In VV, the following markers of inflammation and endothelial damage were significantly increased in comparison to systemic blood: hsCRP (2,05 ± 1,84 mg/L vs. 1,96 ± 1,90 mg/L, p =0,05), IL-6 (3.62 ± 2.74 ng/L vs. 2.30 ± 1.80 ng/L, p < 0,001), IL-8 (21,15 ±15,28 ng/l vs. 18,74±12,30 ng/L, p=0,025), vWF (118.4±27 % vs. 83.2±22 %, p< 0.05), NGAL (316,93±88,68 mg/mL vs. 299,18 ± 107,33 ng/mL, p=0,002), TNFR1 (0,52±0,13ng/mL vs. 0,50 ± 0,15 ng/mL, p=0,021), D-dimer (247,56 ± 410,24 ng/mL vs. 67,66 ± 325,74 ng/mL, p =0,019) and PAI-1 (3,32±3,56 IU/ml vs 2,99 ± 3,36 IU/ml, p=0,043).

Conclusions: In the blood of VV, some inflammatory markers and indicators of endothelial dysfunction are increased. This is most probably the consequence of deteriorated blood flow in dilated and tortuous superficial veins and increased venous pressure. Damage to the venous wall, which causes a chronic inflammatory response, together with the procoagulant properties of local blood may promote further progression of the disease and thrombotic complications.

PB 854 | Intracranial Hemorrhage in an Infant with Factor V Deficiency

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Background: Congenital deficiency of factor V is a rare, autosomal recessive hereditary coagulation disorder. Affected patients become symptomatic in early childhood with spontaneous or post-traumatic bleeding complications. Bleeding usually occurs when the factor V level is below 20% of normal.

Aims: In this report, the case of an infant with an intracranial hemorrhage due to congenital factor V deficiency is reported.

Methods: Two months old girl infant, presented with recurrent seizures. At admission, her coagulation parameters were as follows: activated partial thromboplastin time was 173.9 seconds (control 33 seconds), prothrombin time 65.6 seconds (control 14 seconds) with international normalized ratio of 5.6. Hemoglobin level was 5.7 gr/dl, platelet count 615.000/mm3.

Results: Subsequent coagulation assays revealed a plasma factor V activity of %9 with all other coagulation factors in the normal range. All other blood and chemistry tests were normal. Computerized tomography scan showed a parenchymal hematoma at the right superior fronto-parietal region, She was managed conservatively with fresh frozen plasma (FFP) and supportive management. Transfusions of FFP and platelet concentrate caused a temporary normalization of coagulation profile and then the hematoma was drained. Postoperatively, she was treated with FFP daily for two weeks.

Conclusions: We also wanted to take note that a patient with factor V deficiency can be seen with intracranial bleeding. Prolonged PT and PTT and normal platelet count would be an initial pointer towards an inherited disorder of coagulation such as factor V deficiency. As there is no specific concentrate available, the mainstay of treatment for severe factor V deficiency is FFP. Platelets also contain factor V, though their use should be reserved for life threatening bleeding and prior to surgery. Regular infusions of FFP are required in patients with a history of severe bleeding complications.

PB 855 | Detection of Specific and Non-specific Inhibitors in Patients of Hemophilia A

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Background: Inhibitors are antibodies that the immune system develops because it sees the infused clotting factor as a foreign substance that needs to be destroyed. People with severe hemophilia A are more likely to develop inhibitors.

Aims: To screen and measure the F.VIII antibodies, Dilute Russell's viper venom test (DRVVT) and anticardiolipin-antibodies (ACLA) in patients of moderate to severe Hemophilia A(HA).

Methods: This cross sectional and descriptive study was conducted at NIBD Karachi in accordance with Declaration of Helsinki. Inhibitor screening was performed by activated partial thromboplastin time (APTT), mixing studies using normal pool plasma immediately and after 2 hours incubation at $37C^0$ after receiving request of clinicians. Bethesda assay for quantification of factor VIII inhibitors, DRVVT, ACLA (IgM/IgG) were performed on samples which were positive with screening tests.

Results: Total 19 patients were included in this cross sectional with mean age 13 ± 8 SD. All these patients had prolonged APTT were later requested for inhibitors screening in lab .Out of 19, 11 patients (58%) were positive for specific inhibitors and results noted after 2hrs.

The remaining 8 patients (42%) were positive for non-specific inhibitors including 5 patients with positive ACLA IgM antibodies and 3 had DRVVT. Patients with positive specific inhibitors (36%) were low responders (< 5 Bethesda units) with a mean Bethesda units of 2.3+1.1 SD, while 7 patients (64%) were high responders (>5 Bethesda units) .Bleeding manifestation was moderate to severe in all patients with positive inhibitors.

Conclusions: The inhibitor development in patients with hemophilia A receiving recombinant factor VIII concentrates therapy is more crucial in first fifty therapies. The development of inhibitory antibodies in patients with HA still remains a major complication of therapy.

PB 856 | Interim Safety of a Long-acting Recombinant Factor VIIa (MOD-5014): A Phase 1 Study in Adult Subjects Following Subcutaneous Administration

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Background: OPKO is a clinical-stage company developing long-acting versions of therapeutic proteins utilizing CTP technology. This involves fusion of the hCG C-terminal peptide to proteins. The technology was clinically validated for several drugs while maintaining their biological activity. MOD-5014 is a long-acting form of recombinant FVIIa (rFVIIa-CTP) being tested for the first time in healthy volunteers using subcutaneous (SC) administration.

Aims: To assess the acute safety, tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) profiles of single SC administration of escalating MOD-5014 doses in healthy subjects.

Methods: A single-dose, open-label, dose-escalating study was performed at MOD-5014 doses of 100, 200, 400 and 600 µg/kg, with 6 subjects in each dose group on active treatment and two receiving placebo. Dosing of all subjects in each cohort was followed by a 7-, 14- and 30-day safety observation period. Safety assessments included regular monitoring of adverse events (AEs), injection site reactions, vital signs and physical condition, as well as laboratory assessments such as hematology, biochemistry, coagulation panel and immunogenicity.

Results: The interim analysis included safety data for all subjects that were doses with 100, 200 and 300 µg/kg. The reported AEs were all considered unrelated or unlikely related to study medication and none led to premature study discontinuation. Laboratory assessments supported the tolerability of MOD-5014 treatment, and no significant overall changes were observed in hematology or chemistry profiles. There was no consistent or meaningful pattern for changes in any coagulation parameter during the study attributable to the study intervention.

Conclusions: MOD-5014 demonstrates promising safety and tolerability profile following single SC administration of escalating MOD-5014 doses (100, 200 and 400 µg/kg) in healthy subjects with no unexpected AEs considered to be related to MOD-5014.

PB 857 | Replacement Therapy in ALL Patients Treated with L-asparaginase

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Background: L-Asparaginase (L-Asp) therapy is associated to both thrombotic and hemorrhagic events during treatment of acute lymphoblastic leukemia (ALL). In this setting, balance between the risk of bleeding and thrombosis requires a careful approach. In 2016 our group adapted the protocol described by Biddeci et al. for prophylactic replacement therapy with Fibrinogen (FI) Concentrate and Antithrombin (AT) Concentrate.

Aims: To evaluate transfusion needs and its impact after protocol implementation.

Methods: From 2014, a total of 26 patients (15 males, 11 females; median age 35 years, 22 Bprecursor-ALL, 4 T-ALL) were treated with intensive chemotherapy (22 HOVON 100, 4 CLCG protocol) comprising scheduled doses of L-Asp. During L-Asp treatment, Prothrombin Time, activated Partial Thromboplastin Time, FI and AT were determined in all patients. After 2016, we also introduced anti-Xa and Maximum Clot Firmness (MCF) at FIBTEM to help transfusion decision.

FI Concentrate was transfused when FI was < 100mg/dL (Clauss test) and MCF at FIBTEM thromboelastogram ≤5 and AT Concentrate was given when AT was < 50% and anti-Xa levels were < 0.2 with Low Molecular Weight Heparin (LMWH).

Results: 10 of the 26 patients were enrolled after the protocol implementation. There were no differences between the 2 groups (before and after protocol) concerning age, gender, ALL phenotype, risk, hyperleukocytosis, LDH level, SNC involvement, coagulation at diagnosis and treatment response. All patients but 1 started prophylactic LMWH during L-Asp therapy in the protocol group but only 7 (44%) were given LMWH before 2016. A total of 5 thrombotic and haemorrhagic events were recorded (only 1 in the protocol group, χ^2 p=0.35). In the protocol group AT levels were inferior (median 0.50 vs 0.64, p< 0.05) and these patients were less transfused, namely with plasma.

TABLE 1 Replacement therapy transfusion differences between the 2 groups. SD = standard deviation

	Before Protocol	After Protocol	
	(mean ± SD)	(mean ± SD)	t-test
Fibrinogen Concentrate (g)	3.6 ± 3.7	4.4 ± 4.7	0.664
Plasma (units)	1.1 ± 2.2	5.3 ± 7.2	0.046
Antithrombin Concentrate (UI)	3000 ± 2000	5100 ± 5850	0.204

Conclusions: Although there were no differences in haemorrhagic or thrombotic events, there was a trend to transfuse less when MCF at FIBTEM and anti-Xa levels were determined.

PB 858 | Hemarthrosis in Rare Factor Deficiencies

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Background: Hemarthrosis is severe and debilitating manifestations occurring in patients with rare factor deficiencies(RFDs). Despite its clinical importance, little has been published on the frequency and complication.

Aims: The aim of this study was to retrospectively collect data on patients affected with RFDs who had hemarthrosis.

Methods: In this study, our purpose is to assess 17 patients retrospectively, who were diagnosed and treated rare factor deficiencies presenting hemarthrosis between 1990 and 2016. Information of patients with hemarthrosis were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005.

Results: We identified 24 episodes of hemarthrosis in 17 patients. Eleven (65%) of the patients were male and 6 (35%) were female. The median age of the group was 8 (range, one month to 24 year). The presenting ages were ranging between one month to 17 years. There were 9 patients with FVII deficiency, 3 patients with FX deficiency, 3 patients with afibrinogenemia, one patient with FV+FVIII deficiency, one patient with FXIII deficiency. All patients had one bleeding episode, except seven patients with six FVII and one FX deficiency. The levels of Factor:C were as follows: 10 (59 %) patients; < 1%, 3 (17.5%) patients; 1-5% and 4 (23.5 %) patients; >5%. Among 24 hemarthrosis, knee localization were the most common site (n=15, 62.5%). It is followed by ankle (n=5, 20.9%), elbow (n=2, 8.%) and shoulder (n=2, 8.3%). A history of trauma was documented in two cases (8.3%). Recurrent hemarthrosis were observed in five patients. All patients were submitted to replacement therapy. Prophylaxis was applied to 8 patients (47%).

Conclusions: Hemarthrosis is less common than hemophilia, although the characteristics of joint destruction are similar in the RFDs. In our study, we discussed the treatment hemarthrosis in RFDs and evaluated the conditions which required prophylaxis.

PB 859 | Amyloidosis Induced Abnormal Coagulopathy Associated with Multiple Coagulation Factor Deficiencies

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Background: When amyloidosis leads to a coagulopathy, it is most often acquired factor X deficiency, but there are rare reports of amyloidosis being associated with other acquired factor deficiencies.

Aims: Herein, we aimed to report an amyloidosis induced abnormal coagulopathy associated with multiple coagulation factor deficiencies.

Methods: 51 years old women had applied to our clinic for investigation of her bleeding diathesis.

Results: 8 months before the admission, she had abnormal bleeding episode during a surgical procedure in another hospital. After this procedure, she also had gingival bleeding and extensive bleeding during upper gastrointestinal endoscopy. She had 8 healthy children with no history of abortion. She had hypermenorrhoea for last 8 months. In her laboratory test in our clinic was resulted as; hemoglobin 10 gr/dl, white blood cell $13x10^3$/µl, platelet $653x10^3$/µl, transferrin saturation %6, APTT 34 s, APTT mixing 28 s, INR 1.4, ALT 11 u/l, AST 26 u/l, creatinine 1.4 mg/dl, factor VII 61%, factor X 68%. Her thrombocyte aggregation was abnormal with ADP and epinephrine. In her blood film was normal. In her urine protein electrophoresis (PE) glomerular proteinuria was detected. Her serum PE was non-specific. Rectal biopsy resulted as focal active colitis, renal biopsy revealed renal amyloidosis. Colchicine was started for our patient.

Conclusions: In systemic AL-amyloidosis with acquired factor X deficiency, and the baseline factor X level is not predictive of bleeding risk. Although amyloidosis is frequently associated with factor deficiency, combined factor deficiencies are rarely seen with amyloidosis. Our patient had F-VII and F-X deficiencies as well as platelet aggregation disorder which lead to bleeding diathesis. To conclude, herein we have presented an amyloidosis induced abnormal coagulopathy case associated with multiple coagulation factor deficiencies.

PB 860 | Protein S Inhibits Apoptosis of Islet β Cells via Regulating BIRC3 Expression and AKT Signaling

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Background: Protein S (PS) is a vitamin K-dependent anticoagulant protein. It functions as a cofactor of activated protein C to inactivate FVa and FVIIIa. PS can also regulate inflammatory responses and apoptosis. The effect of PS on glucose tolerance is unknown.

Aims: To evaluate the effect of PS on streptozotocin(STZ)-induced diabetes by *in vitro* and *in vivo* studies.

Methods: *In vivo* experiments were performed using human PS transgenic and wild type(WT) mice . Diabetes was induced by i.p. injection of STZ for 5 consecutive days. Mice were categorized into 4 groups; WT/SAL, PS/SAL,WT/STZ and PS/STZ. Glucose tolerance test and glucose-induced insulin secretion test were performed. Mice were killed after 4 weeks of injection. Immunostaining of insulin was performed and apoptosis of β cells in islets was assessed by TUNEL

method. *In vitro* experiments were performed using MIN6 cells, murine β cell line. Apoptosis was induced with STZ in the presence of hPS or SAL. Apoptotic cells were assessed by flow cytometry after staining with annexin-FITC and PI. Phosphorylation of Akt/PKB and IκB was assessed after treating MIN6 cells with and without hPS (20 μg/ml) by flow cytometry and Western blot. Expression of anti-apoptotic proteins including BIRC3 were assessed by RT-PCR.

Results: Significant high insulin secretion and amelioration of glucose tolerance were observed in PS/STZ compared with WT/STZ. The insulin stained area was significantly increased, whereas the area with apoptotic cells was significantly decreased in PS /STZ compared with WT/STZ. Apoptosis of MIN6 cells induced by STZ was significantly suppressed in the presence of hPS. There was increased phosphorylation of Akt/PKB and IkB treated with hPS compared with controls. The expression of BIRC3 was significantly increased by hPS compared to controls.

Conclusions: hPS protects pancreatic islet β-cells from apoptosis and attenuates STZ-induced diabetes via stimulating the activation of the AKT pathway and by enhancing the expression of BIRC3 anti-apoptotic protein.

PB 861 | Spatiotemporal Modulation of the Protease Activated Receptors Signaling Protects against Diabetic Nephropathy

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Background: Podocytes are terminally differentiated and form a crucial component of the glomerular filtration barrier. Cytoskeletal changes in these specialized epithelial cells lead to gross morphological changes in the podocyte foot processes and subsequent proteinuria in diabetic nephropathy (DN).

Aims: We recently established that the coagulation protease activated protein C (aPC) protects mice from diabetic nephropathy. aPC's cytoprotective response is achieved through its modulation of G protein coupled receptor-protease activated receptor (GPCR-PAR) signaling.

Methods: Here we show that PAR's cellular response can be regulated spatiotemporally by aPC. Upon ligand binding, PAR2 and PAR3 are activated at the plasma membrane and initiate downstream signaling as seen in IP-WB. Upon internalization into early endosomes (observed in PLA and colocalization analyses) their trimeric G protein signaling is maintained for an extended period of time leading to the activation of RhoAGTPases, which is detrimental to the complex cytoarchitecture of the podocytes.

Results: Sustained signaling of PARs leads to enhanced albuminuria, glomerular basement membrane (GBM) thickening and podocyte foot process effacement.Further details regarding the endocytotic signaling of PARs in podocytes will be presented.

Conclusions: The current studies are expected to provide new insight into the endosomal GPCR-G protein system, potentially identifying therapeutic approaches which are more effective than global targeting of cell surface signaling.

PB 862 | Overexpression of Protein S Exacerbates Carbon Tetrachloride-induced Liver Injury and Fibrosis in Mice

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Background: Protein S (PS) is a vitamin K-dependent plasma protein with anticoagulant, anti-inflammatory and anti-apoptotic properties. PS regulates the inflammatory response and apoptosis by binding and activating the (Tyro-3, Axl, Mer) TAM family of receptor tyrosine kinases. We have recently shown that protein S is involved in alcohol-induced liver injury by activating and inhibiting apoptosis of liver natural killer T cells. However, the effect of protein S on liver fibrosis is unknown.

Aims: The purpose of this study was to investigate the effect of protein S on liver fibrosis by comparing the degree of fibrosis induced by carbon tetrachloride (CCl4) between protein S overexpressing transgenic mice and wild type mice.

Methods: To induce liver fibrosis CCl4 was injected intraperitoneally twice a week for six weeks in wild type and protein S transgenic mice both under C57BL/6 background. The experimental protocol was approved by the Institutional Committee for Animal Investigation and followed approved international guidelines.

Results: Compared with wild-type mice, human protein S transgenic mice were more susceptible to both acute and chronic CCl4-induced liver injury, as shown by higher levels of plasma transaminases, greater numbers of apoptotic hepatocytes, and more extended necroinflammatory foci. Human protein S transgenic mice also displayed a greater degree of liver fibrosis after chronic CCl4 injection compared to wild-type mice. Wild-type mice infused with human protein S exhibited exacerbated liver inflammation and fibrosis.

Conclusions: Protein S is involved in acute and chronic liver injury and subsequent liver fibrosis induced by CCl4.

PB 863 | Signal through the Regulatory Subunits of PI3Kinase Endows Coagulation Protease Activated Protein C with Hormone Like Function

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Background: Impaired insulin signaling in renal epithelial cells (podocytes) disrupts the physiological unfolded protein response (UPR) causing maladaptive endoplasmic reticulum (ER) processing and renal dysfunction. Pathways rescuing impaired insulin signaling remain poorly understood.

Aims: Given the nephroprotective effect of the coagulation protease activated protein C (aPC) in acute and diabetic kidney disease (DKD), we here investigated the role of aPC in regulating maladaptive ER-signaling which is mechanistically linked to DKD.

Methods: Compensatory cytoprotective effect of aPC in DKD was evaluated in mouse models with podocyte specific genetic deletion of the insulin receptor (INSR) and its signaling intermediates PI3K p85α and p85β. After 26 weeks of persistent hyperglycaemia in both type-1 (Streptozotocin-induced) and type-2 (db/db mice) diabetic mice, markers of DKD were determined and tissue samples were isolated for *ex vivo* analysis.

Results: We find that aPC rescues impaired renal insulin signaling by restoring insulin-dependent ER-homeostasis. In mice lacking the INSR in podocytes aPC efficiently restores ER-homeostasis by selectively targeting INSR downstream signaling intermediates p85α and p85β. Akin to insulin, aPC signaling through p85-subunits promotes nuclear translocation of the cytoprotective ER-transcription factor sXBP1. Genome-wide mapping of XBP1-transcriptional regulatory patterns demonstrated induction of concordant UPR-configurations involved in maintenance of ER-proteostasis by insulin and aPC.

Conclusions: These results demonstrate that coagulation protease signaling co-opts components of the insulin signalosomes, p85α and p85β, to combat insulin-resistance through ER-reprogramming.

PB 864 | Rivaroxaban Ameliorates Neuroinflammation

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Background: Multiple Sclerosis (MS) is a demyelinating, autoimmune disorder in humans, characterized by immune cell trafficking into the central nervous system (CNS). Pathophysiological analyses revealed MS lesions with cellular infiltrates that lead to demyelination and axonal damage. As a consequence, studies concerning pathogenesis and therapy focused on these cells so far. While the triggers of MS have not yet been identified, recent studies suggest that the coagulation system might be involved in MS development. For instance, it was shown that various coagulation factors like fibrinogen, thrombin and factor XII are involved in neuroinflammation.

Aims: Factor X (FXa) is another central constituent of the coagulation cascade, which is also known to be involved in inflammation and several diseases, e.g. inflammatory bowel disease. Within this project, we therefore further addressed the role FXa in CNS autoimmunity, using its direct, highly selective inhibitor rivaroxaban in a MS animal model, experimental autoimmune encephalomyelitis (EAE).

Methods: Therefore, Lewis rats were immunized with myelin basic protein and subjected to rivaroxaban-containing diet (2.1 mg/kg body weight) 7 days prior immunization over the course of the experiment.

Results: Treatment with rivaroxaban renders rats less susceptible to EAE and was accompanied by reduced infiltration of T cells, as well as microglia activation within the CNS, as shown by immunohistochemistry. However, flow cytometry analysis showed no alterations in the peripheral immune compartment.

Conclusions: Altogether, our study further supports the hypothesis of a cross-link between coagulation and inflammation and identifies FX as a possible therapeutic target in neuroinflammatory diseases, like MS.

PB 865 | Novel Role of Protein S in Clot Retraction: Induction of Apoptosis in Activated Platelets

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Background: Protein S (PS) is a gamma-carboxyglutamate domain-containing anticoagulant. PS also binds to phosphatidylserine residues of the cell membrane and, in so doing, promotes survival, apoptosis, and efferocytosis. Phosphatidylserine has a number of vital functions in platelet biology, including activation, clot formation, and clot retraction. In the current work, we elucidated a novel function of PS, *i.e.*, PS induces an apoptotic-like pathway in activated platelets that promotes clot retraction.

Aims: To investigate a role for PS in regulating activated platelets and clot retraction.

Methods: Platelet activation, ELISA, immunoblotting, FACS, clot retraction assay.

Results: We used an ELISA assay to demonstrate activation of platelets with extracellular ADP or with Par4 and convulxin. Activation of platelets induced mitochondrial depolarization, and a PS antibody reversed the mitochondrial depolarization. Further, PS induced an apoptotic-like pathway in the activated platelets, which we identified by immunoblotting and probing for phosphorylated p53, cleaved caspase, and cleaved PARP. This apoptotic-like pathway was inhibited by PS antibody. Clot retraction assays confirmed that the PS-mediated apoptotic-like pathway in the activated platelets is essential for clot retraction.

Conclusions: Our results demonstrated that, upon activation, platelets secrete PS into the extracellular milieu, it enhances phosphatidylserine exposure on the platelet surface, and PS induces an apoptotic-like pathway that is essential for clot retraction.

PB 866 | Thrombomodulin Dependent PC Activation Protects from a Senescence-like Tubular Phenotype in Diabetic Nephropathy (dNP)

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Background: The coagulation protease activated protein C (aPC) protects against glomerular damage in dNP. While the importance of tubular damage in diabetic nephropathy (dNP) is increasingly recognized, the role of thrombomodulin (TM) dependent protein C activation for tubular cell damage in dNP remains unknown. Tubular cell damage in dNP is characterized by a proliferative arrest, senescence and hypertrophy. Whether these hallmarks are regulated by TM-dependent PC activation remains unknown.

Aims: To evaluate aPC's effect on tubular damage (tubular fibrosis, hypertrophy and senescence) in dNP.

Methods: Wild type (Wt) and $TM^{Pro/Pro}$ (mice with severely reduced TM-dependent protein C activation) mice were used in this study. Type-1 diabetes (DM) was initiated using streptozotocin (STZ) and maintained for 22 weeks, followed by tubular morphometrical and ex-vivo analyses.

Results: While detailed histological analyses did not reveal a basal phenotype in non-diabetic $TM^{Pro/Pro}$ mice, tubular damage was increased in diabetic $TM^{Pro/Pro}$-DM mice as compared to Wt-DM mice. In addition, $TM^{Pro/Pro}$-DM mice displayed increased expression of the tubular injury marker KIM-1 and prominent fibrosis (Masson´s Trichrome staining). Enhanced tubular damage in $TM^{Pro/Pro}$-DM mice is associated with increased tubular hypertrophy (higher tubular surface area and tubular size, but constant number of nuclei/tubule). The proliferation marker (Ki-67) was decreased in WT-DM mice as compared to non-diabetic WT mice, and was even further reduced in $TM^{Pro/Pro}$-DM mice. Tubular senescence (SA-βgal. staining and p21 expression) was enhanced in $TM^{Pro/Pro}$-DM mice.

Conclusions: These data shed the light on the tubular-specific protective effect of coagulation protease aPC. Furthermore, they establish a mechanistic relevance of aPC´s for the premature ageing-like phenotype in the diabetic renal tubular cells.

PB 867 | Coagulation Proteases are Potential Regulators in the Development of Exercise-associated arterial Endofibrosis

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Background: High performance athletes can develop symptomatic arterial flow restriction during exercise, caused by endofibrosis. The latter characterized by fibrosis of the external iliac artery (EIA). Pathophysiological processes involved in the development of endofibrosis are unknown. However, evidence shows that coagulation enzymes such as thrombin and factor Xa (FXa) can influence pro-fibrotic processes, mediated through activation of protease activated receptors (PARs).

Aims: To determine immunohistochemical characteristics of endofibrosis to elucidate the potential role of coagulation factors and PARs in development of endofibrosis.

Methods: 19 arterial endofibrotic specimens were collected during endarteriectomy. As control 20 arterial segments of the EIA were collected post mortem from individuals, with no medical history of cardiovascular disease. Acquired arteries were used for immunohistochemical staining. Data were analyzed using a Mann-Whitney U test. Data is presented as median [IQR]. A 2-tailed $p< 0.05$ was considered as statistically significant.

Results: Endofibrotic segments contained a neo-intima, resulting in intraluminal stenosis (42%[33-51]). Compared to controls, endofibrotic lesions were highly positive for collagen (47%[31-63] vs 17%[10-19]) and elastin (41%[28-46] vs 14%[11-21]). These findings were accompanied by significantly increased alpha smooth muscle actin (51%[45-58] vs 38%[23-47) (Fig 1), which morphologically appeared to be myofibroblasts in endofibrotic lesions; which were hardly present in controls. In addition, PAR1 (55%[35-65] vs 23%[11-28]) and PAR4 (41%[38-55] vs 13%[10-16]) were significantly upregulated and pro-enzyme form of their activators, prothrombin (18±9% vs 3±2%) and factor X (23%[16-28] vs 5%[4-6) were abundantly present.

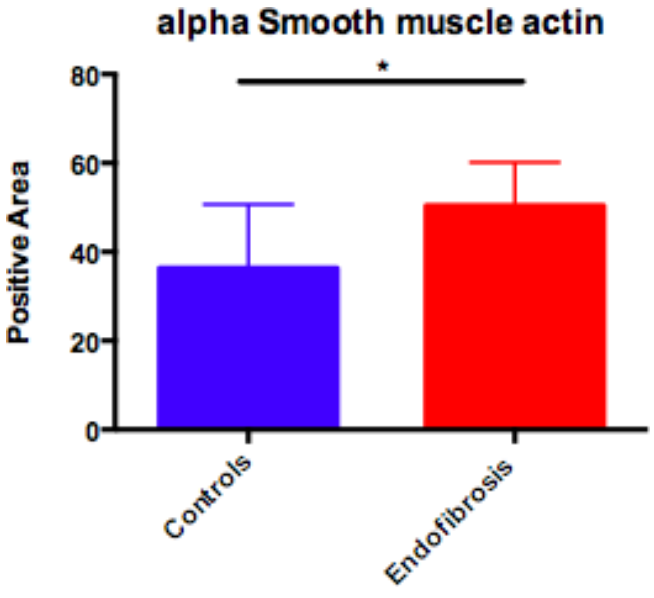

FIGURE 1: Significantly increased alpha Smooth muscle actin expression in endofibrotic lesions. *p<0.05

Conclusions: This is the first study to show that myofibroblasts might be a key factor in the development of endofibrosis. The special association suggests that these processes may be regulated through activation of PARs by coagulation proteases.

PB 868 | Biochemical and Pharmacological Differentiation of APC and Recombinant Thrombomodulin with Reference to the Effect on the Hemostatic and Fibrinolytic Processes

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Background: Both the thrombomodulin and activated protein C play an important role in the regulation of hemostasis. Recombinant version of both the thrombomodulin (rTM) and activated protein C (rAPC) have been developed for clinical applications. Marked differences in their biochemical and pharmacological profiles exist.

Aims: The aim of this study is to demonstrate differences between a clinically developed rTM (ART-123, Asahi Kasei Pharma, Tokyo, Japan) and a plasma derived APC (Haematologic Technologies, Burlington, VT, USA).

Methods: The anticoagulant profile of both agents was investigated in native whole blood using the ACT, TEG and global anticoagulant profile using TT, PT and APTT assays at a concentration range of 0-10 ug/ml. The global anticoagulant profile and antiprotease (Xa and IIa) inhibitory studies were also carried out. A modified TEG system was used to study the modulation of fibrinolysis by these agents.

Results: In the ACT assays APC produced a much stronger anticoagulant effect in a concentration range of 0-10 ug/ml with a doubling time at 3 ug/ml. Whereas rTM produced marginal effects on this parameter. The TEG profile of APC exhibited much stronger anticoagulant effects in comparison to rTM. APC also facilitated fibrinolysis in contrast to rTM. rTM showed a concentration dependent inhibition of fibrinolysis. In the APTT assay APC produced a much stronger anticoagulant effect whereas TM produced relatively weaker effects. Both agents did not produce any modification of agonist induced platelet aggregation. In terms of anticoagulant potency the APC exhibited 70 ± 6 USP U/mg whereas the rTM was much weaker < 10 USP U/mg.

Conclusions: These studies demonstrate that APC is a much stronger anticoagulant in comparison to rTM. In addition both agents produced different modulation of fibrinolytic and hemostatic processes. These observations suggest that at therapeutic levels each of these agents may have differential impact on hemostatic balance.

PB 869 | Plasminogen-deficient Patients

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Background: Congenital plasminogen (Plg) deficiency is a rare autosomal recessive disorder that leads to the development of ligneous membranes on mucosal surfaces.

Aims: Here we report our experience with local and intravenous fresh frozen plasma (FFP).

Methods: Our cohort consisted of 14 patients and their 8 first-degree relatives. The patients have been diagnosed between 3 months and 18 years of age, and the median age at the time of first clinical manifestation was 4.5 months (range 3 days to 12 months).

Results: Conjunctivitis is the main complaint, hydrocephalus and hearing loss follow. In 10 patients, ligneous membranes were surgically removed but all recurred. Nine patients were treated with intravenous and conjunctival FFP. Two patients had no complaints after treatment. Most patients needed transfusion with FFP every three weeks. Only one patient had severe endophthalmitis and lost vision in one eye before treatment. Two female patients and one male patient had undergone multiple surgeries for ligneous conjunctivitis despite being treated with FFP. The response rate to FFP treatment was 6/9 (66%). Another 8-year-old female with severe bronchial membranes was treated with FFP and t-PA through bronchoscopy. Venous thrombosis did not occur in any of the patients. Nine have consanguineous parents. The genetic evaluation of our patients revealed heterogeneous mutations as well as polymorphisms.

Conclusions: The diagnosis and treatment of Plg deficiency is challenging, and there is no consensus on treatment. Topical and iv FFP may be used with good clinical outcome.

PB 870 | Direct Factor Xa Inhibitor Apixaban Prevents Endothelial Activation and Damage Associated with Chronic Kidney Disease

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Background: Chronic kidney disease is a multisystemic condition resulting in endothelial dysfunction and enhanced cardiovascular risk. We have established a model of endothelial dysfunction in uremia characterized by a proinflammatory and prothrombotic phenotype and enhanced oxidative stress. Apixaban is a direct oral anticoagulant inhibiting factor Xa. Anti-Xa strategies may exert anti-inflammatory actions on some cell lineages.

Aims: To explore the protective effect of apixaban on the endothelial dysfunction associated with uremia.

Methods: Endothelial cells were exposed to media containing sera from healthy donors or uremic patients, in the absence and presence of apixaban (from 10 to 100ng/mL), to evaluate changes in: the expression of VCAM1 and ICAM1 on the surface, presence of von

Willebrand Factor (VWF) on the extracellular matrix (ECM), presence of intracellular eNOS, and the production of reactive oxygen species (ROS).

Results: Exposure of cells to uremic media resulted in an increased expression of VCAM1 and ICAM1 at their surface (of 47% and 65% vs controls); production of ECM enriched with VWF (increase of 30% vs control); decrease of intracellular eNOS (of 35% vs control) and higher production of ROS (% of increase of 35% vs control). Preventive treatment of cells with apixaban inhibited the endothelial damage caused by uremic media. Levels of VCAM1, ICAM1, VWF and ROS were diminished to control levels and eNOS was normalized ($p< 0.01$ for all parameters with 60ng/ml apixaban).

Conclusions: Apixaban prevented endothelial damage triggered by the uremic milieu, exhibiting anti-inflammatory and antioxidant properties. The protective effect of apixaban was noticeable at concentrations lower than those currently used for anticoagulation. Apixaban may provide an alternative therapeutic approach to prevent the endothelial dysfunction caused by uremia, and also by other pathological conditions with elevated cardiovascular risk.

PB 871 | A Factor X Activator from Caterpillar Triggers Cell Survival on Fibroblasts in Response to Serum Deprivation by Inhibition of Apoptosis

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Background: Fibroblasts are the main cellular component of connective tissues and play important roles in health and disease through the production of extracellular matrix (ECM) components. rLosac is a recombinant hemolin with factor X (FX) activation activity from the caterpillar *Lonomia obliqua*. In HUVECs, rLosac induced proliferation and cell survival.

Aims: To study the effect of rLosac on cell survival and cell migration of fibroblast submitted to serum deprivation.

Methods: For cell survival: MTT assay. For cell cycle, apoptosis, production of reactive oxygen species (ROS) and mitochondrial membrane potential (MMP): flow cytometer. For morphological changes: immunocytochemistry and scanning and transmission electron microscopy. For cell migration: wound scratch test assay.

Results: A reduction of Losac's activity on FX lead to a reduction on cell survival activity. rLosac has activity in serum deprivation conditions but not when nutrient is sufficient and caused an apparent dose-dependent increase in cells in the S phase of the cell cycle and a significant reduction of cells with fragmented DNA. Furthermore, treatment with rLosac results in a significant decrease in the production of ROS and MMP and up-regulation of Bcl-2 and a down-regulation of Bax protein levels. rLosac treatment reduces the morphological changes induced by prolonged serum deprivation (apoptotic bodies, nucleus fragmentation, cytoplasmic vacuolization and loss of ECM organization). Moreover, rLosac could enhance wound healing *in vitro*.

Conclusions: Altogether, these findings suggest that rLosac strongly induces cellular protection in conditions of stress by serum deprivation by inhibiting apoptosis. This finding opens a new perspective to further understand the role of this FX activator during cellular processes such as cell migration. Funding Agencies: Fapesp, Capes.

PB 872 | Protective Effect of Protein S against the Development of Diabetic Nephropathy in Mice

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Background: Diabetic nephropathy (DN) has long been recognized as the leading cause of end-stage renal disease, but the efficacy of available strategies for the prevention of DN remains poor. Protein S (PS) is a vitamin K-dependent anticoagulant protein. It functions as a cofactor of activated protein C to inactivate activated factor V (FVa) and activated factor VIII (FVIIIa). PS can also regulate inflammatory responses and apoptosis.

Aims: We evaluated the effect of PS on STZ -induced DN.

Methods: In one experiment, human PS (hPS) transgenic mice and wild type (WT) mice underwent unilateral nephrectomy and after 4 weeks of recovery, diabetes was induced using intraperitoneal injection of STZ. Mice were categorized into 4 groups: WT/saline (WT/SAL), hPS/saline (hPS/SAL), WT/STZ, hPS/STZ groups. After 8 weeks of hyperglycemic condition, the mice were sacrificed.

In a separate experiment, WT mice underwent unilateral nephrectomy and were made diabetic with STZ or kept as controls with saline after 4 weeks of recovery. Mice were then treated with either hPS or saline subcutaneously through osmotic minipumps for 4 weeks before they were sacrificed. Mice were categorized into 4 groups: saline/pump saline (SAL/p-SAL), saline/pump hPS (SAL/p-hPS), STZ/pump saline (STZ/p-SAL), STZ/pump hPS (STZ/p-hPS).

Results: In one experiment, compared with WT/STZ group, plasma creatinine concentration and the renal tissue concentration of hydroxyproline were significantly lower and renal fibrosis was histologically improved in hPS/STZ group.

In another experiment, the plasma creatinine concentration and the renal tissue concentration of collagen and hydroxyproline were significantly lower and renal fibrosis was histologically improved in STZ/p-hPS group compared with STZ/p-SAL group. Relative renal mRNA expression of TGF-β1 was significantly elevated, whereas that of podocin was reduced in STZ/p-SAL group compared with STZ/p-hPS.

Conclusions: Administration of exogenous PS or kidney overexpressing PS can prevent the development of DN.

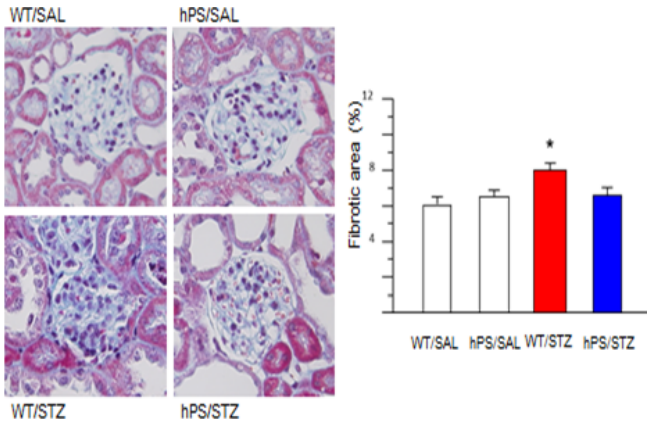

FIGURE 1 Overexpression of hPS is protective against DN. Collagen deposition was assessed by Masson trichrome stain. *P<0.05 vs. hPS/STZ group

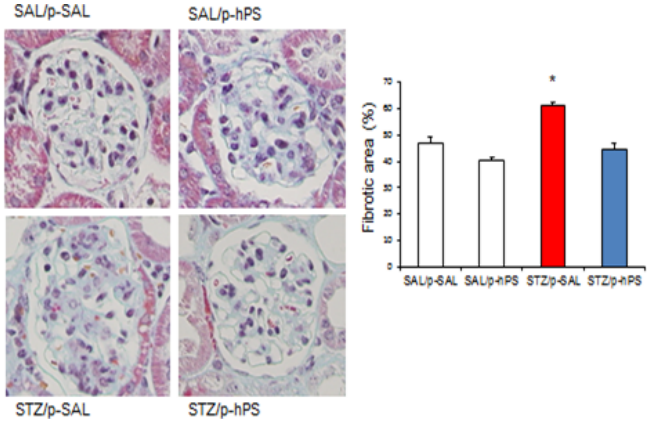

FIGURE 2 Exogenous hPS attenuates the development of DN. Collagen deposition was assessed by Masson trichrome stain. *P<0.01 vs. STZ/p-hPS group.

PB 873 | A Significant Change in Laboratory Markers of Thrombosis Very Early in the First Trimester of Pregnancy May be Linked to a Concurrent Increase in Estradiol

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Background: Pregnancy is associated with an increased risk of venous thrombosis. Previous data have shown that levels of laboratory markers of thrombotic risk change during normal pregnancy, but it is unknown exactly how early in pregnancy these changes occur. Furthermore, pregnancy associated thrombosis may be secondary to high estrogen levels, resembling users of exogenous estrogen, however estrogen levels remain uncharacterised in very early pregnancy.

Aims: To characterise laboratory markers of thrombosis and estradiol during the early first trimester.

Methods: Blood samples were taken from 22 women, just prior to conception and five times during very early pregnancy (up to Day 59 gestation), who were undergoing natural cycle *in vitro* fertilization and gave birth at term following a normal pregnancy.

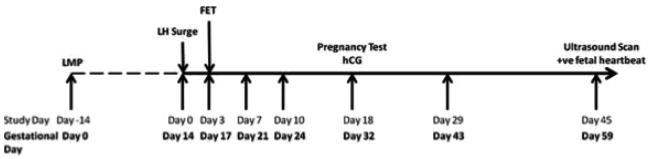

FIGURE 1 Timeline of blood sampling

The study was approved by the local medical ethics committee and informed consent obtained. Analysis included thrombin generation, Protein S, factor VIII, D-dimer, fibrinogen and estradiol. To counter inter-individual variability, the change between the pre-pregnant and pregnant state in each parameter was measured over time.

Results: Three thrombin generation parameters (Endogenous Thrombin Potential, Peak thrombin, Velocity index), Protein S and fibrinogen significantly changed from baseline by approximately Day 32 gestation ($p < 0.01$) and D-dimer by Day 43 ($p < 0.01$), which for all, persisted to Day 59 ($p < 0.0001$). Factor VIII increased by Day 59 ($p < 0.0001$). Estradiol significantly increased by Day 43 gestation ($p=0.003$) with a non-significant mean increase of 67% and 130% by Day 24 and Day 32 respectively. All laboratory markers of thrombosis correlated significantly with estradiol ($p < 0.001$).

Conclusions: We are the first to demonstrate that laboratory markers of thrombosis begin to change during the 5th gestational week, and that this change may be linked to a concurrent early rise in estradiol. Women at high risk of thrombosis should commence thromboprophylaxis as early as possible in pregnancy.

PB 874 | Analysis of Estrogen Mediated Expression of TFPI-2 by miRNAs in Breast Cancer Cells

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Background: Tissue factor pathway inhibitor type 2 (TFPI-2) has been linked with breast cancer pathogenesis. We have recently reported that estrogens significantly upregulated TFPI-2 expression in a breast cancer cell line (MCF-7) through the involvement of estrogen receptor alpha (ERα). Accumulating evidence indicates that activation of the

ERα signalling by estrogens may modulate the expression of target genes indirectly through microRNAs (miRNAs).

Aims: To identify miRNAs involved in the estrogenic regulation of TFPI-2.

Methods: miRNA prediction algorithm was used to identify miRNAs potentially regulating *TFPI2* expression. Pearson correlation analysis of *TFPI2* and miRNA expression (SurePrint G3 Human GE 8x60K one-color microarray, Human miRNA Microarray Kit, Agilent Technologies) was performed in a breast cancer data set of 151 patients. Functional studies of selected miRNAs will be performed in MCF-7 cells in order to examine their effect on *TFPI2* expression using qRT-PCR. Potential effects of 17β-estradiol and/or the ERα antagonist fulvestrant on relative miRNA levels in MCF-7 cells will also be assessed by qRT-PCR. To investigate the interaction of selected miRNA(s) with their predicted target sites within the *TFPI2* 3‘ untranslated region (UTR), a luciferase reporter construct containing the full length *TFPI2* 3‘ UTR will be co-transfected with miRNA mimics and luciferase activity will be measured.

Results: Negatively correlated miRNAs having potential target sites in the *TFPI2* 3‘ UTR and also previously reported to be dysregulated by ER were found in tumors of ER positive breast cancer patients (miR-15a, miR-16, miR-23a, miR-93, and miR-195).

Conclusions: The identification of several miRNAs within the 3‘ UTR of *TFPI2* as being negatively correlated with the TFPI-2 level indicates their involvement in the regulation of the gene. Cell experiments are in progress and the results will be presented.

PB 875 | Comparative Studies on the Inhibition of Thrombin by Recombinant Thrombomodulin in Activated Protein C and Whole Blood Plasma

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Background: Recombinant thrombomodulin (rTM) and activated protein C (APC) are important modulators of coagulation and fibrinolysis. Both agents have been developed for various clinical indications. Beside their direct effect on coagulation, these agents also modulate other components of hemostatic processes to mediate their pharmacologic actions.

Aims: The aim of this study is to compare the differential inhibition of thrombin generation (TG) by a clinically available rTM (ART-123, Asahi Kasei Pharma, Tokyo, Japan) and a plasma derived APC (Haematologic Technologies, VT, USA).

Methods: Native blood from healthy volunteers (n=5) was supplemented with rTM and APC at levels of 0-2.5 ug/ml. TG was triggered by adding diluted APTT (Triniclot) and reaction was stopped after 3 minutes. TG markers such as F1.2 and TAT were measured using ELISA methods. In the citrated plasma studies both agents were supplemented at a 0-2.5 ug/ml. TG studies were carried out using a fluorimetric assay (TGA, Techniclone, Vienna, Austria). Lag time peak TG and area under the curve (AUC) were measured. The IC50 values for each of the agents was calculated.

Results: In the whole blood systems both the APC and rTM produced a concentration dependent inhibition of TG markers such as F1.2 and TAT. APC produced a slightly stronger inhibition of TG as measured by F1.2, IC50 = 0.55 ug/ml compared with thrombomodulin, IC50 = 0.70 ug/ml. In the plasma based system the IC50 for peak thrombin inhibition was 0.46 ug/ml for APC and 0.97 ug/ml for rTM. Additional differences in the lag phase and AUC profiles were also noted.

Conclusions: Beside the direct anticoagulant effects both the APC and rTM are capable of inhibiting the TF mediated thrombin generated in whole blood and plasma based systems. APC exhibits slightly stronger inhibitory effects in TG assays in both plasma and whole blood. These studies suggest that modulation of thrombin and other proteases contribute to the clinical effects of both rTM and APC.

PB 876 | Activation of the Hepatocyte Growth Factor/c-met Pathway during Experimental Envenomation of Rats with Bothrops Jararaca or Crotalus Durissus Terrificus Snake Venoms

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Background: Hepatocyte growth factor (HGF)/c-met pathway regulates cell growth, motility, and morphogenesis of various types of cells, protecting epithelial and non-epithelial organs via anti-apoptotic and anti-inflammatory signals. Thrombin is the main physiological activator of this pathway, and in some pathological conditions such as disseminated intravascular coagulation, increased plasma concentration of active HGF have been reported.

Aims: Since consumption coagulopathy and thrombin generation are usual phenomena in viperid snakebites, we hypothesized that circulating HGF would be increased in plasma of rats envenomated with Crotalus durissus terrificus (Cdtv) or Bothrops jararaca (Bjv) venoms.

Methods: Three experimental groups (n=5, each) of male adult Wistar rats were injected (s.c.) with 500 µL containing (a) 0.9% NaCl solution or sub-lethal doses (1.6 mg/kg) of Cdtv (b) or Bjv (c). After 3 hours, hemostatic function of recalcified whole blood samples was analyzed by means of the INTEM profile of the thromboelastometric assay. Simultaneously, plasmatic fibrinogen (colorimetric assay) and total HGF (ELISA) were evaluated.

Results: Total HGF concentrations were almost 6-fold higher ($p < 0.05$) in both Bjv- and Cdtv-injected groups, when compared with control values (68 ± 18 pg/mL). While in Bjv group these increased levels of total HGF correlated well with severe coagulopathy and hypofibrinogenemia, in the Cdtv group only moderated hypofibrinogenemia was observed.

Conclusions: Presence of thrombin-like toxins in these venoms and of prothrombin and factor X activators in Bjv would explain at least in part the significant increase in plasma concentration of HGF. In addition, active factor X is reported to release soluble HGF from

granulocytes. This study would shed new light on some aspects of the pathophysiological mechanisms induced by these venoms.

PB 877 | Targeted Inhibition of Activated Protein C by an Exosite Antibody to Treat Hemophilia

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Background: Activated Protein C (APC), a plasma serine protease, can have antithrombotic and cytoprotective functions.

Aims: To test the hypothesis that anti-APC antibodies (Abs) specifically inhibiting its anticoagulant function but not cytoprotective activities have desirable therapeutic effects for hemophilia therapy

Methods: Surface plasmon resonance (SPR) and X-ray crystallography were used for the Ab binding and epitope mapping studies. Ab activity was measured using activated partial thromboplastin time (APTT) and thrombin generation assay (TGA). Ab efficacy was tested in induced hemophilia A cynomolgus monkeys.

Results: The 2 types of anti-APC monoclonal Abs selected (type I and type II) bound to APC at ~10 nM binding affinity (KD) by SPR. X-ray crystallography studies reveal that they bind to distinct epitopes on APC (type I on active site vs type II on exosite). The type I Ab was shown to fully block APC enzyme activities and promote thrombin generation by TGA and plasma clotting by APTT. The type II Ab was shown to interfere with APC-mediated cleavage of the physiologic substrates activated factor VIII (FVIII) and activated factor V, thereby preserving both cofactors' activities in APC-mediated inactivation assays using purified systems as well as plasma-based assays. Most importantly, the type II Ab preserved APC's cytoprotective function as measured by histone-mediated cytotoxicity assay in primary human umbilical vein endothelial cells. In normal monkeys, the type I Ab was found to cause adverse effects in the vascular systems. In contrast, the type II antibody was well tolerated and showed prophylactic and dose-dependent efficacy at 3, 10, and 30 mg/kg in anti-FVIII Ab-induced hemophilia A monkeys in a 10-day study.

Conclusions: Our data suggest that cytoprotective function of APC is important and that the type II anti-APC exosite Ab can specifically inhibit APC's anticoagulant function without compromising its cytoprotective function, offering therapeutic opportunities for persons with hemophilia.

PB 878 | Silencing of Anticoagulant Protein C Evokes Low Incident but Spontaneous Atherothrombosis in Apolipoprotein E Deficient Mice

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Background: Mouse atherosclerosis models do not spontaneously develop atherothrombotic complications.

Aims: To investigate whether acute silencing of natural anticoagulation allows pre-existing atherosclerotic plaques to progress towards an atherothrombotic phenotype.

Methods: In atherosclerotic apolipoprotein E (*Apoe*-/-) deficient mice on a Western-type diet, protein C was acutely lowered using small interfering (si) RNA (si*Proc*).

Results: Upon lowering of protein C levels, in a pilot study 1 out of 4 *Apoe*-/- mice displayed a large, organized, and fibrin- and leukocyte-rich thrombus on top of an advanced atherosclerotic plaque in the aortic root. Although again at low incidence, similar thrombi at the same location were observed in a second independent experiment (3/25 mice). In addition, 7/25 si*Proc* mice featured clots in the left atrium of the heart. Within this group of 25 mice, platelets numbers ($1629x10^9$ platelets/L ±70 vs. $1832x10^9$ platelets/L ±58; $P>0.05$, si*Proc* treated mice without thrombi vs. si*Proc* treated mice with thrombi, resp.), fibrinogen levels (178 U/dL ±8 vs. 189 U/dL ±14; $P>0.05$), and thrombin-antithrombin complex levels (45 ng/L ±3 vs. 43 ng/L ±5; $P>0.05$) were comparable. Other organs (liver, lungs, kidneys) did not show thrombotic lesions. Moreover, aortic plaques in si*Proc* mice with thrombi had collagen content (18.6% ±1.3 vs 15.6% ±1.9; $P>0.05$), necrotic area (20.9% ±1.1 vs. 23.3% ±3.9; $P>0.05$) and macrophage content (3.7% ±0.3 vs. 4.0% ±0.9; $P>0.05$) comparable to plaques in si*Proc* mice without thrombi. Albeit again at low incidence, in a third independent experiment identical thrombi in the aortic root (6/20 mice) and clots in the left atrium (5/20) were observed.

Conclusions: Transient silencing of *Proc* in *Apoe*-/- mice creates a condition that allows the occurrence of spontaneous low-incidence atherothrombosis. Lowering natural anticoagulation in atherosclerosis models may help to discover factors which increase atherothrombotic complications.

PB 879 | Protection of the Endothelial Protein C Receptor (EPCR) against Inactivation by P.*falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) Domains with a Non-inhibitory Anti-EPCR Antibody

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Background: Expression of PfEMP1 variants during *P. falciparum* infection that bind to EPCR is associated with severe malaria complications. The inhibition of (activated) protein C ((A)PC) binding to EPCR by PfEMP1 domains is considered to be a major contributing factor. Thus, approaches to protect normal EPCR functions may attenuate severe malaria symptoms.

Aims: To identify a functional non-inhibitory EPCR antibody (Ab) that specifically blocks PfEMP1 binding but permits normal EPCR-dependent functions of (A)PC.

Methods: PfEMP1 domain CIDRa1.1 binding to EPCR was determined by ELISA, on EA.hy926 endothelial cells (EC) and in human EPCR (hEPCR) transgenic mice. EPCR function was determined by PC activation, PAR1 cleavage and barrier function on EC.

Results: Of the 19 anti-EPCR Abs screened, 4 Abs demonstrated significant inhibition of CIDRa1.1 binding to sEPCR with negligible effects on APC binding. Of the 4 Abs, #19 showed the most favorable dose response for selective inhibition of CIDRa1.1 but not APC binding to sEPCR and EC. Ab #19 restored EPCR-dependent protein C activation on EC that was inhibited by CIDRa1.1. Ab #19 also improved APC-mediated PAR1 cleavage on EC. In EC barrier assays as a measure of EPCR-dependent APC cytoprotective effects, #19 had no effect on APC's barrier protective activity in the absence of CIDRa1.1 and protected EPCR against inhibition by CIDRa1.1, thus permitting normal APC barrier protective effects. In vivo, infusion of CIDRa1.1 in hEPCR mice resulted in rapid accumulation of CIDRa1.1 in the lung that could be detected up to 2 days later. A single bolus of #19 (5 mg/kg) reduced CIDRa1.1 accumulation in the lung >3-fold when given 48 hrs before CIDRa1.1 and >5-fold when given 1 hr before.

Conclusions: Ab #19 selectively inhibited CIDRa1.1 to EPCR and protected normal EPCR functions against inhibition by CIDRa1.1. In vivo, #19 provided long lasting protection of EPCR against CIDRa1.1 binding, suggesting that EPCR targeting to attenuate severe symptoms in malaria may be feasible.

PB 880 | Characterization of an In vivo Mouse Model for the Binding of P. *falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) Domains to the Endothelial Protein C Receptor (EPCR)

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Background: Severe malaria due to *P. falciparum* infection is associated with PfEMP1's binding to EPCR. In vitro, PfEMP1 domains inhibit (activated) protein C ((A)PC) binding to EPCR required for PC activation and APC cytoprotective effects. Proof for the contribution of a defective PC system to severe malaria in vivo is hampered by the lack of a rodent model for the PfEMP1-EPCR interaction.

Aims: To develop a mouse model for the PfEMP1-EPCR interaction, and to validate the model using the soluble (s) E86A-EPCR decoy strategy.

Methods: Human EPCR (hEPCR) transgenic and WT mice were injected with the EPCR-binding PfEMP1 domain CIDRa1.1 and non-EPCR-binding CIDRa1.2. Accumulation of PfEMP1 domains in organs and plasma was determined by ELISA, W-blot and histology.

Results: PfEMP1 domains do not bind to mouse EPCR, therefore PfEMP1 domains were injected i.v. in hEPCR transgenic mice. CIDRa1.1 accumulated preferentially in the lung and to a lesser extent in the spleen and liver of hEPCR but not WT mice, a pattern matching the hEPCR expression in these organs. Accumulation of CIDRa1.1 (0.4-2.0 mg/kg) was dose- and time-dependent and could be observed as early as 5 min and as late as 2 days after injection. No accumulation of CIDRa1.2 (2.0 mg/kg) was observed. Histology confirmed vascular accumulation of CIDRa1.1. Clearance of CIDRa1.1 was ~3 min in WT mice but extended 4-fold in hEPCR mice due to binding to soluble (s) hEPCR. The non-(A)PC binding E86A-sEPCR restored EPCR-dependent (A)PC functions on cells in the presence of CIDRa1.1. In vivo, E86A-sEPCR (0.4 mg/kg) reduced CIDRa1.1 (0.4 mg/kg) accumulation in the lung 5-fold when given 5 min before and 3-fold when given 15 min after CIDRa1.1.

Conclusions: This mouse model showed high affinity binding of PfEMP1 domains to EPCR in vivo and permitted testing of the efficacy of EPCR protective strategies in vivo. The model also provides a framework for studies on the contribution of the PfEMP1-EPCR interaction to the pathogenesis of severe malaria.

PB 881 | Antithrombin and Protein C Plasma Levels at Admission Are Associated to Mortality at Six Months in Patients with Pulmonary Embolism with and without Malignant Disease

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Background: The association between hemostasis parameters and death in patients with pulmonary embolism (PE) is unknown.

Aims: To investigate the association of haemostasis parameters at admission and the mortality at 6 months in patients with PE.

Methods: Two hundred and thirty four consecutive patients with PE who were admitted in the intensive care unit of the tertiary

hospital were enrolled in the study. Plasma activity of antithrombin (AT), Proteins C (PC), factor II, VII, VIII and fibrinogen concentration were determined in the majority of patients (between 165-186 depends on the parameter) after admission without the storage of plasma. The results were split into the tertiles for the estimation of significant difference at 6-month mortality. The results were presented in whole group and in subgroups with and without malignancy.

Results: The 6-month mortality was 15/60 (25.0%) and 15/59 (25.4%); 6/63 (9.5%) and 8/59 (13.6%); 7/63 (11.1%) and 5/61 (8.2%) across the tertiles of AT (I< 0.75IU/L, III>0.97IU/L) and PC (I< 0.81IU/L and III>1.2IU/L) with Log Rank test p_{AT}=0.031 and p_{PC}=0.039, respectively. The significant difference between the mortality at six months were found across the tertiles of AT (I-22.6% vs II-3.8% and III-7.1%, p=0.009) for subgroup without malignancy (159 patients). However, in patients with malignancy (26 patients), mortality at six months was significantly different only across the tertiles of PC (I-70% vs II-44.4% and III-0.0%, p=0.030).

Conclusions: The low plasma levels of AT and PC are associated to mortality at six months in patients with PE. In the PE patients with malignant disease only low PC activity is associated to 6-month death.

PB 882 | Positive Correlation of Plasma Protein S Levels to Apolipoprotein C-II in Middle-aged Obese Women and Non-obese Young Women

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Background: Protein S is a vitamin K-dependent plasma glycoprotein functions as a non-enzymatic co-factor to activated protein C in the degradation of factors Va and VIIIa. In plasma, protein S is present in two forms, free form and in a complex with C4b-binding protein, and has been reported to associate with triglyceride-rich lipoproteins but not with LDLs and HDLs.

Aims: To elucidate the physiological relevance of the association of protein S and lipoproteins, we performed correlation studies of plasma protein S levels with atherosclerosis-related factors including adiposity indicators, metabolic parameters, and inflammatory markers

Methods: The relationships between plasma total and free protein S antigen levels and adiposity indicators, blood pressure, estradiol, lipoproteins, glucose, insulin resistance, adipocytokines, inflammattory markers, and hemostatic factors were examined in middle-aged obese women (n=62) and non-obese young women (n=160).

Results: Total and free PS antigen levels in middle-aged obese women correlated negatively with estradiol and positively with triglyceride, total cholesterol, LDL cholesterol, apoA-II, apoB, apoC-II, apoC-III, apo E, HbA1c, and protein C, whereas there was no correlation with HDL cholesterol, apoA-I, body mass index, visceral fat area, blood pressure, or factor VII. Stepwise multiple linear regression analyses revealed that protein C, apoC-II, and fibrinogen were significant predictors of total protein S antigen levels, accounting for 51.9% of variance. ApoC-II was selected as a single significant predictor for free protein S antigen levels, accounting for 18.6% of variance. In non-obese young women, apoC-II was also selected as a significant predictor of total protein S antigen levels, but not of free protein S antigen levels.

Conclusions: Correlation studies revealed a strong positive relationship between plasma protein S antigen levels and apoC-II, an essential cofactor for lipoprotien lipase present mainly on triglyceride-rich lipoproteins.

PB 883 | Hepatocyte Growth Factor Reduces Protein C Inhibitor Expression in HepG2 Cells via MEK and PI3 Kinase

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Background: Protein C inhibitor (PCI) regulates the anticoagulant protein C pathway by neutralizing activated protein C (APC) and thrombin-thrombomodulin complex in the human hemostatic system. Hepatocyte growth factor (HGF) plays an important role in tissue repair and regeneration, is activated by HGF activator (HGFA), and then promotes proliferation of hepatocyte *via* c-Met. Recently, we indicated that PCI regulates liver regeneration by inhibiting HGFA *in vitro* and *in vivo*, but it is unclear whether HGF itself affects the proHGFA (HGFA precursor) and PCI expressions in hepatocytes.

Aims: We investigated the effect of HGF on proHGFA and PCI expressions in hepatocytes, and elucidated the detailed signal transduction process of the HGF-induced change of their expressions.

Methods: Confluent HepG2 cells were treated with HGF, or HGF plus various signal transduction inhibitors, and then culture supernatants were collected by centrifugation at 15,000 rpm for 10 min. The concentrations of proHGFA and PCI in culture supernatants of HepG2 cells were determined by specific ELISA. The mRNA expressions of proHGFA and PCI in HepG2 cells were evaluated by specific Real-time PCR analysis.

Results: HGF had no effect on proHGFA production in hepatocytes and hepatoma cell line, HepG2 cells. However, it decreased the PCI production in both cells. Real-time PCR analysis showed that this HGF-induced change of PCI production in HepG2 cells is transcriptionally regulated. Furthermore, NFkB inhibitor did not recover the HGF-induced decreased PCI expression in HepG2, and inhibitors of p38MAPK and JNK also did not do so. However, c-Met, PI3 kinase and MEK inhibitors did recover HGF-induced decreased PCI expression in HepG2 cells. In addition, ERK inhibitor had no effect on HGF-induced decreased PCI expression in HepG2 cells.

Conclusions: These findings suggest that HGF decreases PCI expression in HepG2 cells *via* c-Met, which is subsequently followed by PI3 kinase, and MEK signaling whose downstream is not ERK activation.

PB 884 | Differential Modulation of Plasminogen Activator Mediated Thrombolysis by Recombinant Thrombomodulin and Activated Protein C

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Background: Urokinase (UK) and tissue plasminogen activator (tPA) mediate thrombolytic actions by activating endogenous plasminogen. Thrombomodulin (TM) complexes with thrombin to activate Protein C and thrombin activatable fibrinolysis inhibitor (TAFI). Activated Protein C (APC) modulates coagulation by digesting factors V and VIII and activates fibrinolysis by decreasing PAI-1 functionality. Recombinant versions of TM (rTM) has been developed for therapeutic purposes.
Aims: To compare the effects of rTM and APC on urokinase and tPA mediated thrombolysis utilizing thromboelastography.
Methods: Native whole blood was activated using a diluted intrinsic activator (APTT reagent, Triniclot). The modulation of thrombolysis by tPA and UK (Abbott, Chicago, USA) was studied by supplementing these agents to whole blood and monitoring TEG profiles for 3 hours. To investigate effect of APC (Haematologic Technologies, VT, USA) and rTM (Asahi Kasai Pharma, Tokyo, Japan) these agents were also supplemented to the activated blood. The modulation of tPA and UK induced thrombolysis by APC and rTM was also studied.
Results: In comparison to rTM, APC produced a stronger anticoagulant effect in terms of r time, k time, angle and MA. Fibrinolysis was assessed in terms of LY30(%) and LY60 (%). At concentrations of up to 2.5ug/ml rTM and APC did not produce any direct fibrinolytic effects. APC also produced strong augmentation of the lytic of effects of tPA and urokinase in a concentration dependent fashion. rTM at concentrations of less than 10ug/ml produced stabilization of clot resisting fibrinolysis.
Conclusions: These studies demonstrate the differential anticoagulant and procoagulant effects of APC and rTM. APC is a stronger anticoagulant than rTM and facilitates thrombolysis. rTM is a much weaker anticoagulant and modulates clot stability branding it resistant to fibrinolysis. These observations suggest that while APC may impair hemostasis, rTM restores hemostasis via TAFI activation.

PB 885 | G-quadruplex-Directed Aptamer Selection against Activated Protein C (APC)

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Background: Aptamers are single stranded DNA/RNA molecules which are able to bind to different target molecules with high affinity and specificity. Aptamers are typically selected from randomized libraries of nucleic acids using a procedure termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX). The procedure is completed by identification and evaluation of individual aptamer sequences.
Aims: To identify aptamers that bind to distinct epitopes of APC that can be used for selective and/or bivalent targeting of APC.
Methods: To figure out the effect of the composition of the starting library on the structure of the selected aptamer(s), two distinct ssDNA libraries, namely a completely randomized as well as a G-rich one, were used in capillary electrophoresis-based SELEX (CE-SELEX) against APC. The enrichment of high-affinity aptamers during selection was assessd using next generation sequencing (NGS). Selected aptamers were characterised in terms of binding to APC, its zymogen Protein C, and the structurally related serine proteases thrombin and activated factor VII. In addition, competition experiments were performed to find out the binding site of the aptamers on APC.
Results: Enrichment of 3 aptamers (NB1, NB2, and NB3) demonstrating high binding affinities to APC (0.2 to 20 nM) was confirmed by NGS analysis. Structural and fluorescence analysis using G-quadruplex (G4)-binding thioflavin T suggest that NB3 consists of a distinct G-quadruplex structure while NB1 and NB2 were predicted to form highly conserved stem-loop structures. Binding experiments revealed high APC-specificity of the NB-aptamers and identified the heparin binding site of the enzyme as the common target region.
Conclusions: The identified aptamers represent distinct high-affinity ligands specifically binding to APC. Further characterization will reveal their characterisitcs with respect to the modulation of APC functional activity as well as the development of diagnostic APC assays.

PB 886 | The Protective Effect of Activated Protein C as the Baised Agonism in Activation of Protease-activated Receptor 1

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Background: Activated protein C (APC) isn't only a main component of anticoagulant system, but it plays important role in inflammatory, neurodegeneration, proliferation and etc. Protease-activated receptor 1 (PAR1) is a common receptor for thrombin (Th) and APC, via which these proteases regulate cell functions. Why Th and APC demonstrate different effects via PAR1 isn't clear.
Aims: In the present work, we studied the biased activation of PAR1 by APC, we hypothesized that PAR1-dependent signaling by APC

involves a novel cleavage of the receptor's N-terminal domain, differing from that of thrombin.

Methods: The work was performed on different models: neuronal exitotoxicity, ischemia in vitro (astrocyte culture), acute inflammation and proliferation of keratinocytes. Thrombin, APC and peptides of tethered ligand with different amino acid sequences were used for activation of PAR1.

Results: It has been shown recently that the multidirectional effects of thrombin and APC may be due to biased agonism under the action of thrombin and APC proteases on the PAR1 in endothelial cells. Under conditions of biased agonism, APC-specific noncanonic cleavage at Arg46 has been identified in the PAR1 exodomain instead of the canonic cleavage by thrombin at Arg41.

We have shown for the first time that a noncanonical PAR1 ligand (NPNDKYEPF amide) protects neurons at glutamate-induced toxicity, mast cells at acute inflammation, astrocytes at ischemia and stimulates the proliferative activity of keratinocytes, like protease APC. These effects of new peptide were abolished by blockage of PAR1. Canonical peptide (TRAP6) has demonstrated proinflammatory and toxic effects on astrocytes, mast cell and neurons, similar to Th.

Conclusions: Thus the new peptide-agonist PAR1 simulates APC-induced but not thrombin-induced signaling on different cell types.

This work was supported by Russian Foundation for Basic Research № 16-04-01869.

PB 887 | Function and Prevalence of Host-derived Coagulation Factors on the Virus Surface

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Background: Numerous viruses are known to alter the hemostatic system. To explain this link, we have shown that the envelope of three herpesviruses acquire the initiators of coagulation from the host, tissue factor (TF) and anionic phospholipids (aPL). Viral TF and aPL act as cofactors for clotting factor VIIa-mediated factor X (FX) activation, ultimately leading to clot formation and cell signaling. Using herpes simplex virus 1 (HSV1) as a model envelope virus, virus TF was previously shown to enhance infection. HSV1 encoded glycoprotein C (gC) has also been implicated in FX activation.

Aims: The aims of the current research are to:

1) dissect the role of gC on HSV1-mediated FX activation; and

2) investigate the ubiquity of viral TF and aPL on enveloped viruses.

Methods: TF^+/TF^- HSV1 variants and dengue virus (DENV) propagated in cell culture were purified and characterized. The FX activating roles of viral TF and a soluble form of gC were assessed by chromogenic and plasma clotting assays. Immunogold electron microscopy was used to simultaneously visualize TF, aPL, and a virus-encoded marker on the virus surface.

Results: In plasma, HSV1 and DENV induced TF-mediated clotting. Viral TF was required for optimal FX activation, and was essential for HSV1 gC-mediated enhancement. This was confirmed using soluble gC and purified membrane-bound TF. Both TF and aPL were incorporated into HSV1 and DENV particles.

Conclusions: Virus surface TF function is enhanced by gC to contribute to FX activation and clot formation. Identification of TF on both HSV1 and DENV demonstrates the viral acquisition of host constituents and suggests the ubiquity of TF on enveloped viruses. Combined with the prior observation that virus TF enhances infection, the data presented here may support targeting viral TF as a broad-spectrum anti-viral agent.

PB 888 | Decryption of Hepatocyte Tissue Factor Procoagulant Activity by Bile Acids Requires Non-apoptotic Phosphatidylserine Externalization

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Background: Liver parenchymal cells (i.e., hepatocytes) express tissue factor (TF) in an encrypted form that lacks procoagulant activity. TF-dependent coagulation occurs in cholestatic liver disease, where bile flow is disrupted. However, the timing and mechanism of decryption of hepatocyte TF procoagulant activity is not known.

Aims: We tested the hypothesis that exposure of hepatocytes to pathologic levels of bile acids rapidly increases TF procoagulant activity.

Methods: Ligation of the common bile duct (BDL) was utilized to induce obstructive cholestasis in wild-type mice. Coagulation was assessed 15 minutes after BDL or sham surgery. The impact of pathologically relevant concentrations of the bile acid sodium taurocholate (TCA) on TF procoagulant activity was examined in primary mouse hepatocytes.

Results: Increased hepatic fibrin(ogen) deposits indicated intrahepatic coagulation within 15 minutes of BDL. This occurred without detectable liver injury. Treatment of primary mouse hepatocytes with 1 mM TCA increased TF-dependent procoagulant activity within 15 minutes in the absence of necrosis or apoptosis. TCA driven TF procoagulant activity was not affected by inhibitors of transcription nor recapitulated by direct activation of the bile acid nuclear receptor farnesoid X receptor. Inhibitors of PI3K, ERK1/2, p38 and PKC failed to inhibit TCA-triggered TF procoagulant activity. TCA treatment triggered rapid externalization of phosphatidylserine (PS), independent of caspase 3 activation, and the TCA-mediated increase in TF activity was blocked by addition of the high-affinity PS-binding protein lactadherin.

Conclusions: The results indicate that TCA causes rapid decryption of hepatocyte TF activity through a mechanism involving PS

externalization. This suggests a mechanism whereby nontoxic bile acids could play a key role in triggering intrahepatic coagulation in disease settings associated with cholestasis.

PB 889 | Clustering of CD98 on Monocytic THP1 Cells Increases Tissue Factor Procoagulant Activity

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Background: We have previously shown that rabbit antithymocyte globulin (ATG) rapidly activates monocyte tissue factor (TF) in a complement- and thiol-disulfide exchange-dependent manner. Full TF activation by ATG required lipid raft integrity, intact surface-located protein disulfide isomerase (PDI) activity, and phosphatidylserine (PS) membrane exposure, suggesting that concerted interactions between heterotypic proteins were involved.

Aims: Since ATG is a polyclonal rabbit IgG targeting ubiquitous epitopes, we aimed to identify additional proteins involved in the regulation of cellular TF procoagulant activity (PCA).

Methods: Cell surface expression of CD98 and PS was analyzed by flow cytometry. TF activity was measured by single-stage clotting and Xa generation assays.

Results: Preincubation of THP1 cells with ATG abolished binding of a fluorescently labeled CD98 monoclonal antibody (mAb), indicating that CD98 is recognized by ATG on cell surfaces. Cross-linking of the CD98 heavy chain with mouse anti-CD98 mAbs LT98 or UM7F8 followed by goat-anti mouse IgG $F(ab')_2$ increased PS externalization and cellular TF PCA or TF-mediated Xa generation by 2- to 6-fold. This effect was not prevented by complement (anti-C5) or PDI (rutin) inhibition. Similarly, cross-linking of ATG with goat-anti rabbit IgG $F(ab')_2$ increased PS exposure and TF-dependent Xa generation 4-fold on THP1 cells, but significantly attenuated ATG-induced TF PCA in the clotting assay from 39- to 3-fold, suggesting that blocking ATG Fc portions by the $(Fab')_2$ prevented complement activation for full cellular TF procoagulant function. CD98 clustering did not increase TF PCA in solid cancer cell lines with relatively low CD98 expression.

Conclusions: Our findings implicate CD98, a heterodimeric transmembrane protein involved in amino acid transport and signaling of integrins, in the regulation of monocyte TF PCA.

PB 890 | A First-in-Human (FIH) Study of the Safety, Tolerability, Pharmacokinetics (PK) and Pharmacodynamics (PD) of PF-06741086, an anti-TFPI Monoclonal Antibody, Following Administration of Single Subcutaneous or Intravenous Doses in Healthy Adult Male Volunteers

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Background: Tissue factor pathway inhibitor (TFPI) is a protease inhibitor of tissue factor-activated coagulation factor VII (FVIIa) and activated factor X (FXa). PF-06741086 is a monoclonal antibody that targets TFPI to reduce inhibition of the extrinsic coagulation pathway and increase clotting activity in bleeding disorders such as hemophilia.

Aims: This study (protocol B7841001, approved by the comité d´éthique hospitalo-facultaire Erasme - ULB) was a randomized, double-blind, sponsor-open, placebo-controlled, single intravenous (IV) or subcutaneous (SC) dose escalation study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of PF-06741086, done in compliance with the Declaration of Helsinki.

Methods: Volunteers who signed informed consent were assigned to cohorts with ascending dose levels. A single SC dose level was studied in a cohort of Japanese volunteers. Safety endpoints included treatment emergent adverse events (TEAEs), infusion/injection site reactions, and coagulation laboratory parameters. PK and PD endpoints included concentrations of PF-06741086 in plasma and measures of PF-06741086 pharmacology.

Results: Forty-one volunteers were recruited overall. Thirty-two were dosed with PF-06741086 at escalating IV or SC dose levels, beginning with 30 mg SC. All doses were safe and well-tolerated. TEAEs were mild or moderate in severity, laboratory abnormalities were transient, there were no infusion/injection site reactions and no stopping criteria were met.

Plasma exposures of PF-06741086 increased greater than proportionally with dose under the same dosing route. Coagulation pharmacology was demonstrated via Dilute Prothrombin Time, D-Dimer, Prothrombin Fragment 1.2 and Thrombin Generation.

Conclusions: Single doses of PF-06741086 at multiple dose levels were safe and well tolerated in a healthy adult male population. The safety, PK and PD data from this study support progression to a multiple dose study in hemophilia patients.

PB 891 | Hypoxia Augments Tissue Factor Expression in Inflammatory Stimulated Macrophages but Not in Smooth Muscle Cells or in Endothelial Cells

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Background: We recently reported that tissue factor (TF) and hexokinase II (HK-II) expression in coronary arteries correlated with coronary

thrombus size. However, the underlying conditions in atherosclerotic plaques remain unclear.

Aims: First, to identify TF and HK-II expression under hypoxia and/or inflammatory stimuli in plaque cells. Second, to demonstrate expression of TF and HK-II in thrombotic plaque components in aspirated coronary thrombi from patients with acute coronary syndrome (ACS).

Methods: TF and HK-II expression were assessed with THP-1 macrophages, human umbilical artery smooth muscle cell (HUASMC)s, and in human umbilical vein endothelial cell (HUVEC)s. Cultured cells were stimulated by tumor necrosis factor-a (20ng/mL) and interferon-g (10ng/mL) under normoxic (21% O_2) or hypoxic (1% O_2) conditions. Human coronary thrombi were aspirated from culprit lesions during percutaneous coronary intervention for ACS (n=11, age range:53-85, male:10, female:1). The paraffin sections were immunohistochemically stained with anti-CD68, smooth muscle actin (SMA), TF and HK-II antibodies.

Results: The hypoxic condition augmented TF mRNA and protein expression and TF activity in the cytokine-stimulated THP-1 macrophages, but not in HUASMCs or HUVECs. The hypoxic condition mildly enhanced TF expression in non-stimulated THP-1 macrophages, and markedly enhanced HK-II expression and lactate production in the stimulated and non-stimulated THP-1 macrophages but not in HUASMCs or HUVECs. All thrombotic plaques expressed TF and HK-II in CD68-positive macrophages in the aspirated thrombi. TF expression was also noted in necrotic tissue in the plaques. There were no smooth muscle cells in the aspirated thrombi and plaques.

Conclusions: Hypoxic and inflammatory conditions in atherosclerotic plaque may markedly enhance TF and HK-II expression in macrophages but not smooth muscle cells or endothelial cells, and contribute to symptomatic coronary events.

PB 892 | BAY 1093884 Binds to the Kunitz 1 and 2 Domain Interface of Tissue Factor Pathway Inhibitor and Inhibits its Function

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Background: BAY 1093884 is a fully human monoclonal antibody against tissue factor pathway inhibitor (TFPI) developed as a bypass agent for patients with hemophilia with or without inhibitors. BAY 1093884 restores a sufficient thrombin burst which leads to stable clot formation in hemophilic conditions and effectively stops bleeds in vivo.

Aims: Here we investigated the mechanism by which BAY 1093884 inhibits TFPI activity.

Methods: BAY 1093884-TFPI interactions were studied by crystallography and surface plasmon resonance (SPR). Functional assessment was performed via thrombin generation assay and dilute prothrombin time clotting assay.

Results: Cocrystallization of BAY 1093884-TFPI complex revealed that BAY 1093884 binds at the interface of the Kunitz 1-Kunitz 2 (K1-K2) domains of TFPI. These observations were supported by SPR data which showed BAY 1093884 competes with TFPI binding to factor VIIa (FVIIa) bound to soluble tissue factor (TF). In functional assays, BAY 1093884 restored the amidolytic activities of both factor Xa (FXa) and FVIIa bound to soluble TF, corroborating the biophysical observations. Further, BAY 1093884 dose-dependently improved thrombin generation in hemophilia A plasma, both in the presence and absence of endothelial cell surfaces. The maximum amount of thrombin generated by BAY 1093884 was significantly higher than that of BAY 1093889, another antibody that binds only to the K2 domain of TFPI. This was reflected as faster clotting times in dilute prothrombin time clotting assays.

Conclusions: Our data suggest that BAY 1093884 binds to TFPI via the K1-K2 domains of TFPI and inhibits its interactions with both FVIIa and FXa. Further, targeting K1-K2 domains of TFPI may have an advantage over targeting only the K2 domain of TFPI by increasing the thrombin peak in patients with hemophilia.

PB 893 | mTOR Inhibitors Reduce Expression of Tissue Factor Isoforms in Neuroendocrine Tumors of the Pancreas

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Background: Full-length Tissue Factor (flTF) and alternatively spliced TF (asTF) contribute to malignant growth. flTF and asTF are overexpressed in pancreatic ductal adenocarcinoma (PDAC). Interactions between asTF and β1 integrins promote activation of Akt and p42/44 MAPK, potentiating cancer cell migration and proliferation. It is unknown whether, aside from PDAC, flTF and/or asTF also contribute to the pathobiology of pancreatic neuroendocrine tumors (pNET).

Aims: Assess expression of TF in pNET and potential utility of targeting intracellular pathways activated by TF in pNET.

Methods: 6 human pNET specimens were immunostained for flTF and asTF. Human pNET cell lines QGP1 and BON (gift of Dr. Courtney Townsend, UTMB) were evaluated for TF expression and responsiveness to mTOR inhibition using the mTORC1/2 ATP-site competitive inhibitor MLN0128. TF cofactor activity was assessed via two-step FXa generation assay. BON were stably transfected with an inducible asTF expression construct (BON-asTFi) to assess the efficacy of MLN0128 in the context of asTF overexpression.

Results: All pNET tissue specimens stained positive for flTF and asTF; normal pancreas tissue exhibited minimal TF staining. BON and QGP1 expressed both TF isoforms with BON expressing much higher levels of flTF and asTF mRNA and protein. Treatment of QGP1 and BON with MLN0128 markedly suppressed the levels of flTF and asTF protein and reduced phosphorylation of several mTOR targets including Akt, S6K1, 4E-BP1, and ULK1. Treatment of BON with 100 nM and 400 nM MLN0128 reduced TF cofactor activity by 64% and 83%, respectively. Treatment of BON-asTFi with MLN0128 significantly reduced secretion of asTF.

Conclusions: TF isoforms are expressed in pNET. In BON cells, MLN0128 reduces TF protein levels, TF cofactor activity, and asTF secretion. Further studies are thus warranted to evaluate the functional significance of tumor derived TF isoforms in pNET, and the utility of MLN0128 as a therapeutic agent for pNET management.

PB 894 | High Plasma Concentration of Activated Factor VII-antithrombin Complex is Associated with an Increased Activated Factor X Generation

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Background: activated factor VII-antithrombin (FVIIa-AT) complex has been proposed as an indicator of tissue factor (TF) exposure to the blood, thus being a potential biomarker of prothrombotic diathesis. High plasma levels of FVIIa-AT have been associated with an increased thrombin generation and predicted total and cardiovascular mortality in patients with coronary artery disease (CAD).

Aims: to evaluate in CAD patients the relationship between FVIIa-AT levels and activated factor X generation (FXaG) at low TF concentration, and by inhibiting tissue factor pathway inhibitor (TFPI) or activated protein C (APC) with the aim to highlight procoagulant components.

Methods: within the framework of the Verona Heart Study we selected 40 male CAD patients (mean age 62.4±10.0 years) not taking anticoagulant drugs and with data of FVIIa-AT levels measured by ELISA. FXaG in plasma was evaluated at 1 pM TF by addition of a specific FXa fluorogenic substrate, and also in the presence of an anti-TFPI RNA aptamer (TFPI-aptamer) or an anti-APC DNA aptamer (APC-aptamer).

Results: among the parameters estimated from the analysis of FXaG curves only the area under the curve (FXa-AUC) showed a significant correlation with FVIIa-AT levels. The FXa-AUC increased progressively across FVIIa-AT quartiles, from 4031±210 Rfu in the lowest to 4268±84 Rfu in the highest (P=0.007 by ANOVA with polynomial contrasts for linear trend, confirmed after adjustment for traditional cardiovascular risk factors). The addition of either TFPI-aptamer or APC-aptamer produced a marked increase of FXaG with shorter time parameters (P< 0.001). The increase of FXa-AUC across FVIIa-AT quartiles was confirmed in both TFPI-aptamer or APC-aptamer assays (TFPI-aptamer, P=0.019; APC-aptamer, P=0.014).

Conclusions: in male CAD patients high plasma levels of FVIIa-AT are associated with an increased FXaG at low TF concentration. The increase of FXa-AUC across FVIIa-AT quartiles is still maintained after potentiation of procoagulant pathways by inhibiting TFPI or APC.

PB 895 | Renin-angiotensin System Blockade Downregulates Tissue Factor Expression Induced by γ-Glutamyltransferase in Human Peripheral Blood Mononuclear Cells Exposed to High Glucose

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Background: Consistent epidemiological studies demonstrate the power of gamma-glutamyltransferase(GGT) as cardiovascular risk predictor suggesting its causal role in the development of acute thrombotic events to which Tissue Factor(TF), a major regulator of blood coagulation, contributes. In agreement with that possibility, GGT upregulates TF expression in human peripheral blood mononuclear cells(PBMCs)(Thromb J. 2016;14:45),a non-enzymatic,NFkB-dependent behavior susceptible to modulation by pharmacological renin-angiotensin system (RAS)blockade or high glucose (HG)conditions, as reported for other proinflammatory agents.

Aims: To assess the effect of RAS blockade by Aliskiren(ALI),a direct renin inhibitor, Zofenopril(ZOF),an ACE inhibitor and Olmesartan(OLM),an AT1R blocker,on GGT-induced TF expression in PBMCs exposed to either normal (NG,10mM) or HG (30mM)concentrations.

Methods: PBMCs obtained from healthy donors incubated with enzymatically inactive human recombinant GGT(0.5ng/μl),either alone or in presence of RAS blockers (10^{-6}M).TF PCA(1-stage clotting assay, arbitrary units),ag(ELISA,ρg/mL)and mRNA (real-time PCR,normalized-fold expression)were the evaluation variables.

Results: HG amplified GGT-induced TF expression (mRNA:from 0.05±0.04 to 0.4±0.1,n=8,p< 0.001; PCA:from 0.3±0.2 to 1.6±0.9, n=8,p< 0.001; TFag:from 251±101 to 457±149,n=8,p< 0.001 vs NG) that ALI (mRNA: -11±8%,n=4,p< 0.05; PCA: -25±19%,n=6,p< 0.05; TFag:-24±23 %,n=6,p< 0.05), ZOF(mRNA: -12±3 %,n=4,p< 0.001; PCA:-39±22%,n=6,p< 0.001; TFag:-40±26 %,n=6,p< 0.01) and OLM (mRNA: -12±2%,n=4,p< 0.001; PCA:-49±33%,n=6,p< 0.01; TFag:-43±30%,n=6,p< 0.01) downregulated.

Conclusions: GGT-induced TF expression is sensitive to modulation by HG and is inhibited by RAS blockers. While confirming the direct TF stimulating effect of GGT, the data unveil insofar unknown links with RAS, a behavior close to that reported for other proinflammatory agents whose pathophysiological and clinical implications need further studies.

PB 896 | Over-activation of Src1 Mediates Tissue Factor-induced Apoptosis in Endothelial Cells

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Background: Accumulation of tissue factor (TF) within cells can impede the release of TF in cell-derived microvesicles and can lead to cellular apoptosis through a mechanism that involves the activation of p38. However, the signalling process linking TF and p38 activation is unknown.

Aims: In this study we examined the ability of Src1 and Rac1 to mediate TF-signalling in the activation of p38 and cellular apoptosis.

Methods: The pCMV6-AC-TF-tGFP plasmid was mutated to express TF with a Ser253 to Ala substitution to prevent TF release. Human dermal blood endothelial cells (HDBEC; 1.5×10^5) were transfected to express the TF_{Wt}-tGFP, TF_{Ala253}-tGFP, tGFP or used un-transfected. The cells were then activated with PAR2-agonist peptide (SLIGKV-NH; 20 μM), harvested at intervals up to 120 min and the phosphorylation of Src1 and Rac1 assessed by western blot. Samples of transfected cells were then pre-incubated with pp60c Src inhibitor (TSTEPQpYQPGENL; 0-500 μM) or a control peptide (TSTEPQWQPGENL) prior to activation with PAR2-AP. The phosphorylation of p38 and cellular apoptosis was then analysed. Samples of cells were also co-transfected to express TF_{Ala253}-tGFP together with a specific Src1 siRNA or a control siRNA, activated as above and the cellular apoptosis measured.

Results: Activation of PAR2 in HDBEC led to the phosphorylation of Src1 and Rac1 proteins at 60 min regardless of TF expression. Moreover, Src1 phosphorylation was prolonged up to 100 min in the presence of TF, with a significantly higher magnitude following mutation of Ser253. Either inhibition of Src1 function, or suppression of Src1 expression reduced p38 phosphorylation and prevented cellular apoptosis in the activated cells, expressing the mutant TF.

Conclusions: Our data suggest that Src1, but not Rac1 mediates TF-induced activation of p38 and can promote cellular apoptosis in endothelial cells. This identifies a further step in the mechanism of TF-induced apoptosis particularly during inflammatory conditions such as cancer and vascular disease.

PB 897 | Indoleamine 2,3-dioxygenase 1 Enhances Tissue Factor Expression in Coronary Atherosclerotic Plaque

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Background: Unstable plaque morphologies with tissue factor (TF) expression are crucial factors for the development of atherothrombosis. Clinical studies revealed that alteration of kynurenine (Kyn) pathway, a tryptophan (Trp) metabolism, is associated with cardiovascular events. However, the roles of the kynurenine pathway on vascular wall thrombogenicity remain unknown.

Aims: This study is to identify the role of indoleamine 2,3-dioxygenase 1 (IDO-1), a rate-limiting enzyme of the Kyn pathway, on thrombogenicity in human coronary atherosclerotic plaques and cultured macrophages.

Methods: We immunohistochemically evaluated expression and localization of IDO-1 and TF in coronary atherosclerotic lesions in the patients with stable angina pectoris (SAP) and unstable angina pectoris (UAP). Trp and Kyn levels in the cultured medium, expression of IDO-1, thrombogenic factors were assessed with THP-1 macrophages activated with interferon (INF)-g and tissue necrosis factor (TNF)-a. TF activity was measured with factor Xa generation.

Results: IDO-1 expressed mainly in CD68 positive macrophage and closely localized with TF. Immunopositive areas for TF, IDO-1, CD3 positive T lymphocyte were significantly larger in coronary plaques in patients with UAP than those with SAP. INF-g and TNF-a markedly upregulated IDO-1 expression and increased Kyn/ Trp ratio, and enhanced TF expression and activity, but not tissue factor pathway inhibitor expression. Epacadostat, an IDO-1 inhibitor, significantly reduced Kyn/Trp ratio, and inhibited the cytokine induced TF expression and activity in the activated macrophages.

Conclusions: Enhanced IDO-1 expression in coronary atherosclerotic plaque may contribute to atherothrombosis through TF upregulation in activated macrophages.

PB 898 | Application of a Quantitative Systems Pharmacology (QSP) Model of Human Blood Coagulation to Understand the Response of Clinical Biomarkers from a Recent Anti-TFPI Trial in Healthy Subjects

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Background: We have developed and validated a mechanistic QSP model of the human blood coagulation system which allows simulation of the effects of pro- and anti-coagulant therapies. The current model can simulate both *in vivo* biomarkers (e.g., prothrombin fragment 1+2 or PF12) and *ex vivo* biomarkers such as the thrombin generation assay (TGA) parameters of peak thrombin, TGA lag time, activated partial thromboplastin time (aPTT) and dilute prothrombin time (dPT) clotting times.

Aims: To validate the QSP model of the human coagulation system with clinical data for *in vivo* and *ex vivo* biomarkers from a recently conducted clinical trial (NCT02531815).

Methods: The model was used to simulate the effect of single dose administration of an anti-TFPI antibody therapy on clinical biomarkers. The inhibition of TFPI in this clinical study was predicted using

drug and drug-complex pharmacokinetics modeling. Inhibition of TFPI was then used as an input into the QSP model to simulate *in vivo* and *ex vivo* biomarkers.

Results: A comparison of model predictions at different observation times showed that the results matched the clinical observations for TGA peak thrombin, TGA lag time, and dPT clotting times very well. Additionally, the model did not predict any significant changes in aPTT, a measure of modulation of the intrinsic pathway, which matched the observations in the clinical study.

Conclusions: Predictions from a computational model of human blood coagulation matches results obtained from a recently concluded clinical trial using an anti-TFPI compound. These results provide us with confidence in the ability of the model to estimate variability likely to be observed in a future clinical trial using techniques such as sensitivity analyses.

PB 899 | Coagulation and Angiogenesis Parameters in Essential Thrombocythemia

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Background: Recent studies suggest that VEGF may be involved in the thrombotic process, by stimulating the expression of tissue factor (TF) in vascular endothelial cells. It also known that TF can stimulate the growth of transcription of the gene encoding VEGF.

Aims: The aim of the study was to evaluate selected coagulation and angiogenesis parameters in ET patients.

Methods: The study group consisted of 130, newly diagnosed patients with essential thromobocythemia (mean age 61 years; F/M=84/46). Patients were recruited from the Department of Hematology and Malignant Diseases of Hematopoietic System, Bydgoszcz, Poland. Essential thrombocythemia was diagnosed according to the World Health Organization (2008) criteria. The control group consisted of 25 healthy volunteers (mean age 51 years; F/M=17/13). All parameters were measured using immunoenzymatic method (ELISA).

Results: The median concentration of TF Ag was 3 fold higher in ET patients than in the controls (Me=457.26 pg/ml vs Me=145.34 pg/ml, p< 0.000001), and the activity of TF was more than 15 fold higher than in controls (Me=49.13pM vs Me=3.22 pM, p< 0.000001). There were no statistically significant differences in the values of TFPI concentration and activity between ET patients and controls. VEGF-A was significantly increased in patients with ET (p < 0.000001) and the median in ET patients was more than 5 times higher than found in the control group (Me= 137.33 pg/ml vs Me= 23.00 pg/ml). Analysis of correlations revealed a positive correlation between VEGF-A concentration and TF Ag (R= 0.27; p= 0.09) as well as a positive correlation between VEGF-A concentration and TFPI activity in the group of ET patients (R= 0.26; p=0.01).

Conclusions: The simultaneous increase of TF concentration/activity and VEGF-A concentration as well as a positive correlation between TF and VEGF-A indicate activation of TF-dependent blood coagulation and its relationship with angiogenesis in ET patients.

PB 900 | Simulation of Factor X Activation by Extrinsic Tenase on Phospholipid Vesicles: Size Matters!

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Background: It is widely reported that TF-rich circulating microparticles have increased concentration in bloodstream in various pathological conditions such as sepsis, cancer, etc. On the surface of such vesicles TF and FVIIa could assemble into the enzyme - extrinsic tenase. Its substrate, factor X, can reversibly bind to negatively charged phospholipid membrane. Thus circulating microparticle makes a potential constant source of active factor Xa in the bloodstream. To analyze the role of the stream in the activation of blood coagulation by circulating microparticles the diffusion of factors, their mixing and their distribution between the membrane and solution have to be taken into account.

Aims: Construction of a comprehensive computational model of factor X activation on the surface of a 3D microparticle moving in a bloodstream.

Methods: The model was a set of partial differential equations in three dimensions based on a scheme of reactions including extrinsic tenase formation and factor X delivery via two-dimensional diffusion on the membrane.

Results: The model was validated using reported experimental data (Hathcock et al. Biochemistry, 2005).

It was found that apparent equilibrium dissociation constant (K_d) for interaction of FX/FXa with phospholipid membrane of microparticles in bloodstream rises with the vesicle radius. In the approximation of collision limit theory (Abbott et al. Biochemistry, 1987) the apparent association constant is inversely proportional to the vesicle radius, so K_d could rise with the vesicle radius. As a result the factor X activation is faster on smaller vesicles than on the large ones while keeping the total lipid concentration constant (many small vesicles or a few large vesicles).

Conclusions: The binding of FX/FXa is better for smaller vesicles than for larger ones. Thus the big population of 30nm-diameter TF-containing circulating microparticles would lead to 25% faster FX activation than small population of large vesicles.

PB 901 | Rutin (quercetin-3-rutinoside) Modulates Hemostatic Disturbances and Reactive Species Levels in Experimental *Bothrops jararaca* Envenomation

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Background: Snakebites are a major public health problem worldwide. In Brazil, patients bitten by *Bothrops* snakes manifest bleeding disorders associated with thrombocytopenia, consumptive coagulopathy, increased plasma tissue factor (TF) levels, and oxidative/nitrosative stress (ONS), a secondary complication of envenomation. Specific antivenom does not combat ONS, which in turn may induce additional hemostatic disorders. Thus, it is important to search for new complementary treatments, such as rutin, a natural antioxidant that also inhibits protein disulfide isomerase (PDI), an enzyme that has been proposed to control TF encryption/decryption.

Aims: To investigate the potential of rutin as an ancillary therapeutic agent for snake envenomation, and its effects on ONS and hematological/hemostatic disturbances evoked in mice injected with *B. jararaca* venom (BjV).

Methods: Swiss mice were allocated in 5 experimental groups (n=6/group/time) and were injected s.c. with: saline (group 1), propylene glycol (group 2), rutin (14.4 mg/kg b.w., group 3), BjV (1.6 mg/kg b.w., group 4) or BjV and rutin (group 5). After 3, 6 and 24 h, blood and tissues were collected for biochemical and hemostatic assays. Furthermore, *in vitro* assays were carried out to test whether rutin inhibits the activity of snake venom metalloproteinases, serine proteinases, and L-amino acid oxidases.

Results: As expected, envenomation evoked hematological/hemostatic disturbances and ONS. Interestingly, incubation of BjV with rutin (a) inhibited the activity of important venom proteins that engender envenomation; (b) shortened the recovery time in red blood cell destruction and plasma fibrinogen consumption; (c) inhibited the generation of reactive species; and (d) altered TF and PDI protein expression in heart and skin.

Conclusions: Rutin abbreviated the hemostatic and ONS disturbances, suggesting that it and may be used as a supplementary treatment for snakebites. Financial support: São Paulo Research Foundation (FAPESP), CNPq and CAPES.

PB 902 | Measurement of Tissue Factor Molecules and Factor Xa Generation on Vascular Cells

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Background: Tissue factor (TF) is a transmembrane protein that initiates coagulation. It is expressed constitutively on extravascular cells (fibroblasts, smooth muscle cells (SMC)) and induced by inflammatory stimuli on endothelium and monocytes. The plasma membrane modulates the pro-coagulant activity of TF by providing a source of phosphatidylserine (PS) and phosphatidylethanolamine (PE).

Aims: The number of TF molecules on individual cells is not well defined and, although PS/PE are readily demonstrated on activated platelets, there are limited data on the amount of PS/PE present on TF bearing cells.

TABLE 1 Expression of TF molecules on vascular cells compared to FXa generation and externalisation of PS/PE measured by Annexin V binding

	TF molecules/cell Mean (SEM)		FXa generation (nM/min) by 104 cells Mean (SEM)		Percentage of cells binding annexin V (SEM)	
Cell Type	Unstimulated	TNFα	Unstimulated	TNFα	Unstimulated	TNFα
Fibroblasts (HCA2)	70260 (6955)	Not done	0.48 (0.07)	Not done	2.50 (0.25)	Not done
Smooth Muscle Cells (HPASMC)	3591 (352)	Not done	0.14 (0.03)	Not done	0.31 (0.09)	Not done
Edothelial cells (HUVEC)	414 (41)	4534 (907)	0.03 (0.01)	0.12 (0.01)	0.20 (0.03)	4.47 (0.22)
Primary Monocytes	0.0	1175 (156)	0.0	0.01 (0.005)	0.13 (0.02)	3.90 (0.49)
THP-1 cells (monocytic)	6769 (29)	13968 (115)	0.04 (0.004)	0.06 (0.01)	1.30 (0.01)	1.83 (0.23)

Methods: The number of TF molecules/cell was measured by flow cytometry on live cells. Quantum™ Simply Cellular® beads (Bangs Laboratories) were used to determine the antibody binding capacity (ABC) of an anti-TF mouse IgG monoclonal antibody on cells and the number of TF molecules/cell determined from a standard curve. Annexin V and lactadherin were used to detect externalised PS/PE. TF-FVIIa mediated FXa generation was measured using Z-GGR-AMC substrate (Bachem).

Results: Results are shown in the table. Fibroblasts had the most TF molecules/cell while smooth muscle cells (HPASMC) and THP-1 also expressed high levels of TF constitutively. TF was not detected on resting monocytes and was very low on resting HUVEC. After stimulation with TNFa there was a marked increase in TF molecules/cell on monocytes and HUVEC and similar results were seen with IL-1β (data not shown). Only a low proportion of TF bearing cells were positive for Annexin V or Lactadherin and this did not change substantially on stimulation.

FXa generation correlated better with the number of TF molecules/cell (r^2=0.9) than the proportion of annexin V positive cells (r^2=0.04).

Conclusions: We report a novel method for measuring the number of TF molecules on individual healthy cells. Externalised PS/PE on TF-bearing cells was below the limit of detection for our assay on almost all cells, however, despite this TF-initiated FXa generation was supported.

PB 903 | Tissue Factor Pathway Inhibitor Polymorphism 399C>T Predisposes to Idiopathic DVT in Young Indians

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Background: Low Tissue factor pathway inhibitor (TFPI) is a predisposing factor of Deep Vein Thrombosis (DVT) in the West. However, its role in Idiopathic Indian DVT is not yet defined. Furthermore, the role of gene polymorphisms affecting its plasma levels is controversial.

Aims: To look for association of TFPI with the Idiopathic DVT in Indians and to elucidate the role of TFPI polymorphisms with TFPI plasma levels.

Methods: Doppler proven idiopathic DVT patients (age< 45 years) and age and sex matched healthy controls were studied. Exclusion criteria included patients on anticoagulants or those with history of malignancy, pregnancy or sepsis. Plasma TFPI levels were determined by ELISA and its normal range determined (Mean±2SD of TFPI levels in controls). TFPI gene polymorphisms (-33 T>C, 536 C>T) were detected by PCR-RFLP and 399 C>T by allele specific PCR.

Results: 90 DVT subjects, median age 35 years range (20-43 years) and M: F (59:31) were recruited. DVT was seen in lower limb in 78 patients and upper limb in 11 patients. Mean TFPI levels in DVT patients, (33.55±23.4 ng/ml) was significantly lower than controls (48.05±27.2 ng/ml, p=0.001). Moreover, 17 patients (18.8%) with DVT had low TFPI (< 20.85 ng/ml), whereas no control had low TFPI. CT and TT genotype of 399C>T polymorphism were found in 35.5% of patients and 12.2% of controls (p=0.001). TC and CC genotype of -33T>C polymorphism were found in 50% of patients and 67.7% of controls (p=0.038). TFPI 536 C>T polymorphism was absent in our population. Relation between the TFPI levels and their genotypes in subjects are shown in table.

TABLE Distribution of TFPI levels according to their genotypes in subjects

	TFPI levels ng/ml Mean±SD	
Polymorphisms	Patients (n=90)	Controls (n=90)
TFPI 399 C>T: CC, CT+TT	30.0±13.5, 35.4±10.2; p=0.021	49.1±14.0, 40.1±7.10; p=0.032
TFPI -33 T>C: TT, TC+CC	27.0±8.10, 40.0±11.2; p= <0.001	38.9±8.93, 52.0±13.8; (p=<0.001
TFPI 536 C>T: CC, CT+TT	-	-

Conclusions: As in the West, low TFPI level predisposes to DVT and -33 T>C polymorphism has a protective role in Idiopathic DVT in Indians. However, unlike earlier studies, CT and TT genotype of 399 C>T polymorphism may be associated with low levels of TFPI and DVT. Thus TFPI 399C>T polymorphism detection may help in identifying young Indians having high risk of DVT.

PB 904 | Role of Tissue Factor 603A>G and 5466A>G Polymorphisms in the Development of DVT in Young Indians

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Background: Deep Vein Thrombosis (DVT) is a multifactorial disease and leading cause of morbidity and mortality worldwide. Tissue factor (TF) is a transmembrane protein considered to be responsible for the initiation of coagulation. Elevated levels of TF have been associated to cardiovascular disease whereas its association with DVT is controversial. In addition, the role of TF with DVT in India is not yet studied.

Aims: To analyze the distribution of TF polymorphisms and their impact on TF plasma level, and to assess the association between TF and risk of DVT in young Indians.

Methods: 90 Doppler proven idiopathic DVT patients (< 45 years) and equal number of age and sex matched healthy controls were studied. Exclusion criteria included patients on anticoagulants or those with history of malignancy, pregnancy or sepsis. Plasma TF levels were determined by ELISA. TF gene polymorphisms (603A>G and 5466A>G) were detected by PCR-RFLP.

Results: Mean TF levels (pg/ml) in DVT patients, (84.95±17.16) was significantly higher than controls (70.55±15.87, p=0.001. 603A>G polymorphism was found in 64.4% of patients and 46.6% of controls (p=0.001). The distribution of 5466A>G polymorphism was not significant between patients (16.6%) and controls (7.7%, p=0.118). TF plasma level was significantly higher in patients and controls with G allele than A allele of 603 A>G polymorphism (patients; AG+GG: 93.4±12.55, AA: 69.22±13.03 p=< 0.001, controls AG+GG: 76.42±17.16, AA: 64.90±12.22 p=< 0.001). However 5466A>G polymorphism was not associated with TF plasma level (patients; AG+GG: 83.05±18.24, AA: 85.36±17.01 p=0.607, controls; AG+GG: 68.75±18.2, AA: 70.70±15.7 p=0.739).

Conclusions: High plasma level of TF was significantly associated to DVT in young Indian. TF 603 G allele was associated with its high plasma levels and risk of DVT. Whereas 5466A>G polymorphism neither associated with TF levels nor DVT. A study with larger sample size is required to confirm these findings.

PB 905 | Tissue Factor Gene Polymorphisms (A603G, C1322T, C1812T, G1442C) in Healthy Individuals and Patients with Erysipelas in Transbaikalian Region

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Background: It is known that the erysipelas is characterized with disorders of haemostasis and development of immune response. Considering that the skin represents the most powerful macrophage system and its activation accompanied by expression of tissue factor which leads to hypercoagulability in erysipelas. Genetic polymorphism of tissue factor is reflected in the quantitative and qualitative indicators of macrophage activation.

Aims: The aim was to study the genetic polymorphism of TF (A603G, C1322T, C1812T, G1442C) in healthy individuals and patients with erysipelas in Transbaikalian region.

Methods: The study was performed at 96 patients with erysipelas and 82 healthy residents of Transbaikalian region. Tissue factor gene polymorphism was detected by PCR method.

Results: At was established that of homo- and heterozygous SNP of TF gene were spreading to Hardy-Weinberg equilibrium (p>0,05). It was founded that alleles and genotypes of SNP TF A603G, C1322T, C1812T, G1442C did not differ in healthy individuals and patients with erysipelas. Alleles and genotypes of SNP of TF gene A603G, C1322T, C1812T, G1442C didn´t effect on tissue factor expression.

Conclusions: Thus, SNP of TF (A603G, C1322T, C1812T, G1442C) not influence on TF activity in healthy individuals and patients with erysipelas.

PB 1682 | Chemical Footprinting Reveals Conformational Changes Following Activation of Factor XI

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Background: Coagulation factor XI (FXI) is activated by FXIIa or thrombin resulting in a conformational change that converts the catalytic domain into its active form and exposing exosites for FIX on the apple domains. Although crystal structures of FXI and the catalytic domain of FXIa are available, the structure of the apple domains and hence their interactions with the catalytic domain in FXIa are unknown.

Aims: Identify regions involved in stability or enzymatic activity of FXIa.

Methods: We employed chemical footprinting on recombinant FXI(a). Equal amounts of FXI and FXIa were labeled with lysine-directed isobaric tandem mass tag (TMT)-126 or -127, respectively. After proteolysis by chymotrypsin, peptides were analyzed by mass spectrometry.

Results: Fifty-two unique peptides were identified, covering 37 of 41 lysine residues present in FXI. TMT-modified peptide TLRLCKM with lysine residue 357 had the highest TMT-127/TMT-126 ratio indicating that this peptide is more accessible following cleavage of FXI. In the catalytic domain, we identified peptide QKAKIPLVTNEECQKRY with lysine residues 516, 518 and 529 to have a TMT-127/TMT-126 ratio < 1, suggesting that this peptide is hidden in FXIa. The crystal structures of FXI(a) suggest that K516 interacts with E380 and that this region is covered by the N-terminus of the light chain of FXIa. To study the role of the identified regions, we generated FXI variants K357A, K516A, K516E, E380K, and E380K/K516E. Cleavage by thrombin or FXIIa of these FXI variants was similar or slightly improved, except for FXI-K516E (by thrombin) and FXI-K357A (both by thrombin or FXIIa). Interestingly, in a purified system the cleaved FXI variants K516E, E380A, and E380K had reduced chromogenic activity, while this was rescued in the double variant E380K/K516E.

Conclusions: We have identified several regions in FXI that undergo a conformational change upon activation.

PB 1683 | Acute Lowering of Factor XII Induces Accelerated Spontaneous Venous Thrombosis in Mice: New Evidence for the Contact System that "Idles"?

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Background: We reported on an unexpected prothrombotic effect upon acute lowering of coagulation factor XII (FXII), which has been introduced as a novel therapeutic target to prevent thrombosis, in a mouse model for spontaneous venous thrombosis (VT, Heestermans et al. *Blood*, 2016).

Aims: To investigate the mechanism underlying the prothrombotic response upon impact acute lowering of FXII. We hypothesized that acute inhibition of FXII affects the clearance of its target protein coagulation factor XI (FXI), in a similar fashion as shown for prekallikrein (PK), another target of FXII (Revenko et al. *Blood*, 2011).

Methods: C57BL/6 mice without FXII (*F12*-/-) or mice treated with siRNAs against *F12* (si*F12*) were analyzed for (1) their response to siRNAs targeting protein C and antithrombin (which trigger spontaneous VT), (2) tissue factor (TF) and ellagic acid induced thrombin generation, and (3) plasma levels and activity of components of the contact system.

Results: Unlike si*F12* treated mice, *F12*-/- mice did not demonstrate an earlier onset of spontaneous VT (*F12*+/+ controls: 9 out of 11 vs. *F12*-/-: 9 out of 11, *P*=1.0. For siNEG: 0/8 vs. si*F12*: 7/8 after 2 days, *P*< 0.01). Interestingly, in si*F12* mice, thrombin generation (induced

by 1pM TF) was significantly increased compared to siNEG, *F12*+/+, and *F12*-/- mice (peak heights siNEG: 69.8±3.4 nM thrombin, si*F12*: 88.1±3.8 nM, *F12*+/+: 66.2±9.4 nM, *F12*-/-: 63.7±1.7 nM, *P*< 0.01). This coincided with mildly increased plasma PK levels (140.0±17.0% of siNEG (100±14.9%), n=3) and higher plasma FXI activity (165.5±50.1% of siNEG (100±58.0%), *P*=0.01). In addition, spontaneous VT in mice appeared responsive to FXI deficiency (*F11*+/+: 9/11 vs. *F11*-/-: 5/11 and acute weight loss: 2.1g ±0.52 vs 1.2g ±0.33, *P*< 0.01).

Conclusions: In mice, acute lowering of FXII induces an unexpected prothrombotic shift, characterized by high FXI activity. This suggests that FXII, in absence of a challenge, modulates plasma FXI (and PK), thus providing new evidence for a contact system that "idles".

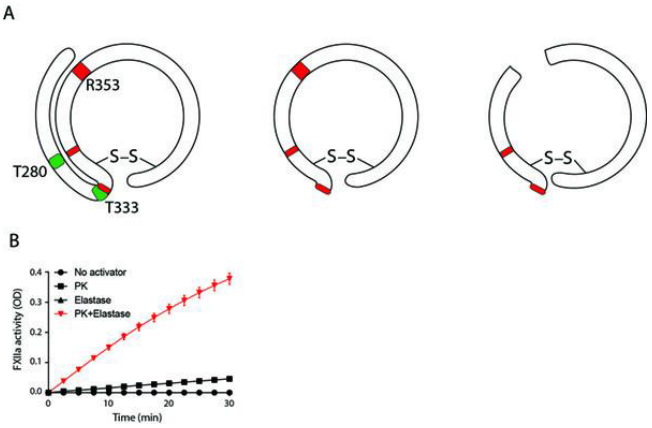

FIGURE 1 A) Two-step activation model. Cleavage sites for Elastase in Green, for PK in red. B) Synergism between elastase and PK in FXII activation.

PB 1684 | A Two-step Mechanism for Factor XII Activation in Solution

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Background: The plasma contact system is named after its interaction with negatively charged surfaces. Under specific conditions, this system also becomes activated in solution. We previously identified that pathogenic mutations in the proline-rich region of factor XII (FXII) enhance its activation in solution, resulting in uncontrolled bradykinin production. We hypothesized that this reflects a physiological fluid-phase activation mechanism for FXII without mutations.

Aims: To uncover a general mechanism for fluid-phase activation of FXII.

Methods: FXII-variants were produced by transient transfection in HEK cells and affinity-purified. FXII activation was monitored by chromogenic substrates and with an in-house developed capture ELISA. FXII fragmentation was investigated by western blotting.

Results: Detailed investigation of the pathogenic mutant FXII-T309K by plasmin revealed that this actually is a two-step process: first, plasmin cleaves the proline rich region to remove a large shielding sequence. This does not directly activate FXII, but primes it for a subsequent activating cleavage (after R353). In *silico* predictions indicated several cleavage sites for various enzymes in the proline-rich region of FXII. These enzymes include neutrophil elastase (NE), cathepsin K, MMP-9 and calpain 1. Exposure of FXII to NE in buffer and plasma cleaves FXII into two fragments, which destroys its procoagulant activity. As before, this cleavage does not directly activate FXII. The resulting truncated FXII zymogen (single-chain, but lacking its surface-binding domains), becomes highly susceptible to fluid-phase activation by low levels of plasma kallikrein (Fig. 1). Identical data were obtained with cathepsin K.

Conclusions: We propose a unified two-step model for fluid-phase activation of FXII. In the first step, FXII becomes primed for activation by enzymatic removal of a large sequence that includes the surface-binding domains. This exposes site R353 for rapid cleavage by plasma kallikrein, culminating in FXII activation.

PB 1685 | Cell Receptor Interactions of the Contact System

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Background: Complement receptor gC1qR, coagulation factor XII (FXII) and kininogen (HK) interact and play important roles in innate immunity, thrombosis and inflammation. Complement receptor gC1qR is present on the surface of endothelial cells, platelets and in platelet microparticles. High molecular weight kininogen (HK) is an important co-factor for the contact pathway localising factors to the correct surface. Domain 5 of HK binds to endothelial cell surface through the gC1q-R receptor, whilst domain 6 interacts with the proteases prekallikrein and factor XI. gC1qR also interacts with factor XII. gC1q-R is a trimeric, acidic receptor that binds HK and factor XII at the cell surface.

Aims: To understand the molecular mechanism of receptor interaction of the contact factors HK and FXII and thus understand cell surface localisation an contact activation.

Methods: Surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) was used to obtain affinity binding constants for protein interactions and protein crystallography was used to determine crystal structures.

Results: We characterised in detail the binding interaction between recombinant FXII and domain 5 of HK and identified the interaction with a gC1q-R trimer as having multiple binding sites. Truncations of domain 5 along with point mutations within the acidic loop regions of gC1q-R have been made in an attempt to pinpoint the exact binding interface between these two proteins. We also report the first complex structure of a domain from FXII with gC1qr showing the detailed receptor interactions.

Conclusions: These studies on HK D5 together with experiments characterising the interaction with FXII define gC1qR as a unique zinc ion sensing trimeric receptor landing platform capable of assembling the contact system.

PB 1686 | Endothelial Cell Serpins, Including PAI-1, Form a Complex with Fxia Blocking its Activity and Inducing the Clearance, Internalization and Degradation of FXIa

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Background: Platelets can bind factor XI (FXI) supporting FXI activation. In contrast, it has been suggested that endothelial cells can not bind FXI nor promote its activation or enzymatic activity.

Aims: Characterization of FXI activation on the surface of endothelial cells.

Methods: To measure FXIa activity we used a FXIa chromogenic assay. Human umbilical vein endothelial cells (HUVEC) were incubated with vehicle or FXIa for 2h at 4°C. Immunofluorescence microscopy was used to measure FXIa binding to HUVECs; FXIa from either the supernatant (SN) or the cell lysate (CL) was immunoprecipitated and complexes were detected by western blot (WB) using an anti-FXIa catalytic domain or light chain (LC) antibody or an anti-plasminogen activator inhibitor 1 (PAI-1) antibody.

Results: FXIIa was able to activate FXI bound to the surface of the empty wells or platelets, but not to HUVECs. FXIIa was able to activate prekallikrein bound to the surface of either the empty wells, platelets or HUVECs. HUVECs blocked the activity of FXIa but not the activity of either kallikrein or FXIIa. FXIa was able to bind to the surface of HUVECs and the FXIa-LC, which has a molecular weight of ~30 kDa, formed a complex with two serpins generating two bands at ~80 kDa and ~70 kDa. After incubation of HUVECs with FXIa at 4°C, cells were incubated at 37°C for different times. The formation of FXIa-serpin complexes disappeared from the surface of HUVEC and only the band at ~80 kDa appeared in the SN in a time-dependent manner. WB for PAI-1from the SN and CL detected a band at ~80 kDa while the expression of PAI-1 on the HUVEC surface decreased after incubation with FXIa. Immunofluorescence analysis found that FXIa bound to the surface of HUVECs disappeared after 2h at 37°C.

Conclusions: Our data suggest that FXIa forms a complex with PAI-1 and a yet-unidentified serpin on the surface of endothelial cells inducing the release of the complex to the SN while also inducing internalization and degradation of FXIa.

PB 1687 | Characterization of Polyphosphate in Endothelial Cells under Cellular Stress

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Background: Polyphosphate polymers are stored in platelet dense granules and released upon platelet activation, whereupon they act as a scaffold for coagulation factor assembly and activation. Compared to platelet polyphosphate, little is known about the role of endothelial cell polyphosphate and its function in the vasculature under normal or disease conditions.

Aims: This project seeks to identify and localize endothelial cell polyphosphate and determine if cellular stress can up- or down-regulate the production of polyphosphate in endothelial cells.

Methods: Polyphosphate binding domain (PPX_Δ12) was generated as a recombinant deletion mutant of E. coli exopolyphosphatase (PPX) containing domains 3 and 4 only. In HUVECs or EA.hy926 endothelial cells, polyphosphate was probed with PPX_Δ12 and visualized with confocal immunofluorescence. To determine polyphosphate chain length, cell lysates were characterized via urea polyacrylamide gel electrophoresis. To assay the functional role of endothelial polyphosphate, EA.hy926 or ECV304 cells were exposed to cold or heat shock for one hour to determine changes in polyphosphate content, characterized by malachite green assay for monophosphate content.

Results: Polyphosphate was visualized in endothelial cells in the cytosolic compartment in a punctate pattern and was resistant to digestion with shrimp phosphatase. Endothelial cell polyphosphate was shown to migrate on urea acrylamide gels as a long smear of chain length greater than 400 phosphate units that was resistant to digestion by shrimp phosphatase but partially digestible by PPX. Heat or cold shock did not up- or down-regulate polyphosphate expression in endothelial cells.

Conclusions: Significant amounts of polyphosphate of long chain length is present in endothelial cells and was not demonstrated to respond to conditions of cellular stress; thus, further studies are warranted to interrogate the functional role of endothelial polyphosphate in the vasculature.

PB 1688 | Identification of *F11* Gene Variations Involved in the Interindividual Variability of Factor XI in Subjects with Congenital Deficiency Caused by Recurrent Mutations

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Background: High FXI levels increase the risk of venous thrombosis, while its deficiency may protect against it. This and the huge interindividual variability of FXI levels have encouraged us to search new elements involved in their control. Current data only show *F11* SNVs with mild and conflictive functional role.

Aims: We identified 183 subjects with FXI deficiency, carrying 4 recurrent mutations, with a remarkable variability of FXI, even in carriers of the same family. We aimed to analyze gene variations of *F11* in these patients and new mechanisms involved in the variability of FXI levels.

Methods: Plasma FXI was evaluated by 4 clotting assays and Western Blot in 85 individuals with FXI deficiency. The whole *F11* gene was sequenced by NGS (Ion Torrent platform).

Results: 114 SNVs were identified, 22 new (all intronic and mostly restricted to isolated cases). The p.E226R (MAF: 0.002) reported as nonfunctional SNV was identified in a family carrying the p.C398Y mutation. Interestingly, compound heterozygous displayed about 50% lower FXI levels compared to single carriers.

TABLE Carriers of F11 mutations identified in our study. The variability of FXI levels of each group is indicated

			Whole cohort		This study	
HGVSc	HGVSp	References	Subjects (families)	%FXI:C Mean±SD (range)	Subjects (families)	%FXI:C Mean±SD (range)
c.166T>C	p.Cys56Arg (p.Cys38Arg)	Zivelin et al, Blood 2002;99:2448-54.	105 (23)	42.1±10.1 (21-66)	26 (20)	41.2±11.2 (21-66)
c.1247G>A	p.Cys416Tyr (pCys398Tyr)	Mitchell el al, Blood. 2005;105:4671-3.	19 (7)	39.7±14.4 (22-72)	14 (6)	42.8 ±14.3 (22-72)
c.1693G>A	p.Glu565Lys (pGlu547Lys)	Quélin et al, Haematologica. 2005;90:1149-50.	27 (6)	46.6±13.9 (28-75)	16 (6)	48.6 ±13.7 (30-75)
c.1613C>T	p.Pro538Leu (pPro520Leu)	Mitchell et al, Br J Haematol. 2005;129(6)734-745.745.	32 (2)	51.6±9.1 (34-67)	29 (2)	51.7±9.3 (34-67)

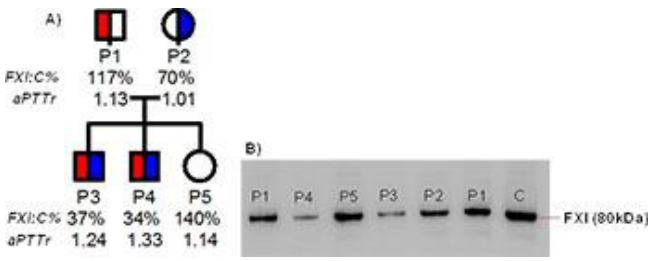

FIGURE Compound heterozygosity. A) Pedigree. Heterozygous for p.Cys398Tyr (Blue) or p.Glu226Arg (Red). B) FXI levels in control (C) and family members

rs2289252 and rs2036914, associated with high levels of FXI in non-deficient subjects, also increased (5%) FXI levels in deficient carriers. rs925451 and rs4253399, in linkage disequilibrium D=0.8, were significantly associated with higher (17%) FXI levels (p= 0.02). This effect was particularly strong in p.C56R, a CRM- type 3 with the lowest FXI levels.

Conclusions: The study of *F11* gene in congenital FXI deficiency carriers is an excellent and sensitive model to find new elements and mechanisms involved in the modulation of FXI levels. We suggest that rs925451 and rs4253399, both previously assotiated with stroke, increase FXI levels. Moreover, the dominant negative effect of p.C398Y mutation may be exacerbated if the other allele carry the p.E226R mutation, revealing that the functional consequences of a mutation may depend on the genetic profile.

Funding: SEHH. PI15/00079; CB15/00055 (ISCIII, FEDER); 19873/GERM/15.

PB 1689 | Long-chain Polyphosphate Promotes FXII-dependent Platelet Consumption under Shear Flow

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Background: Bacterial sepsis is a prevalent cause of hospital mortality with terminal complications that include development of consumptive coagulopathy and thrombocytopenia leading to organ hypoperfusion and bleeding. Bacterial constituents, including long-chain polyphosphates (polyP), have been shown to activate the contact pathway of coagulation in plasma. Recent work has shown that activation of the contact pathway is capable of promoting thrombin generation and platelet activation and consumption in whole blood distal to thrombus formation under shear *ex vivo* and *in vivo*.

Aims: Determine whether long-chain polyP-induced contact pathway activation promotes platelet activation and consumption in the bloodstream.

Methods: Human whole blood was collected into citrate, recalcified and studied in open and closed systems. Platelet activation, microaggregation and consumption in the bloodstream were assessed by FACS and thrombus growth was visualized by microscopy.

Results: Presence of long-chain polyP in whole blood enhanced platelet aggregation on immobilized collagen surfaces under shear flow, enhanced fibrin formation and shortened clotting times. Long-chain polyP promoted platelet P-selectin expression, microaggregate formation and platelet consumption in the bloodstream under shear in a FXII-dependent manner. Moreover, long-chain polyP accelerated thrombus formation on immobilized collagen surfaces under shear flow. Distal to the sites of thrombus formation, platelet consumption was dramatically enhanced in the presence of long-chain polyP in the bloodstream. Inhibiting contact activation of the coagulation pathway reduced fibrin formation on collagen as well as platelet consumption in the bloodstream distal to the site of thrombus formation.

Conclusions: This study demonstrates that bacterial-type long-chain polyP promotes FXII-mediated thrombin generation and platelet activation in the flowing blood and could exaggerate sepsis-associated consumptive coagulopathy and thrombocytopenia.

PB 1690 | Fibrin is a strong activator of FXII

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Background: FXII has recently been shown to participate in a positive feedback mechanism involving the intrinsic pathway, which generates thrombin that influences the structure of the clot. Polyphosphate from activated platelets has been reported to be the trigger of FXII. We have previously demonstrated that clotting of whole blood and plasma generates FXIIa-antithrombin (AT) complexes, while FXIIa-C1inhibitor (C1INH) are detected after blood contact with different material surfaces (i.e. glass, kaolin and polyphosphate). The latter confirms that b-XIIa preferentially is inactivated by C1INH. FXIIa-AT is also generated when platelets in platelet-rich plasma are activated by Thrombin/TRAP and if fibrin is added to plasma.

Aims: Identifying the functional requirement for FXII activation in the clot.

Methods: In order to investigate the mechanism of FXIIa-AT generation, the interaction of FXII with fibrin and fibrinogen was studied using QCM-D, activation assay, ELISA and Simple western.

Results: The results show that FXII, α-FXIIa and β-FXIIa bind to fibrin, in close to equimolar ratio. After binding to fibrin, FXII shows comparable AT-binding capacity as pre-activated FXIIa, indicating that native FXII has been activated to FXIIa by the fibrin. By contrast, neither FXII nor any of the FXII activation products bound to fibrinogen. The enzymatic activity of FXII was assessed by using the chromogenic substrate S-2302, confirming that strong activation occurred upon contact with fibrin and glass, but very little activation occurred on polystyrene. When measuring FXII-serpin complex formation after incubation of normal human plasma on fibrin-coated surfaces, high levels of FXIIa-AT and moderate levels of FXIIa-C1INH were found.

Conclusions: Our results show that fibrin is a strong binder and activator of FXII, thereby being an candidate for triggering FXII activation in the clot. The study also shows that activation of FXII by fibrin is regulated by AT and that FXIIa-AT is a biomarker for blood clotting.

PB 1691 | Increased Kallikrein Generation Potential in Patients Suffering from Hereditary Angioedema

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Background: Hereditary angioedema (HAE) type 1 & 2 is an autosomal dominant disease resulting in deteriorated function of C1-esterase inhibitor, the main inhibitor of the contact activation and complement systems. HAE patients experience potentially fatal swellings in various body parts. Previous studies suggest contact activation as the cause of the swellings, as these have been associated with increased plasma levels of kallikrein and FXIIa-inhibitor complexes and bradykinin.

Aims: In this study, we measured the plasma kallikrein generation potential (KGP) of HAE patients in the resting state of the disease, and compared the results to the KGP of normal individuals.

Methods: Plasma from both groups (n = 6+6) was investigated using the calibrated kallikrein generation method.

Results: The KGP in HAE patients was considerably higher than in healthy individuals. The area under the curve, i.e. the total kallikrein activity generated over time, was significantly higher in plasmas from HAE patients compared to controls (p = 0.001) (Fig. 1). Furthermore, the median peak height, indicating the highest kallikrein activity measured, was significantly higher than the median peak height of the controls (p = 0.041). Lastly, the median lag time, indicating the time until kallikrein activity could be measured, was borderline significantly lower in HAE patients (p = 0.07).

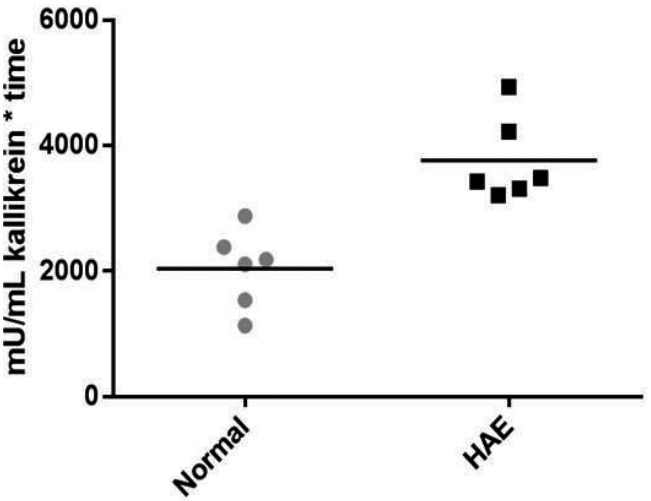

FIGURE 1 The area under curve values obtained from the two groups. A significant difference between the groups was observed (p = 0.001).]

Conclusions: The KGP in plasma from HAE patients, in the resting state of the disease, is significantly increased compared to normal individuals. This suggests that the KGP could be a valuable marker in studies of patients suffering from HAE and possibly other bradykinin-mediated edemas.

PB 1692 | Red Cell and Platelet Microvesicles Activate the Contact System of Coagulation

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Background: Contact system (CS) activates coagulation via the intrinsic pathway and is comprised of factor XII (FXII), prekallikrein and high molecular weight kininogen (HMWK). Although the CS is dispensable for normal hemostasis, animal models suggest that FXII plays a role in thrombosis. In humans, CS contributes to activation of coagulation and thrombosis in the setting of extracorporeal circulation devices.

Aims: Determine how red blood cell (RBC) and platelet (PLT) microvesicles (MV) activate the CS of coagulation.

Methods: Platelet rich plasma (PRP) and RBCs were isolated from whole blood collected in citrate, corn trypsin inhibitor (CTI) and PPACK. Purity of PRP and RBC in buffer was confirmed using an automatic cell counter. PRP or RBC were incubated for 1H with 10µM ionophore. After removal of RBC or PLT, MVs were isolated and washed to remove residual CTI and PPACK. RBC or PLT MVs were counted using flow cytometry. Washed MVs were re-suspended in re-calcified normal pooled platelet free plasma (PFP) or deficient plasmas to assess thrombin generation (TG). They were also re-suspended in a synthetic CS reconstructed by diluting purified FXII, FXI and HMWK at their physiological concentration in buffer. After 1H incubation, 3µM purified C1-inhibitor esterase (C1INH) was added followed by addition of 25µM PPACK 30min later. CS activation was assessed using an in house ELISA assay to detect FXIIa-C1INH complexes.

Results: As shown in Fig.1, both RBC-MVs and PLT-MVs triggered TG in normal pooled and FVII deficient, but not FXI deficient plasma. No TG was observed in FXII deficient plasma containing RBC-MVs while some was still detectable with PLT-MVs. Higher FXIIa-C1INH complexes were detected in the presence of both types of MVs as compared to their absence.

Conclusions: In addition to confirming that RBC- and PLT-MVs activate the contact pathway, this study suggests that RBC-MVs may activate FXII while PLT-MPs may activate FXII and FXI. The mechanism of MV-induced CS activation is currently under investigation.

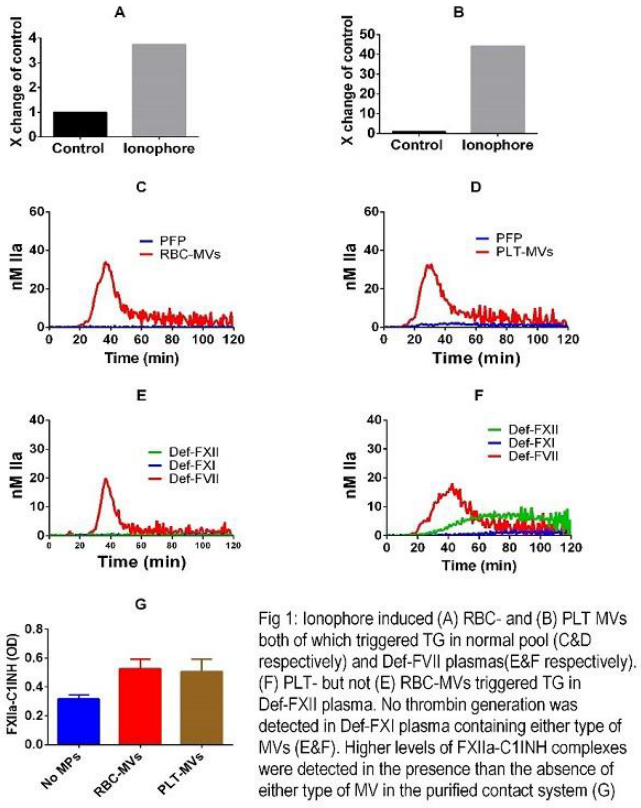

Fig 1: Ionophore induced (A) RBC- and (B) PLT MVs both of which triggered TG in normal pool (C&D respectively) and Def-FVII plasmas(E&F respectively). (F) PLT- but not (E) RBC-MVs triggered TG in Def-FXII plasma. No thrombin generation was detected in Def-FXI plasma containing either type of MVs (E&F). Higher levels of FXIIa-C1INH complexes were detected in the presence than the absence of either type of MV in the purified contact system (G)

FIGURE 1 Procoagulant activity of red blood cell and platelet microvesicles

PB 1693 | Contaminating Silica Particles Contribute Substantially to the Procoagulant Activity of DNA Isolated Using Commercial Kits

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Background: Extracellular DNA and RNA are implicated as initiators of the contact pathway of blood clotting and markers of inflammation and sepsis. Most studies have employed commercial, silica-based spin columns to purify cellular DNA.

Aims: Compare the procoagulant activities of cellular nucleic acids.

Methods: DNA was purified from HEK-293 cells and human neutrophil extracellular traps (NETs) using either phenol/chloroform extraction or DNeasy Blood and Tissue kits (Qiagen). Procoagulant activity was determined by mixing samples with citrated plasma in a 96-well microplate, incubating 3 min at 37°C, then recalcifying and monitoring time to clot formation.

Results: HEK-293 and NET DNA purified using Qiagen kits exhibited substantial (albeit variable) procoagulant activity, while DNA purified by phenol/chloroform extraction had very limited procoagulant activity. The procoagulant activity of kit-purified DNA was only slightly diminished after digestion with nuclease or boiling in acid. When water was applied (in lieu of cellular lysates) to DNeasy columns, the eluates exhibited substantial procoagulant activity. Analyses of these eluates by inductively coupled plasma mass spectrometry identified significant concentrations of silicon. Silica particles and the homogenized matrix material from the DNeasy columns were highly procoagulant.

Conclusions: The clotting activity of cellular DNA that we purified using a commercial kit employing silica-based spin columns appeared to be due to the combination of the very limited clotting activity of DNA plus the much more substantial clotting activity of finely divided silica particles that were found to contaminate such DNA preparations. Some investigators have also used silica-based spin columns to

purify polyphosphate from cells, and these preparations may also be contaminated with silica particles. Conventional means of isolating cellular DNA or polyphosphate (e.g., phenol/chloroform extraction) are recommended when studying the procoagulant activities of these substances.

PB 1694 | Genetic Determinants of Levels of Contact Activation Coagulation Factors

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Background: The complex, interdependent contact activation system has become increasingly interesting for its role in thrombotic disease. Few genetic determinants of the levels of proteins from this system are known.

Aims: To study the influence of *F11*, *F12*, *KLKB1* and *KNG1* variants on FXI activity (C) and FXI, FXII, prekallikrein (PK), and high-molecular-weight kininogen (HK) antigen (ag) levels.

Methods: We analyzed samples from 630 healthy individuals who participated as controls in one of our studies (RATIO), in which 43 tagging single nucleotide variants (SNVs) were genotyped to represent common genetic variation in *F11*, *F12*, *KLKB1* and *KNG1*. Antigen and activity levels were measured with sandwich ELISA-based and one-stage clotting assays. We performed single variant, age-adjusted, linear regression analyses per trait assuming additive inheritance and then conditioned on the lead SNV to identify independent associations. Haplotypes were based on the lead SNV and all independently associated SNVs per trait.

Results: We identified multiple novel associations in addition to replicating existing findings; lead SNVs and results from conditional analyses are shown in Tables 1 and 2. We found that *F11* SNVs rs2036914 and rs1593 influence FXI:C via FXI:ag, whereas rs4253399 was independently associated with FXI:C and FXI:ag. One *F12* SNV was independently associated with FXII:ag and two *KLKB1* SNVs with PK:ag levels. Additional associations observed between *KLKB1* SNVs and FXI levels are likely explained by linkage between *KLKB1* and *F11* SNVs. We identified three associations between *KNG1* SNVs and HK:ag in addition to lead SNV rs5030062. SNV rs5030062 was also associated with FXI:C, FXI:ag, and PK; although the latter two were largely mediated by HK:ag levels. Haplotype analysis revealed a large joint effect of rs5030062 and rs5029980 on HK:ag (ß=25.22, p< 0.0001).

Conclusions: This study adds to current knowledge of how genetic variation influences levels of contact system proteins and clarifies interdependencies.

TABLE 1 Lead variants associated with contact system proteins

Trait	Gene	Lead variant	Major/ minor alleles	Minor allele frequency	$ß_{singleSNV}$	95% CI	p-value
FXI:C	*F11*	rs4253399	T/G	38.9%	9.24	6.69 to 11.79	$3.32x10^{-12}$
FXI:ag	*F11*	rs2036914	C/T	46.3%	-11.38	-14.18 to -8.58	$7.87x10^{-15}$
FXII:ag	*F12*	rs1801020	C/T	25.3%	-41.54	-45.67 to -37.41	$6.85x10^{-67}$
PK:ag	*KLKB1*	rs2304595	G/A	43.2%	10.33	6.65 to 14.02	$5.58x10^{-8}$
HK:ag	*KNG1*	rs5030062	A/C	38.3%	10.23	7.87 to 12.59	$1.63x10^{-16}$

TABLE 2 Independent associations identified in conditional analyses on lead variants

Trait /Gene	Variant	Major/ minor alleles	Minor allele frequen.	Linkage disequilibrium (r^2)	$ß_{singleSNV}$	95%CI	$ß_{cond}$	95%CI	p_{cond}
FXI:C/*F11*	rs1593	A/T	12.2%	0.10	-8.51	-12.25 to -4.76	-5.65	-9.46 to -1.83	0.004
FXI:ag/*F11*	rs1593	A/T	12.2%	0.17	-11.88	-16.13 to -7.63	-7.21	-11.70 to -2.73	0.002
FXI:ag/*F11*	rs4253399	T/G	38.9%	0.60	11.32	8.42 to 12.43	6.20	2.10 to 10.34	0.003
PK:ag/*KLKB1*	rs4253243	T/C	6.9%	0.06	-16.93	-24.19 to -9.67	-12.38	-20.07 to -4.69	0.002
HK:ag/*KNG1*	rs2304456	T/G	11.4%	0.06	-10.81	-14.51 to -7.12	-7.96	-11.60 to -4.32	$2.1x10^{-5}$
HK:ag/*KNG1*	rs5029980	T/C	12.9%	0.00	6.58	2.95 to 10.22	7.02	3.59 to 10.44	$6.4x10^{-5}$
HK:ag/*KNG1*	rs5030039	T/C	25.6%	0.19	-8.30	-10.88 to -5.71	-5.63	-8.32 to -2.95	$4.4x10^{-4}$

PB 1695 | Role of the Platelet Surface in the Activation of FXI by FXIIa

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Background: Activation of coagulation factors on the platelet surface plays an essential role in hemostasis. Yet, select members of the contact activation pathway, such as factor (F)XII, seem to be dispensable for hemostasis despite the fact that targeted inhibition of or deficiency in FXII is protective in thrombosis models. In contrast, a minor bleeding phenotype is associated with FXI-deficiency, suggesting that FXI plays some role in hemostasis. Thus, it remains a mystery as to why activation of FXI by FXIIa contributes to thrombosis but not hemostasis. It is also unknown whether platelets perform a differential role in contact pathway activation.

Aims: Characterize the role of platelets in the activation of FXI by FXIIa.

Methods: Human FXI (30nM) and isolated washed platelets (1.5×10^8 plt/mL; resting or activated with 1nM alpha-thrombin) were incubated with activated FXII (FXIIa; 3nM) for 1h at 37°C in the presence of 10μM Zn^{2+}. Corn trypsin inhibitor (CTI; 20μg/mL) was added to stop the reaction before a FXIa chromogenic substrate was added and FXIa generation was measured at 405nm.

Results: The results show that in the absence of platelets, a linear response in FXIIa-mediated FXIa generation was observed; furthermore, the presence of resting platelets had no effect on the rate of FXIa generation. The presence of activated washed platelets completely abolished FXIa activity. Moreover, the supernatant from activated platelets likewise eliminated FXIa activity. Yet, when purified FXIa was added directly, only the platelet supernatant retained the ability to inhibit FXIa activity, while an increase in FXIa activity was observed in the presence of activated platelets.

Conclusions: Our results suggest that the platelet surface pacifies the activation of FXI by activated FXIIa, while protecting the activity of FXIa. Moreover, our findings suggest that activated platelets secrete inhibitors to block FXI activation by FXIIa or FXIa activity, such as protease nexin 2.

PB 1696 | Contribution of Intrinsic Coagulation Factors on ex vivo Thrombus Formation and Lysis

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Background: Coagulation factor XI (FXI) and FXII are identified as targets for antithrombotic therapy, but the exact mechanisms by which these proteins contribute to clot formation and lysis are still unclear.

TABLE 1 Thrombin generation parameters determined in platelet rich plasma

		Healthy controls n=10	FXII Deficient patients n=4	FXII Deficient patients (corrected 100%) n=4	FXII DEF patients p values	FXI Deficient patients n=6	FXI Deficient patients (corrected 100%) n=6	FXI DEF patients p values
0.5 pM TF	Lagtime (min)	5.8 [4.2-9.4]	5.4 [4.1-9.7]	5.4 [4.0-8.9]	-	5.4 [5.3-6.8]A	4.8 [4.2-5.4]C	A=0.03 C=0.04
	Time to peak (min)	20.0 [13.7-25.9]	16.9 [12.1-24.1]	15.5 [11.5-20.4]	-	16.2 [14.7-21.8]A	11.3 [10.6-13.4]C	A=0.03 C=0.01
	Peakheight (nM)	66 [57-90]	77 [57-106]	88 [71-116]	-	85 [57-115]A	132 [109-204]C	A=0.03 C=0.01
	ETP (nM*min)	1300[1068-1440]	1256 [1240-1415]	1422 [1310-1478]	-	1507 [1230-2013]A	1715 [1274-2421]C	A=0.03 C=0.01
1 μM Sulfatide	Lagtime (min)	6.5 [5.6-10.0]	55.1 [17.0-120.0]B	5.6 [4.6-7.3]	B=0.01	16.8 [9.0-35.7]A,B	5.7 [4.0-6.8]	A=0.03 B=0.01
	Time to peak (min)	13.5 [9.7-16.3]	77.2 [30.5-120.0]B	9.5 [7.9-11.2]C	B=0.01 C=0.01	26 [16.2-60.9] A,B	11.4 [8.0-11.7]C	A=0.03 B=0.01 C=0.01
	Peakheight (nM)	126 [94-160]	32 [0-65]B	206 [152-230]C	B=0.01 C=0.01	95 [24-145]A	158 [110-239]	A=0.03
	ETP (nM*min)	1502 [1252-1612]	280 [0-1340]B	1641 [1560-1932]C	B=0.01 C=0.02	1525 [1457-2372]A	1811 [1457-2372]C	A=0.03 C=0.02

Data are expressed as Median [min-max]. Platelet count was set to 150* 109 /L ETP: Endogenous thrombin potential. A: P <0.05, Factor deficiency vs. 100% corrected Factor deficiency (Wilcoxon); B: P <0.05, Factor deficiency vs. Control (Mann Whitney); C: P <0.05 100% corrected Factor deficiency vs. Control (Mann Whitney).

TABLE 2 Whole blood ROTEM parameters triggered with EXTEM reagent (upper part) and INTEM reagents (lower part) in the presence of tPA.

		Healthy controls n=10	FXII Deficient patients n=4	FXII Deficient patients (corrected 100%) n=4	FXII DEF patients p values	FXI Deficient patients n=6	FXI Deficient patients (corrected 100%) n=6	FXI DEF patients p values
EXTEM	CT (min)	4.8 [2.0-6.7]	5.5 [4.8-12.2]	4.2 [3.8-6.7]	-	3.3 [2.5-4.6] A	2.6 [2.3-3.5] C	A=0.03 C=0.02
	MCF (mm)	48 [37-60]	44 [26-64]	46 [40-58]	-	52 [41-55]	50[46-54]	-
	LT (min)	35.3 [18.6-67.8]	33.5[27.5-110.5]	28.8[23.9 -62.4]	-	47.8[36.0-51.3]	45.7[29.3 -51.0]	-
	FR (% decline/min)	6.0 [2.0-9.2]	7.6 [2.8-10.5]	6.3 [2.6-8.6]	-	3.4 [2.5-6.7]	3.3 [2.8-8.3]	-
INTEM	CT (min)	3.8 [3.2-4.1]	27.9 [17.3-35.9] B	3.1 [2.6-3.8]	B=0.03	5.1 [3.7-12.7] A,B	2.7 [2.2-2.9] C	A=0.01 B=0.01 C=0.01
	MCF (mm)	53 [67-62]	24[9-59]	50[38-42]	-	49[32-55]	50[44-53]	-
	LT (min)	34.2 [28.9-47.9]	36.5[19.0-120]	26.0[13.0-77.0]	-	43.0[35.8-81.5]	49.5[28.6-54.6]	-
	FR (% decline/min)	5.3 [3.7-7.8]	6.2 [4.8-8.0]	10.8 [9.0-17.6] C	C=0.01	3.3 [0.9-7.2]	3.0 [1.7-10.0] C	C=0.03

Data are expressed as Median [min-max]. CT: Clotting time; MCF: Maximum clot firmness; LT: Lysis time; LOT: Lysis onset time; FR: Fibrinolysis rate FR=75/(LT-LOT) A: P <0.05, Factor deficiency vs. 100% corrected Factor deficiency (Wilcoxon); B: P <0.05, Factor deficiency vs. Control ((Mann Whitney); C: P <0.05 100% corrected Factor deficiency vs. Control (Mann Whitney).

Aims: To determine the role of coagulation FXI and FXII in ex vivo clot formation and lysis.

Methods: Blood was drawn from patients with a severe deficiency in FXI or FXII and healthy controls. Thrombin generation (TG) was determined in platelet rich plasma (PRP) using 0.5 pM TF or 1μM sulfatides. Purified FXI or FXII was added to correct deficiency towards normal. ROTEM parameters were determined ± addition of tPA using 15 pM TF or 4μM sulfatides. Fibrinolysis rate (FR, %decline/min) was calculated from the ROTEM parameters lysis onset time and lysis time.

Results: Both in FXII and FXI deficiency TG was delayed, peak and ETP were reduced in FXII deficiency, not in FXI deficiency, upon activation with sulfatides; neither condition had any effect on TG induced by TF(Table1). Addition of 100%FXI led to higher ETP values in patients compared to controls (TF and sulfatides), while addition of 100%FXII higher ETP values are only obtained when activated with sulfatides, but not with TF. In whole blood ROTEM experiments only the clotting time was significantly prolonged in both FXII- and FXI deficients upon sulfatide stimulation(Table2); neither showed any difference in fibrinolysis between patients and controls. Upon correction of the deficiency towards 100%, the FR was higher when initiated with sulfatides in FXII deficient patients and lower in FXI deficient patients versus controls.

Conclusions: TG in PRP was impaired both in FXII and FXI deficient patients after contact activation; correction towards 100% resulted in an overcompensation of ETP. While ROTEM only showed a delay in CT in FXII deficient patients, neither deficiency had impact on fibrinolysis. Correction of FXII enhanced fibrinolysis, in contrast, correction of FXI lowered fibrin degradation probably due to a firmer clot due to more thrombin formation.

PB 1697 | Generation of a Monoclonal Antibody against "Fast Form" Alpha-2-macroglobulin and Development of a Method to Measure Artificial Surface Driven Protease Activation in Plasma

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Background: Human alpha-2-macroglobulin (α_2M) is a 725 kDa broad-spectrum inhibitor, targeting almost any protease in plasma. The mechanism of inhibition conducted by α_2M is a unique and multi-step process resulting in an irreversible conformational change of α_2M into a complexed or "electrophoretic fast-form" (F-α_2M).

Aims: Because of its involvement in coagulation, fibrinolysis, contact activation, and inflammation, we hypothesized that detection of F-α_2M could be a valuable marker for the *in vitro* evaluation of the blood/plasma compatibility of artificial surfaces.

Methods: Generation and characterization of a F-α_2M specific monoclonal antibody without interference with native α_2M and development of a sandwich ELISA to measure F-α_2M levels.

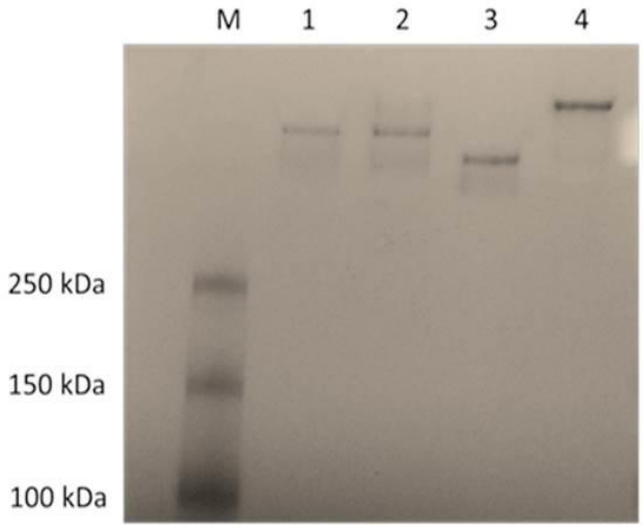

FIGURE 1 Native PAGE of a2M and F-a2M in presence or absence of 16-11-17. 1: α2M; 2: α2M + 16-11-17; 3: F-α2M; 4: F-α2M + 16-11-17.

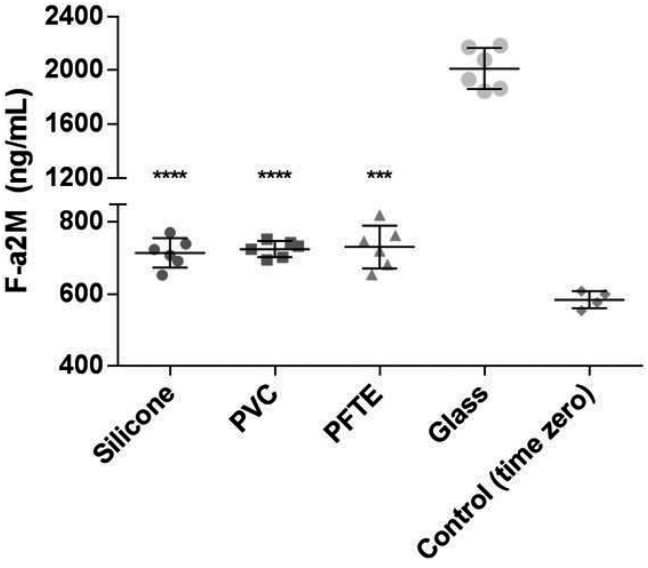

FIGURE 2 F-α2M levels in plasma after incubation with artificial materials. Stars indicate a significant difference compared to the control sample

Results: The specificity of the antibody was evaluated with native PAGE, demonstrating that antibody 16-11-17 recognized F-α_2M, but not native α_2M (Fig. 1).

Furthermore the antibody showed reactivity to recombinant receptor binding domain region of F-α_2M, which is hidden in native α_2M. The F-α_2M levels of plasma samples incubated, in the absence of calcium, with commonly used artificial surfaces were evaluated. F-α_2M levels in plasma samples incubated in silicone (n = 6), polyvinylchloride (PVC) (n = 6), and polytetrafluoroethylene (PFTE) (n = 6) tubings, as well as glass tubes, were significantly higher than control samples ($p < 0.001$) (Fig. 2), indicating activation of calcium independent proenzymes by the artificial surfaces.

Conclusions: A F-α_2M specific antibody was developed and used for F-α_2M measurements in plasma by ELISA. We demonstrate that the F-α2M level in plasma is a useful marker of blood/plasma compatibility of artificial surfaces in the absence of calcium.

PB 1698 | Haemocompatibility Properties of Polyethersulfone Based Haemodialysis Membrane with Different Amounts of Polyurethane

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Background: The study will explore the haemocompatibility of a haemodialysis membrane based on polyethersulphone (PES) when polymerised with different amounts of polyurethane (PU) and comparing the it with control membrane which is of PES/ PVP (Polyvinylpyrrolidone).

Aims: The aim of the study is to find the PES/ PU based membrane with the best haemocompatibility characteristics.

Methods: PU will be blended with PES and fabricated into hollow fibre membrane for haemodialysis application. To test for biocompatibility, flat sheet rather than hollow membrane will be used in the experiment, which would include testing for protein adhesion using the Micro BCA™ Protein Assay Kit, clotting time using automated blood coagulation analyser, thrombin -antithrombin generation and complement activation using enzyme-linked immunosorbent assay (ELISA).

Results: Preliminary data from this study showed that the BSA adsorption amounts increased with the increased PU in the modified membranes. SEM images analysis for a qualitative measure of platelet adhesion on fabricated PES/PVP/PU composite membrane shows the most significant amount of platelet that adhered and spread on the surface of the membrane are not activated. As more PU loading occur, the platelet adhesion on the surface of the membrane was decreased. In addition, membrane incorporated with PU showed lower TAT generation as compared to its control. In addition, clotting time is shown to be prolonged with different PU loading and there is a significant decrease in concentration of activated C3a in PES/PVP with higher PU percentage as compared to the neat PES/PVP membrane.

Conclusions: Based on this study, it can be suggested that membrane incorporated with PU has better anticoagulant property compared to control. However, this study also showed that protein adsorption is increased with higher PU loading, which could be due to the surface roughness of the membrane that are produced during phase inversion processes.

PB 1699 | Comparison of Nine Different Blood Collection Tubes for Contact Activation of Coagulation

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Background: Blood collection induces low grade contact activation of coagulation interfering with tests with low activation triggering.

Aims: To evaluate contact activation differences among collection tubes, we collected nine different and one duplicate tube from 10 healthy volunteers.

Methods: Tubes: Monovette® vacuum and aspiration tubes and Becton Dickinson (BD) plastic tubes all with and without corn trypsin inhibitor (CTI) added immediate after collection, Greiner plastic tubes, coated glass tubes from BD and SCAT® tubes containing citrate + CTI. Tests: NAPTT, thromboelastography with recalcification (NATEM), thrombin generation assay (TGA) with low tissue factor concentration (TGA®-RCH).

Results: CTI increased NAPPT four times; rate of clotting (-CTI) varied among individuals (CV 28%) and variability by tube was smaller (CV 10%). Rate of clotting in the different tubes (- CTI) correlated (R^2 : 0.64-0.93). NATEM showed increased clotting time and decreased alpha angle in the presence of CTI, whereas MCF showed no differences among tubes. TGA showed CTI to be most effective in Monovettes® vacuum and less in BD and SCAT® tubes in reducing signals to nihil. TGA was elevated in some individuals using Monovettes® aspiration tubes with CTI.

In tubes without CTI, time variables (lag, time to peak) were not very different among tubes, but peak height, AUC and in particular velocity to peak showed distinct differences.

Coated glass tubes showed the highest TGA-values, and both B&D and Greiner tubes showed higher values than Monovette® tubes.

Conclusions: We conclude that the rate of thrombin and clot formation is variably affected by the tubes, but the differences are smaller than the differences between individuals. Interestingly, the NAPTT records the individual differences suggesting to be a test for individual differences in potency of the contact system.

TGA is particularly sensitive to tube differences. Monovette® vacuum showed the lowest contact activation among the tested tubes.

PB 1700 | CD40L Induces Association of PRDX-2 and TRAF-2 to CD40 of Platelets from IBD Patients

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Background: Although platelets are well known for their role in hemostasis, there are a rising number of studies supporting their considerable role as inflammatory amplifiers in chronic inflammatory conditions. Indeed, platelets circulate in an activated state in patients with inflammatory bowel disease (IBD), but their role in the pathogenesis of IBD remains unclear. It is well established that the CD40L-CD40 complex, a key regulator and amplifier of immune reactivity, is activated in inflammatory bowel disease (IBD) mucosa.

Aims: We have demonstrated by this study that different signaling pathways are triggered downstream of the CD40L-CD40 complex, involving several intraplatelet proteins.

Methods: Platelets from blood of IBD patients and healthy subjects were freshly isolated to evaluate the involvement of TRAFs in response to CD40L-CD40 interaction.

Results: Tumor necrosis factor receptor-associated factor-1 (TRAF-1), TRAF-2 and TRAF-6 are overexpressed in platelets from IBD patients versus control. Furthermore, CD40L-CD40 interaction induces the association of TRAF-2 and PRDX-2 with platelet CD40.

Conclusions: This study adds new insights into the role of TRAFs and PRDX-2 in platelets downstream of CD40-CD40L complex.Thus, the CD40L/CD40/TRAFs-PRDX-2 axis in platelets may represent a critical regulator of platelet function and inflammation.

PB 1701 | Short-term Increase in Factor (F)XIa-dependent Procoagulant Activity in Citrate Blood

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Background: It has been observed that in the absence of contact pathway inhibition, the procoagulant activity of fresh citrate blood increases within several minutes.

Aims: To compare changes in thrombin generation potential and its response to FXIa inhibition in citrate blood from healthy donors kept for up to 75 min either in standard plastic vacutainer tubes or in polypropylene conical tubes.

Methods: Fresh blood from 4 healthy donors (2 male and 2 female) was collected into standard 3.2% sodium citrate plastic vacutainer tubes (BD catalog #363083). In one set of experiments, blood was kept at room temperature in such tubes for up to 75 minutes with CTI quenching of aliquots every 15 minutes. Alternatively, blood from the same 3 of 4 donors was immediately transferred to 15 mL polypropylene conicals and the same CTI quench scheme was applied. Thrombin generation (WB-TG) and clot formation (ROTEM) were evaluated. For

selected blood draws, FXIa concentration was quantitated from the response to an inhibitory αFXIa antibody.

Results: For blood kept in vacutainer tubes, a time-dependent increase in procoagulant activity was observed which was partially FXIa-dependent. The most pronounced increase in procoagulant activity occurred in the first 15 minutes post-draw to which the addition of CTI prevented any further increase. No increase in procoagulant activity was observed over the entire 75 minute incubation period when blood from the same donors was kept in polypropylene conical tubes in the absence of CTI.

Conclusions: Fresh citrate blood from healthy donors kept in citrate vacutainer tubes at room temperature leads to an increase in contact pathway-dependent procoagulant activity that is partially FXIa-dependent. The incubation of citrate blood from the same individual in a polypropylene conical tube prevents this contact activation and subsequent increase in procoagulant activity.

PB 1703 | Temporal Contribution of the Platelet Body and Balloon to Thrombin Generation

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Background: Ballooned platelets are present in the primary haemostatic plug and subsequent clot after blood vessel injury in human subjects. We recently demonstrated the functional relevance of platelet ballooning to haemostasis and thrombosis, by showing that it coincided with procoagulant microvesiculation and spreading, which massively increased the procoagulant surface area and enhanced thrombin generation.

Aims: It is presently not clear whether the procoagulant nidus of the ballooned platelet is the 'ballooned membrane' or a part of the platelet, termed the 'cap', which we previously described as the remnant 'platelet body'. Here, we aimed to establish the temporal contribution of the platelet body and balloon to thrombin generation.

Methods: 4D fluorescence live-platelet imaging and scanning electron microscopy.

Results: Phosphatidylserine was predominantly expressed on the 'platelet body' or 'cap' at earlier stages of ballooning, whereas the balloon surface itself formed the predominant procoagulant surface at later time points. Both platelet body or 'cap' and balloon form distinct procoagulant 'cauliflower-like' surfaces that support thrombin generation; and unlike classical capping, 'capped platelet' formation is irreversible.

Conclusions: Both ballooned platelet membrane and the platelet body or ,cap' provide important 'cauliflower-like' procoagulant surfaces, but are temporally separated. Furthermore, classical capping is not associated with ballooned platelet formation, therefore the use of 'cap' in the platelet context may need reconsideration.

PB 1704 | Mechanism of Growth and Inhibition of Plasma Clots Growing from a Surface with Immobilised Tissue Factor

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Background: Clot formation in plasma in test tubes usually is started with tissue factor homogeneously mixed in the plasma. Clot formation from a surface coated with tissue factor is another phenotype and can be tested in the thrombodynamics analyser where clot growth is recorded by video.

Aims: We evaluated the characteristics of this growth process and its inhibition.

Methods: Citrated plasma was depleted of microvesicles, and corn trypsin inhibitor and excess (4μM) lipids were added before recalcification was starting the process with exposure to surface-bound tissue factor.

The layer formation can be analysed for several variables:

(a) time and rate of initial clot formation in the first 1-2 minutes,

(b) further growth represented by layer thickness reached in 50 minutes and deceleration of the rate of clot growth.

Results: Contact factor, factor XI deficient plasma and normal plasma collected on CTI behaved similar indicating absence of participation of the intrinsic pathway in the test. In FV deficient plasma growth was absent. Adding activated protein C to normal plasma inhibited growth after 1-2 minutes strongly.

In the first 1-2 min after the initiation of clot growth there was no difference between normal control and deficiency in factors VIII, IX. In factor VIII and IX deficient plasma the layer thickness reached in 50 minutes is about 65% of normal, and further continuation of thickening was reduced.

Inhibition by dabigatran delayed clotting, but effects on growth were nihil; rivaroxaban delayed clotting and showed no growth inhibition in factor IX deficient plasma, only inhibited the factor IX dependent growth. Enoxaparin inhibited growth in normal and factor IX deficient plasma.

Conclusions: It is concluded that growth after tissue factor initiation has a core process dependent of factor IX and X, while inhibitors show unique profiles for inhibition of growth.

The test system can be used to evaluate growth inhibitors directed to activation and activity of factor IXa and factor Xa.

PB 1705 | Co-operative Binding of VWF to the TSP8 and CUB Domains of ADAMTS13 is Required for its Conformational Activation

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Background: The von Willebrand factor (VWF) cleaving protease, ADAMTS13, circulates in a closed conformation, maintained by an interaction between its N-terminal spacer domain and its C-terminal domains. Conformational activation is mediated by the binding of VWF to the ADAMTS13 C-terminal domains, which causes their dissociation from the spacer domain.

Aims: We aimed to characterize the interaction of the ADAMTS13 spacer domain and it C-terminal domains to further understand the molecular basis of ADAMTS13 conformational activation.

Methods: Binding between isolated ADAMTS13 CUB domain fragments (CUB1, CUB2 and CUB1-2) and MDTCS (an ADAMTS13 N-terminal fragment lacking its C-terminal tail) was analysed by surface plasmon resonance (SPR). The auto-inhibitory potential of the CUB domains was determined by examining their ability to inhibit MDTCS, as well as spacer domain variants, in FRETS-VWF73 assays. The binding of ADAMTS13 and its truncated variants to VWF-D4CK (the binding partner of the ADAMTS13 C-terminus) was analysed by SPR.

Results: SPR analysis revealed that both CUB1 and CUB2 bind to WT-MDTCS. Binding of either CUB1 or CUB2 is inhibited by specific point mutations of the spacer domain exosite (R568/F592/R660/Y661/Y665).

Only CUB1 is able to inhibit the conformationally active variant WT MDTCS. Both CUB1 and CUB2 bind to the VWF-D4CK domains. However, the affinity of VWF-D4CK for ADAMTS13ΔCUB2 and ADAMTS13ΔCUB1-2 is similar (K_D of 176.9 ± 31.6 nM and 181.8 ± 25.3 nM, respectively) to its affinity for WT ADAMTS13 (K_D of 148.5 ± 38.1 nM). In contrast, variants lacking the TSP8 domain exhibit a marked reduction in affinity for VWF-D4CK and the in frame deletion of TSP8 abolishes conformational activation of ADAMTS13.

Conclusions: While both CUB1 and CUB2 interact with the spacer domain, only CUB1 is responsible for mediating the partially inactive conformation. Both CUB1 and CUB2 bind to VWF-D4CK. Conformational activation of ADAMTS13 also requires binding of VWF-D4CK to its TSP8 domain.

PB 1706 | α-Synuclein Binds to Human α-Thrombin and Inhibits Thrombin-mediated Platelet Aggregation: Possible Implications in Parkinson's Disease

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Background: α-Synuclein (αSyn) is an intrinsically unfolded protein expressed in the presynaptic terminals of neurons and is the major component of protein deposits in Parkinson›s disease (PD), a neurodegenerative disease characterized by αSyn overexpression. αSyn is also expressed in platelets, where it is associated with the secretory α-granules. α-Thrombin (αT) is the most potent platelet activator and exploits exosite2- to anchor on the GpIbα platelet receptor. After PAR1- cleavage, αT induces α-granule release and platelet aggregation. Notably, αT-induced platelet degranulation is inhibited by αSyn (Park et al. 2002) and clinical evidences show that patients with PD exhibit a reduced tendency to platelet aggregation and are less susceptible to ischemic stroke (Sharma et al. 1991).

Aims: Establish whether αSyn interacts with αT and study the effect of αSyn on αT-induced platelet aggregation.

Methods: αSyn was expressed in *E. coli* and purified by RP-HPLC. Binding measurements were carried out by fluorescence spectroscopy and Surface Plasmon Resonance (SPR). The effect of αSyn on platelet aggregation was studied on whole blood by Multiple Electrode Aggregometry (MEA).

Results: Fluorescence measurements indicate that full-length αSyn and the synthetic αSyn(103-140) peptide bind to αT with similar K_d values (1.04±0.25 μM). Fluorescence and SPR displacement experiments, carried out with αT specific binders at exosite-1 (hirugen), exosite-2 (fibrinogen g′-peptide), and active site (hirudin fragment 1-47), indicate that both αT exosites participate in αSyn binding. Strikingly, MEA measurements indicate that full-length αSyn (20 μM), but not αSyn(103-140), dose-dependently inhibits αT-induced platelet aggregation, up to 80%.

Conclusions: Our results suggest that αSyn impairs platelet activation by competing with platelet GpIbα receptor for binding to αT exosite-2, thus helping to rationalize earlier clinical evidences showing that PD patients display a reduced risk of thrombotic events.

PB 1707 | The Effect of Short Chained Polyphosphates on Coagulation Activation in a Whole Blood Model

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Background: Human platelets contain short chain (SC, 60-100 units) inorganic polyphosphate (SC-polyP) stored in dense granules that is released on the cell surface upon activation and trigger coagulation. SC-polyP is shown to facilitate coagulation activation by promoting FXI activation by thrombin and augmenting FV activity, whereas longer chain polyP, not present in blood under physiological conditions, is able to activate FXII.

Aims: To investigate whether SC-polyP is able to trigger coagulation activation in human blood and reveal underlying mechanisms.
Methods: Blood was collected in lepirudin tubes (50 µg/mL) from healthy fasting individuals. Blood was incubated at 37°C by different concentrations of SC-polyP (0-100 µM) for different time intervals (0-120 min), at physiological calcium concentrations. Coagulation activation was stopped by adding 10 mM EDTA (final concentration) and monitored by measuring prothrombin fragment 1+2 (Enzygnost® F1+2, Siemens). Factor XII (FXII)-dependent activation of coagulation was inhibited by Infestin® (CSL Behring). The effect of SC-polyP on TF synthesis was assessed by TF mRNA by qPCR and TF activity in monocytes by a chromogenic assay. The effect of SC-polyP was also evaluated in platelet free plasma (PFP).
Results: SC-polyP added to whole blood showed a dose- ($p < 0.01$) and time- ($p < 0.001$) dependent increase in plasma F1+2 levels where incubation of 100 µM SC-polyP for 60 min generated 1939 ± 252 pmol/mL F1+2. Pre-incubation of whole blood with FXII inhibitor before SC-polyP stimulation, reduced F1+2 levels by 94±2 % ($p < 0.005$). Incubation of whole blood with SC polyP did not increase the expression of TF mRNA or cell surface TF activity in isolated monocytes. SC-polyP had no effect on coagulation activation in PFP isolated from lepirudin anticoagulated blood.
Conclusions: SC-polyphosphates trigger coagulation activation through factor XII in a whole blood model at physiological calcium concentrations, but not in a plasma system.

PB 1709 | Evidence for Direct and Indirect Modulation of Haemostatic Factors by miR-494

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Background: Oestrogen can modulate Protein S (*PROS1*) expression via miR-494 in *in vitro* models. Several biological functions have been ascribed to miR-494 and we had previously postulated a role for it in oestrogen-mediated acquired Protein S deficiency.
Aims: This study aims to: 1. Characterise the effects of miR-494 on the expression of coagulation factors at the mRNA and protein level. 2. Determine if miR-494 indirectly regulates coagulation factors by directly inhibiting transcriptional factors AP1, SP1 and/or STAT5B.
Methods: HuH-7 human hepatoma cells were transfected with synthetic negative control miRNA (miR-NC) or miR-494, and the levels of mRNA and protein were determined with qPCR and Western blot, respectively, for; PS (*PROS1*); Plasminogen (*PLG*); and Tissue Factor (*F3*) at 48-72h post transfection. *In silico* analyses of 3'UTR sequences of transcription factors AP1 (*JUN*), Sp1 (*SP1*) and STAT5B (*STAT5B*) were performed to identify putative miR-494 binding sites. Computational transcription factor binding analyses (PROMO) was also performed on *PLG* and *F3* promotor regions.
Results: Treatment of HuH-7 cells with miR-494 significantly down-regulated *PROS1*, *PLG*, *SP1* and *STAT5B* mRNA levels, and reduced *PROS1* and plasminogen protein levels, with no changes observed for *JUN*. In contrast, *F3* mRNA expression was significantly increased with miR-494 treatment. *JUN*, *SP1* and *STAT5B* 3'UTRs contained putative miR-494 binding sites, and PROMO analyses identified predicted AP1 binding sites in *PLG* and *F3* promoters, and Sp1 binding sites in the *F3* promoter. No *STAT5B* binding sites were identified.
Conclusions: Coagulation regulation by miR-494 is mediated via direct and indirect targeting of multiple coagulation factors. Direct targets such as *PROS1*, *SP1* and *STAT5B*, results in indirect regulation of *PLG* and *F3*, demonstrating a prothrombotic role for miR-494 and strongly suggests important clinical implications of raised miR-494 levels under high circulating oestrogen levels such as pregnancy.

PB 1710 | Situation-adjusted Anticoagulant Release System with Response to Different Coagulation Factors

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Background: Healthy vascular endothelium is considered the most effective anti-thrombogenic surface, as it can adjust its anticoagulant properties to the local requirements. Implants or biomedical devices in flowing blood always require an anticoagulant fitting of the surface. This ideally also should respond to the actual coagulation situation.

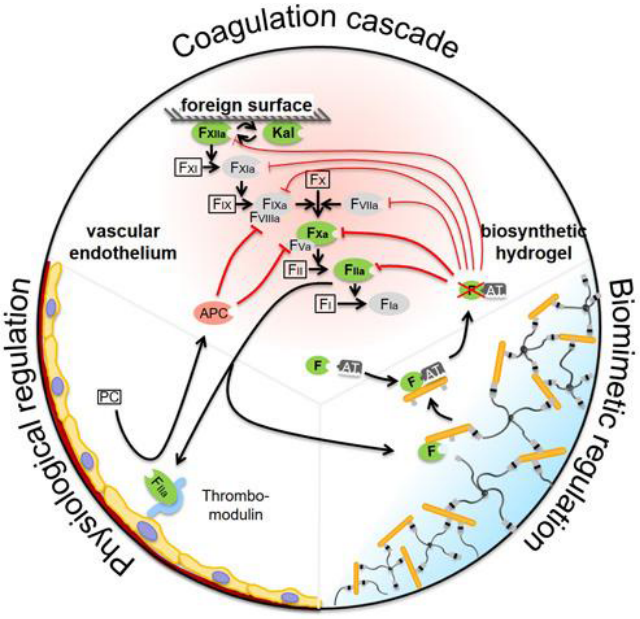

FIGURE 1 Physiological and biomimetic coagulation regulation

A biohybrid hydrogel of starPEG crosslinked with heparin using thrombin-cleavable linker peptides shows such behavior: Thrombin cleaves the linker peptides and releases heparin; this inhibits the thrombin activity and interrupts further degradation of the hydrogel.

Since thrombin is a late enzyme in the coagulation cascade, the timing of the heparin release by this trigger may be suboptimal for thromboprotection.

Aims: Hydrogels with faster release kinetics for a more efficient anticoagulant response.

Methods: starPEG-Heparin hydrogels were prepared using the previously described thrombin-cleavable peptide, a peptide with faster turn-over, a non-cleavable scrambled sequence of the peptide, a factor FXa cleavable, and a kallikrein/FXIIa cleavable peptide as responsive linkers. They were characterized for their general response behavior and for the performance in whole blood.

Results: At equal concentration of the respective enzymes, the kallikrein responsive hydrogel was degraded fastest, followed by the FXa responsive gel and the different thrombin responsive gels. In whole blood, the kallikrein-responsive gel also showed best anticoagulation, associated with highest heparin release, what is attributed to the low feedback effect of heparin on the contact phase system. The FXa- and the standard thrombin-responsive hydrogel had equal anticoagulant properties, but the FXa responsive system achieved this with lower heparin release due to the improved timing of the degradation.

Conclusions: Responsive anticoagulant hydrogels responding on FXa as trigger show a favorable profile of thromboresistance and inhibitor release.

PB 1711 | Liver Hypoxia Regulates Protein S Levels in Obese Patients

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Background: Protein S is a vitamin K-dependent plasma protein, produced mainly in the liver; Protein S circulates in the blood at a concentration of 450 nM. Protein S is an anticoagulant, serving as a cofactor for APC and TFP, and as an inhibitor of Factor IX (FIXa). Protein S deficiency causes deep vein thrombosis (DVT), and, because DVT is a complication commonly observed in obese individuals, Protein S deficiency might be associated with obesity.

Aims: To identify a correlation between Protein S abundance and obesity, and identify the cause(s) for the correlation.

Methods: Immunoblots, ELISA, EMSA, CHIP, aPTT assay, and thrombin generation assay.

Results: By ELISA, we measured a decrease in Protein S level in obese mice compared with wild type mice. In obesity, the liver becomes hypoxic, thus, we hypothesized that hypoxia and hypoxia inducible factor 1 alpha (HIF1α) may regulate Protein S expression in obesity. We found that a high fat diet induced HIF1α stability in mice. HIF1α levels were inversely proportional to Protein S levels, suggesting that HIF1α is a negative regulator of Protein S expression. We further identified a putative HIF1α binding site in the Protein S promoter, and, by using *in vitro* and *in vivo* assays, we demonstrated that HIF1α binds directly to the Protein S promoter and suppresses transcription. We further confirmed HIF1α-mediated Protein S transcriptional regulation *in vivo*, Plasma Protein S levels are increased in the liver-specific HIF1α knockout mouse whereas, liver-specific overexpression of HIF1α reduced the concentration of Protein S in the plasma.

Conclusions: We conclude that HIF1α regulates Protein S expression in mouse liver and in obesity. Inhibition of HIF1α or intravenous injection of Protein S may reduce the occurrence of DVT in obese individuals.

PB 1712 | Thrombin is a Selective Inducer of Heparanase Release from Platelets and Granulocytes *via* Protease-activated Receptor-1

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Background: Heparanase, known to be involved in angiogenesis and metastasis, was shown to form a complex with tissue factor (TF) and to enhance the generation of factor Xa. Platelets and granulocytes contain abundant amounts of heparanase that may enhance the coagulation system upon discharge.

Aims: To identify the inducer and pathway of heparanase release from these cells.

Methods: Platelets and granulocytes were purified from pooled normal plasma and were incubated with ATP, ADP, epinephrine, collagen, ristocetin, arachidonic acid, serotonin, LPS and thrombin. Heparanase levels were assessed by ELISA, heparanase procoagulant activity assay and western blot analysis. The effects of selective protease-activated receptor (PAR)-1 and 2 inhibitors and PAR-1 and 4 activators were studied. An in-house synthesized inhibitory peptide to heparanase was used to evaluate platelet heparanase involvement in activation of the coagulation system.

Results: Heparanase was released from platelets only by thrombin induction while other inducers exerted no such effect. The heparanase level in a platelet was found to be 40% higher than in a granulocyte. Heparanase released from platelets or granulocytes increased factor Xa generation by 3 folds. PAR-1 activation *via* ERK intracellular pathway was found to induce heparanase release.

Conclusions: Heparanase is selectively released from platelets and granulocytes by thrombin interacting with PAR-1. Heparanase derived from platelets and granulocytes is involved in activation of the extrinsic coagulation pathway. The present study implies on a

potential anticoagulant effect, in addition to anti-platelet effect, of the new clinically studied PAR-1 inhibitors.

PB 1713 | Effects of Exercise and Erythropoietin on Thrombotic Risk in Well-trained Cyclists

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Background: Recombinant human erythropoietin (rHuEPO) is forbidden in sports, even though the effects on performance are not indisputably proven. In addition, it is unknown if rHuEPO increases thrombotic risk.

Aims: To investigate the effects of rHuEPO on thrombotic risk in well-trained cyclists.

Methods: Forty-eight healthy, well-trained male cyclists were randomly allocated to subcutaneous rHuEPO (mean dose 6000IU/week) or placebo for 8 weeks. Venous blood was collected at rest and after maximal exercise tests. Effects on erythropoiesis were assessed by measuring haemoglobin (Hb) and haematocrit (Ht). Thrombotic risk was established by determination of activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, D-dimer, beta-thromboglobulin (bTG), E-selectin, P-selectin, Factor VIII (FVIII), platelet factor 4 (PF4), thrombomodulin (TM), von Willebrand factor (vWf), prothrombin activation fragment 1+2 (F1+2) and thrombin:antithrombin (TAT).

Results: Mean Hb (9.7mmol/L vs 9.0mmol/L) and Ht (48% vs 44%) were significantly higher in the rHuEPO group compared to placebo. Treatment with rHuEPO significantly increased baseline levels of E-selectin (+8.6% [2.0;15.7%]) and P-selectin (+7.8% [1.5;14.5%]) as compared to controls. Exercise showed a clear association independent of treatment with haemostatic alterations, as reflected by reduced aPTT (-13% [-14;-11%]) and PT (-2% [-3;-1%]) and increased levels of FVIII (+125% [106;146%]), TAT (+593% [413;836%]), F1+2 (+97% [65;135%]), D-dimer (+46% [24;71%]), fibrinogen (+7% [4;10%]), E-selectin (+6% [3;10%]), vWf (+79% [65;95%]), P-selectin (+13% [10;16%]), bTG (+115% [76;163%]) and PF4 (+216% [152;297%]) after maximal exercise test. Increment in TM after maximal exercise was reduced after rHuEPO treatment (+10% vs +15%).

Conclusions: This study is the first to show that rHuEPO, affects biomarkers of pro-thrombosis in well-trained cyclists, which may be considered an indication for increased risk for thrombosis.

PB 1714 | Fibrinogen Polymorphisms Predict Fibrinogen and Clot-related Phenotypes Independently and through Interactions with IL-6

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Background: The identification of single nucleotide polymorphisms (SNPs) that functionally contribute to fibrinogen-related phenotypes in Europeans has been hindered by high linkage disequilibrium (LD) in the fibrinogen gene cluster. African population data can be utilised to overcome this limitation through its great genetic variability and low LD. Furthermore, apart from independent SNP functionality, fibrinogen SNPs alter the magnitude of interleukin-6 (IL-6)-induced

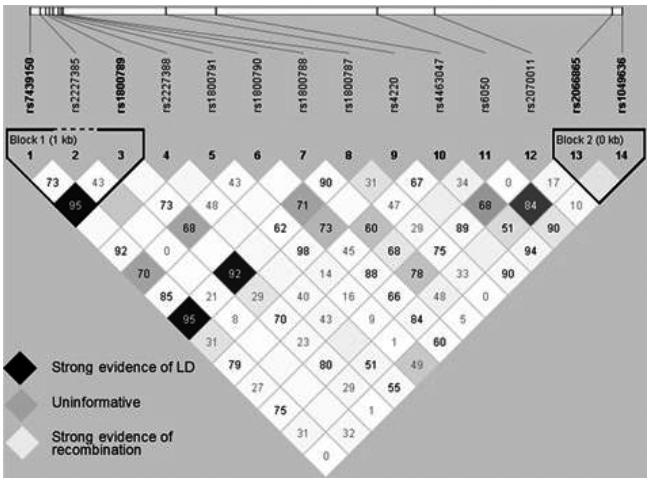

FIGURE 1 Pairwise LD structure of 14 fibrinogen SNPs, illustrated by D' values on an r2 colour scheme

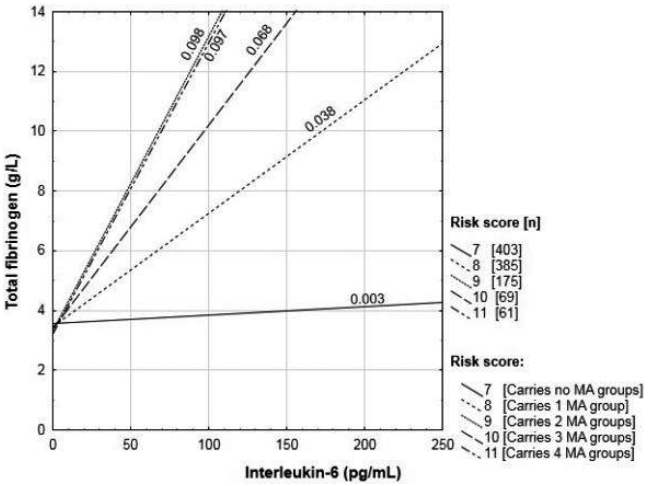

FIGURE 2 Association of total fibrinogen concentrations with circulating interleukin-6 by minor allele (MA) risk score groups

fibrinogen expression. Investigating these relationships in an African population with high basal fibrinogen and IL-6 concentrations could provide valuable insight into expressionrelated fibrinogen SNP functionality.

Aims: To identify SNPs that functionally contribute to fibrinogen's quantitative and qualitative phenotypes independently and through IL-6 interactions.

Methods: Twelve fibrinogen SNPs previously investigated for functionality, and two novel African SNPs were analysed cross-sectionally in 2010 Africans in terms of their independent and IL-6-interactive associations with fibrinogen concentrations and turbidity-derived clot properties.

Results: No complete LD or common European fibrinogen haplotype were observed in this population (Fig 1). SNPs of particular functional relevance were *FGB*rs7439150, $1420_{G/A}$ and $148_{C/T}$. These SNPs significantly associated with fibrinogen concentrations and altered clot properties, with several of these associations influenced by IL6. In addition, harbouring more minor alleles concurrently resulted in greater increases in IL-6-induced fibrinogen expression (Fig 2).

Conclusions: The lack of LD and haplotypes in the fibrinogen genes of this African population allowed for the identification of independent functional SNPs. The additive nature of harbouring risk alleles concurrently, in the presence of IL-6 is shown for the first time. When investigating the phenotypic relevance of fibrinogen genetics, possible additive SNP effects and the contribution of modulating factors should be considered.

PB 1715 | Integrative Analysis of Hemostatic Gene Regulation in the Human Liver

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Background: Most coagulation factors are produced in the liver. Tight regulation of these genes is crucial to avoid thrombus formation which can lead to ischemic stroke. In the present study, we utilized high resolution gDNA-, mRNA-, and DNA methylation sequencing (-seq) of human liver tissue to identify mechanisms regulating hemostatic genes by analyzing allele-specific DNA methylation (ASM) and allele-specific gene expression (ASE). Furthermore, we investigated associations between genetic variants and gene expression (eQTL), genetic variamts and DNA methylation (meQTL), and gene expression and DNA methylation (eQTM). Candidate variants identified in this project will be analyzed for association to ischemic stroke and other thrombotic events.

Aims: To identify novel cis-regulatory variants and DNA methylation patterns regulating coagulation and other hemostatic genes in the human liver.

Methods: We have collected biopsies of human liver tissue from adults undergoing liver surgery at the Sahlgrenska University Hospital (n=70). For the first set of samples (n=19) we isolated genomic DNA (gDNA) and total RNA. We designed a targeted approach in order to enrich and sequence 77 selected genes that are important for hemostasis, including upstream and downstream regions, and their corresponding mRNA transcripts. SNPsplit (Babraham Inst.) was used for ASM analysis and ASE ReadCounter (GATK, Broad Inst.) was used to call ASE. The R package Matrix eQTL was used to assay eQTLs, meQTLs and eQTMs.

Results: 60% of the 77 selected genes displayed an ASM bias of >30% between the two alleles. Genes with the highest number of ASM were PLG and VWF. ASE was identified in 80% of the genes analyzed. Initial results from the QTL analyses revealed approximately 4446 cis-meQTL associations (70 genes), 1172 cis-eQTLs (45 genes), and 247 eQTMs (29 genes).

Conclusions: The present study provides novel insights into the mechanistic relationships between genetic variation, DNA methylation and expression of hemostatic genes in the human liver.

PB 1716 | Effects of the fibrinogen γ' C-terminal Peptide on Thrombin-catalyzed Activation of Factor XI, Factor XIII and TAFI

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Background: Thrombin is a multifunctional enzyme that converts fibrinogen to fibrin and activates platelets, coagulation factors (F) V, VIII, XI and XIII, the anticoagulant protein C and thrombin activatable fibrinolysis inhibitor (TAFI). The two exosites of thrombin are crucial for substrate recognition and for the regulation of thrombin activity. The C-terminus of the fibrinogen γ' chain binds with high affinity to thrombin exosite II, thereby inhibiting thrombin-catalyzed activation of platelets, FV, FVIII and protein C.

Aims: To determine how fibrinogen γ' affects the other functions of thrombin.

Methods: The effects of the fibrinogen γ' chain C-terminal peptide on thrombin-catalyzed activation of FXI (in the presence of dextran sulfate), FXIII (in the absence and presence of fibrinogen) and TAFI (in the presence of thrombomodulin) were investigated in model systems using purified proteins. The involvement of the thrombin exosites in these reactions was probed using aptamers specifically targeting either exosite.

Results: The fibrinogen γ' peptide and both aptamers inhibited FXI activation by thrombin/dextran sulfate and TAFI activation by thrombin/thrombomodulin in a dose-dependent manner. Conversely, the peptide did not affect FXIII activation in the absence or presence of fibrinogen. Aptamer HD1 (targeting exosite I) inhibited FXIII activation only in the presence of fibrinogen, while aptamer HD22 (targeting exosite II) was effective both with and without fibrinogen.

Conclusions: In summary, the fibrinogen γ' peptide inhibits thrombin-catalyzed activation of platelets, FV, FVIII and FXI (anticoagulant effects), protein C (procoagulant effect) and TAFI (profibrinolytic effect),

but does not affect FXIII activation. These findings may help explain the risk of venous thrombosis associated with decreased fibrinogen γ' levels.

PB 1717 | Characterization of GGCX Mutations Identified in the First Clinical Case of a VKCFD Patient

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Background: Vitamin K-dependent coagulation factors deficiency (VKCFD) is an autosomal recessive bleeding disorder resulting from decreased levels of coagulation factors II, VII, IX and X, as well as natural anticoagulants (protein C, S and Z). Two types of VKCFD have been identified based on the mutations of enzymes in the vitamin K cycle. VKCFD1 is associated with functional defects in gamma-glutamyl carboxylase (GGCX), whereas VKCFD2 arises from defects in vitamin K epoxide reductase (VKOR) activity. Although the first clinical case of VKCFD was described in 1966 (PMID: 5936414), the causative mutations in that case have remained unestablished.

Aims: Here, we report identification and characterization of the causative mutations in the first described clinical case of VKCFD.

Methods: Exome sequencing was used to identify mutations in the patient's genomic DNA. Cell based assays, protein biochemistry and fluorescence confocal microscopy imaging were used to characterize the mutant proteins.

Results: Five mutations in the GGCX gene were identified. Two compound heterozygous mutations have not been previously reported. One is a deletion of an adenine at position c.1657 (C.1657ΔA) in exon 12 - which causes a reading frame shift and the early termination of the translation of GGCX. The other is an intronic mutation at c.1889 G-6A in intron 13. Minigene splicing assay results suggest that the c.1889 G-6A mutation causes mis-splicing and retention of 4 intronic bases in exon 14. Functional characterization of these two mutants shows that the c.1657ΔA mutant lacked GGCX activity, while the activity of the c.1889 G-6A mutant was significantly reduced. Importantly, higher vitamin K concentrations were found to restore the carboxylation activity of the c.1889 G-6A mutant up to 80%.

Conclusions: Our results, consistent with the patient's clinical results, suggest that the patient has type I VKCFD and that the c.1889 G-6A mutation in GGCX is the causative mutation for the patient's clinical phenotype.

PB 1718 | Comprehensive Identification of miRNA-gene Interactions Reveals Extensive Regulation of the Plasmatic Coagulation Network

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Background: Through the post-transcriptional fine-tuning of gene expression miRNAs confer robustness to the complex molecular networks regulating biological functions. However despite the ubiquitous involvement of miRNAs in almost all biological processes, their global role in blood coagulation has remained poorly defined, with studies in the field largely focusing on the effects of small numbers of miRNAs on single genes.

Aims: Here we set out to comprehensively identify miRNAs regulating both pro- and anti-coagulatory components of the hemostatic system in an unbiased manner.

Methods: To investigate systemic roles of miRNAs unbiased and extensive methodologies are required. In contrast to widely applied association and in silico studies, we have utilised an integrative assay approach that combines functional aspects of miRNA silencing with physical interaction studies based on RNA pull downs coupled to next generation sequencing and finally validation of specific miRNA-gene interactions.

Results: This analysis initially revealed over one third of the examined hemostatic genes to exhibit significant Dicer-mediated silencing activity, suggestive of targeting by miRNA. Further investigation revealed numerous miRNAs to physically interact with these genes of which luciferase assays confirmed approximately 50 specific miRNA/UTR interactions to result in significant levels of silencing.

Conclusions: These results suggest that the hemostatic system is widely controlled by miRNAs targeting multiple genes that may act as regulatory target hubs within the plasmatic coagulation network. These findings provide the first comprehensive analysis deciphering the role of miRNAs in the systemic control of blood coagulation and provide a foundation for the development of novel therapeutic approaches for the correction of de-regulated hemostasis in the future.

PB 1719 | Resolving the Roles of Platelets and (Anti)Coagulation Pathways in Whole-blood Fibrin Formation under Flow to Phenotype Hemostatic Disorders

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Background: The interactive roles of platelet responses and (anti)coagulation mechanisms in fibrin clot formation under flow are difficult to study *in vivo*.

Aims: Establish key interactions between platelets and coagulation pathways in human blood perfused over an array of adhesive and coagulation-modulating surfaces.

Methods: Human blood was perfused at defined shear rates over microspotted platelet-adhesive and pro/anti-coagulant surfaces, collagen I (COL1), GPO+VWF-BP (collagen mimicking peptide, CMP), collagen III (COL3), rhodocytin+VWF-BP (RHO), laminin+VWF-BP (LAM), thrombomodulin (TM) and activated protein C (APC) with/out tissue factor (TF). Fibrin-thrombus formation was determined by multicolor fluorescence microscopy. Data modelling resulted in a beta matrix of determinants of coagulation pathways.

Results: At high shear rate (1000/s) kinetics of fibrin clot formation on microspots decreased in the order of COL1>CMP>COL3>LAM,RHO with(out) TF. At low shear rate the positions of CMP and RHO reversed, in agreement with high platelet activity of CLEC-2 surfaces. Measurements of time to first fibrin formation showed kinetics in the same order. Regardless of the shear rate, the presence of TF and of PS-exposing platelets were independent determinants of fibrin formation. With TF triggering, both anticoagulant TM and APC prolonged and diminished fibrin clot formation. Inhibition of the extrinsic (active-site-inhibited FVIIai) but not intrinsic (CTI) pathway reversed all TF effects. Without TF, either CTI or FVIIai suppressed fibrin formation in the order of CMP>COL1,COL3>LAM,RHO with limited effect on platelet deposition. Roles of intrinsic, extrinsic and anti-coagulation pathways were verified with blood from patients.

Conclusions: Modelling revealed surface- and shear-dependent parameters that were strongly determined by extrinsic and anti-coagulation pathways. A prediction model was built of surface- and platelet-dependent fibrin formation for phenotyping of patients with bleeding disorders.

PB 1720 | Temperature Effects on Thrombin Generation

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Background: Thrombin generation (TG) is now implemented in more than 1000 (research) laboratories worldwide. TG data are used in a variety of fields from clinical research to mathematical modeling. Mathematical modelers generally assume TG to be chemically controlled, meaning that it abides to the van ´t Hoff rule and doubles the reaction velocity for every 10°C increase in temperature.

Aims: To investigate the temperature dependence of TG, thereby elucidating the rate limiting factors of thrombin formation.

Methods: TG was measured in normal pooled and defibrinated, platelet poor plasma (PPP) at 1, 5 & 10pM tissue factor. A dedicated temperature controller was built into the fluorometer. It consists of a PID controller that steers a resistive heater attached to an aluminum mold. The mold decreases the overall temperature variation throughout the 96 wells plate to < 0.1°C.

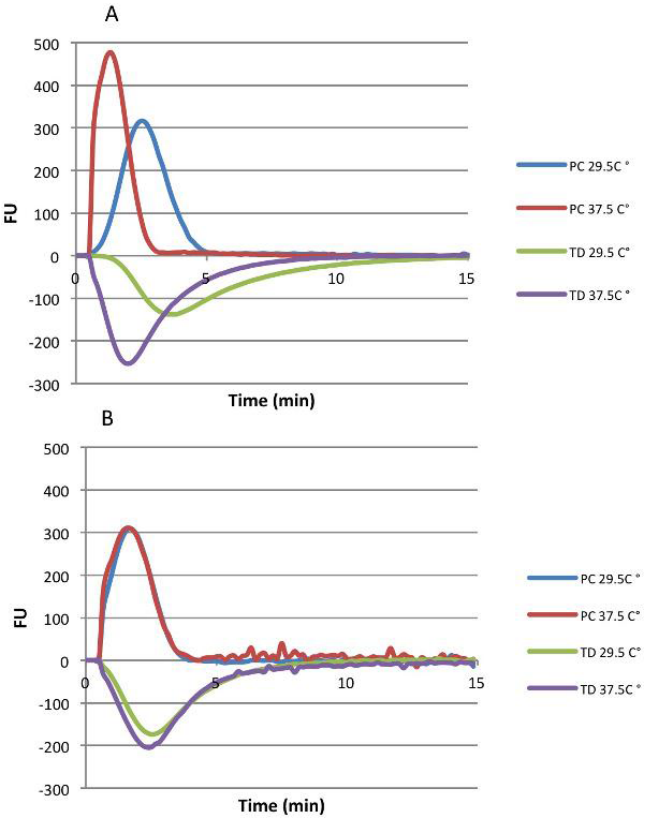

FIGURE 1 Prothrombin Conversion (PC) and Thrombin Decay (TD) in PPP (A) and defibrinated PPP (B) at 37.5C° and 29.5C°

Results: The calibrator curves obey to the rules of chemical control and increase by 10% in activity, for every 1°C increase in temperature over the biological range (29.5 - 37.5°C). Thrombin decay is more affected by temperature in PPP compared to prothrombin conversion, i.e. 84% decrease compared to a 51% increase in through/peak levels at 37.5°C compared to 29.5°C. In defibrinated plasma, the peak of prothrombin conversion was not affected and thrombin decay had a 17% lower through, see figure 1 for details.

Conclusions: Analysis of prothrombin conversion and decay separately reveals that thrombin decay is inhibited stronger by a reduction in temperature then thrombin formation, causing TG parameters to increase at reduced temperatures. Interestingly, this effect is much less apparent in defibrinated plasma with a much less pronounced difference between the parameters for thrombin formation and decay at different temperatures.

PB 1721 | Thrombin Generation in Schizophrenia Patients Treated with Antipsychotic Medications

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[1]*University College Dublin/Conway Institute, Dublin, Ireland*, [2]*St Vincent's Hospital, Dublin, Ireland*, [3]*Royal College of Surgeons in Ireland, Dublin, Ireland*, [4]*Mater Misericordiae University Hospital, Dublin, Ireland*

Background: Schizophrenia patients have reduced life-expectancy, commonly due to cardiovascular disease (CVD). Intriguingly, antipsychotic medications have been reported to reduce death from CVD and inhibit platelet aggregation responses. However, the effect of these medications on plasma thrombin generation (TG) is unknown.
Aims: To compare plasma thrombin generation in schizophrenia patients receiving anti-psychotic medications with matched controls.
Methods: Medicated schizophrenia patients (n=18) and matched controls (n=6) were recruited. TG in platelet rich (PRP) and platelet poor plasma (PPP) was characterized using calibrated automated thrombography. Patients receiving haloperidol/risperidone (H/R) and clozapine/olanzapine (C/O) (n=6 for each drug) were grouped for analysis in view of similar side-effect profiles.
Results: In the absence of a tissue factor (TF) stimulus, lag time to initiation of TG was prolonged (17.6±1.4 v 13.7±0.7min; p=0.015) and velocity index, ETP and peak thrombin were decreased (60±16 v 154±13nmIIa/min, p=0.0014; 1403±193 v 1901±82nmIIa, p=0.012 and 199±37 v 373±18nmIIa, p=0.0002 respectively) in H/R PPP v controls. Peak thrombin was also decreased in C/O patients (291.7±42nmIIa vs 373±18nmIIa; p=0.047) but velocity index and ETP were similar to controls. In the presence of a TF stimulus, lag time was prolonged in both H/R and C/O v controls (7.1±0.2 (C/O) and 8.2±0.4 (H/R) vs 5.8±0.2 min; p=0.0001 and 0.0004 respectively) but velocity index, ETP and peak thrombin were unchanged. In PRP, similar patterns were observed but absolute differences were smaller.
Conclusions: Medicated schizophrenia patients exhibit reduced plasma thrombin generation compared with matched controls. This may represent an additional potential mechanism underlying the observed cardioprotective effect of these medications.

PB 1722 | Blood Clot Contraction is Reduced in Sickle Cell Disease due to Increased Rigidity of Erythrocytes

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Background: Blood clot contraction or retraction, which is the volumetric shrinkage of the clot, has been implicated to play a role in hemostasis and thrombosis. The cellular and molecular composition of the clot, including red blood cells (RBCs), influences the extent and rate of the three phases of clot contraction. Thus, there is a need to examine the effects of diseases where RBCs differ from those of healthy individuals, such as sickle cell disease (SCD). SCD is associated with a hypercoagulable state that contributes to vaso-occlusive events in microcirculation and an increased risk of venous thromboembolism.
Aims: The aim of this study was to examine differences in clot contraction in blood samples from SCD patients compared to healthy donors.
Methods: To follow clot cot contraction, we used an optical tracking methodology that allows for the quantitative tracking of clot size with time. Blood clotting and contraction were initiated using 2 mM $CaCl_2$ and 1 U/ml thrombin and contraction was followed for 20 minutes.
Results: The blood clots of SCD patients were examined and on average had a 53% decrease in the extent of clot contraction, and contracted 2.4 times slower during Phase 2 and 2.7 times slower Phase 3 when compared to healthy subjects. We hypothesized that the reduction in clot contraction could be due to increased RBC stiffness in SCD patients. Consistent with this idea, the addition of naturally rigid llama ovalocytes to human platelet rich plasma and the addition of antibodies to the Wright b epitope, which increase RBC rigidity, resulted in a decrease in extent of clot contraction compared to normal untreated human RBCs.
Conclusions: These results demonstrate that clot contraction is altered in SCD and suggest that the RBC rigidity contributes to the observed reduction in extent of clot contraction. A better understanding of RBC deformability in the process of clot contraction may inform the development of more targeted treatments for thrombosis in patients with altered RBC mechanical properties.

PB 1723 | Tissue Factor-dependent Heme-induced Whole Blood Coagulation Activation: A Thromboelastometry Study

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Background: Recently, free heme emerged as one of the potential triggers of inflammation in hemolytic anemias such as sickle cell disease (SCD). In hemostasis, heme has been shown to exert both pro- and anti-coagulant effects, although the mechanisms underlying these effects are yet to be determined. We have recently demonstrated that heme induces a hypercoagulable state detected by thromboelastrometry and thrombin generation assays.
Aims: Herein, we investigated whether these effects are dependent on tissue factor (TF).
Methods: Citrated whole blood samples from healthy volunteers were stimulated by vehicle or heme (30μM), with or without inhibitory tissue factor antibody or control IgG (10μg/ml), and incubated for 4h at 37°C. Rotem was performed using a non-activated thromboelastometry procedure in a ROTEM®instrument, from which parameters of different stages of clotting were obtained.
Results: When compared to an isotype control, the use of an anti-tissue factor inhibitory antibody abrogated the effect of heme in coagulation activation, resulting in increase in clotting time (Fig1a) and in a decrease in the time to maximal velocities (Fig1c). Inhibition had no

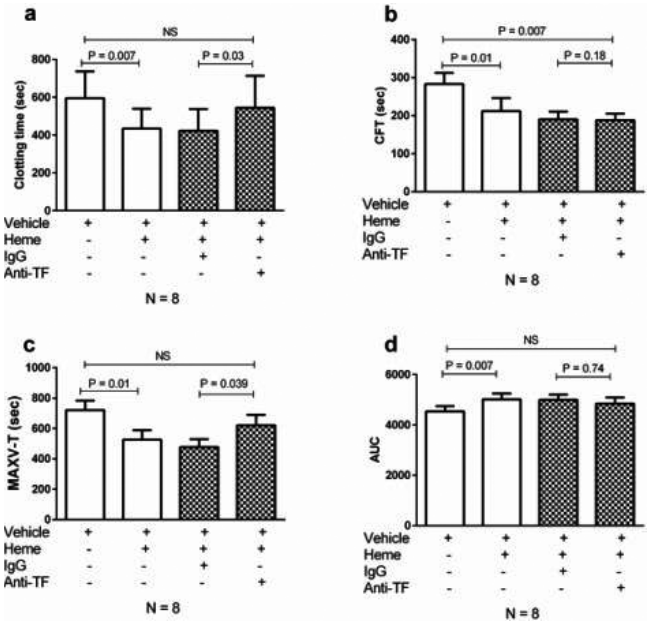

FIGURE 1 Heme triggered-coagulation activation is tissue factor-dependent

effect on clot formation time (Fig1b) or in the area under the Rotem curve (Fig1d).

Conclusions: Using global hemostasis assay capable of detecting clinically relevant changes in hemostasis, we demonstrated that heme was capable of inducing coagulation activation of whole blood in a TF-dependent fashion, influencing predominantly the kinetic of clot formation. These results support the concept that free heme could play a relevant role in coagulation activation in SCD, and in other pathological conditions. Further studies are necessary to elucidate the mechanism of this heme influencing effect in hemostasis.

PB 1724 | Salivary EV: A New Link between Primary and Secondary Hemostasis

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Background: Human saliva contains extracellular vesicles (EV) exposing tissue factor (TF; Blood. 2011; 117: 3172-80). This TF triggers coagulation in platelet-depleted plasma, but whether salivary EV interact with platelets to promote coagulation is hitherto unexplored.

Aims: We hypothesized that the TF-exposing EV from saliva interact with platelets exposing P-selectin, as described earlier for TF-exposing EV from tumor cells in a mouse model of vascular injury (J Exp Med. 2009; 206: 1913-27), thus making the platelets procoagulant.

Methods: To test this hypothesis, we investigated the presence of P-selectin glycoprotein ligand-1 (PSGL-1) and CD24, two known ligands of P-selectin, on salivary EV.

Results: While the presence of PSGL-1 on salivary EV was below the detection limit when analyzed by high-resolution flow cytometry, CD24 was exposed abundantly ($p < 0.05$). Removal of CD24-exposing EV by immune-depletion resulted in the complete removal of the TF coagulant activity as measured by fibrin generation test (n=5), indicating that CD24 and TF are co-localized on EV. This co-localization of CD24 and TF was confirmed by immune-transmission electron microscopy. In a whole blood flow model, salivary EV accumulated on the surface of aggregated platelets, and the deposited EV promoted fibrin generation (n=5). The involvement of EV-CD24 and platelet P-selectin in the interaction is under study by using inhibitory antibodies. Finally, most (70%) of the CD24+ salivary EV originate from granulocytes ($CD66b^+$).

Conclusions: Collectively, we demonstrate that $CD24^+/TF^+$ EV may be a novel link between primary (platelet processes) and secondary (coagulation) hemostasis at a site of vascular injury.

PB 1725 | Heme-regulated Proteins within the Blood Coagulation Cascade: Insights into the Molecular Basis of Protein Binding to Free Heme

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Background: Recently, biochemical research has focused strongly on the role and action of heme as a regulatory effector of proteins [1]. The extracellular release of large amounts of heme cannot be dealt with appropriately by scavenging heme-binding proteins. Consequently, transient binding of free heme to proteins was shown to significantly change a protein´s function. We demonstrated that distinct sequence stretches on the protein surface are responsible for the interaction with heme [2-5].

Aims: Based on the screening of a combinatorial peptide library we predicted motifs for heme binding as well as respective proteins as potential targets [2]. These efforts led us to have a closer look on proteins of the blood coagulation cascade. Indeed, the procoagulant activity of factor VIII was earlier shown to be inhibited by heme [6]. Distinct heme-regulatory motifs, however, have not been characterized thus far.

Methods: UV-vis, EPR, resonance Raman, and NMR spectroscopy studies revealed the existence of different binding modes for heme-peptide/protein complexes [3-5]. Thus, apart from examining the impact of heme on the protein activity structural studies are required to evaluate the heme-binding capacity of the target proteins.

Results: We report attempts to identify and characterize heme binding to Von Willebrand factor (vWF), FVIII and vitamin K dependent protein C. The binding data, structural characteristics and impact on the respective protein activity will be discussed.

Conclusions: The ability of transient heme binding of the target proteins may have serious pathophysiological relevance and needs to be considered in vivo.

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PB 1726 | Heparan Sulfate Chains Contribute to an Anticoagulant Milieu in Malignant Pleural Effusion

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Background: While malignant pleural effusion (MPE) is a common and significant cause of morbidity in cancer patients, current treatment options are limited. Coagulation system is a major contributing factor to tumor growth and angiogenesis. Human heparanase, involved in angiogenesis and metastasis, cleaves heparan sulfate (HS) side chains on the cell surface and in the extracellular matrix, yielding HS fragments of still appreciable size (5-7 kDa).

Aims: Our study explored the coagulation milieu in MPE and infection pleural effusion (IPE).

Methods: Samples of 30 patients with MPE and 44 with IPE were evaluated in comparison to those of 33 patients with transudate pleural effusions, using heparanase ELISA, heparanase procoagulant activity assay, thrombin and factor Xa chromogenic assays and thromboelastography. A cell proliferation assay was performed. A cell line of mouse breast cancer (EMT-6) was injected to the pleural cavity of mice. Peptide 7, inhibiting heparanase activity, was administered subcutaneously.

Results: Levels of heparanase, factor Xa, thrombin and procoagulant activity were significantly higher in exudate than transudate. Thromboelastography detected no thrombus in MPE samples. This effect was reversed when the assay was performed with bacterial heparinase, degrading HS chains to small chains that have no effect in coagulation system. Proliferation of human glioma (U87) and human breast carcinoma (T47D) cell lines was higher with addition of exudate than transudate. IPE induced greater proliferation than MPE ($p<0.001$); however, the difference was reversed when bacterial heparinase was added to MPE. Tumors in the pleural cavity of mice treated with peptide 7 were significantly smaller compared to control ($p<0.0001$).

Conclusions: HS chains, possibly released by heparanase, could create an anticoagulant milieu in MPE, preventing local thrombosis and fibrosis. Inhibition of heparanase might provide a therapeutic option for patients with recurrent MPE.

PB 1727 | Myeloperoxidase (MPO) is a Negative Regulator of Phospholipid-dependent Coagulation

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Background: MPO is a heme protein with positive surface charge abundantly expressed in neutrophils, where it plays a critical role in anti-microbial host defense by catalyzing the production of hypochlorous acid from H_2O_2 and Cl^-. Extracellular MPO also contributes to vascular inflammation, but its direct effects on the blood clotting process are unknown.

Aims: To investigate coagulation in the presence of MPO/H_2O_2.

Methods: We used single-stage clotting, factor Xa and thrombin generation assays to study the effect of purified leukocyte-derived MPO on tissue factor (TF)-dependent and -independent coagulation.

Results: In the presence of H_2O_2, MPO inhibited the procoagulant activity (PCA) of lipidated recombinant human TF (rhTF) in a time- (0-30 min) and concentration-dependent (0-10 µg/mL) manner by >90%. MPO also exerted inhibitory activity towards rhTF in the absence of H_2O_2, albeit to a lesser extent than equimolar concentrations of the phosphatidylserine (PS) binding protein, annexin V. MPO/H_2O_2 synergized with inhibitory TF monoclonal antibody in neutralizing the PCA of monocyte-derived microvesicles isolated from LPS-stimulated whole blood, suggesting that modification of procoagulant phospholipids (PLs) rather than interference with the TF/FVIIa initiation complex was involved. Consistently, MPO/H_2O_2 inhibited the PCA of activated platelets from healthy donors and rabbit brain cephalin, a PL preparation devoid of measurable TF activity. Finally, the PCA of disrupted myelomonocytic HL60 cells and patient-derived myeloblasts was significantly enhanced, when cell lysis was carried out in the presence of the MPO inhibitor, 4-ABAH.

Conclusions: Our findings indicate that MPO is a negative regulator of PL-dependent coagulation and suggest that both the catalytic activity and non-catalytic properties of the enzyme, presumably via electrostatic interactions with the negatively charged head groups of PS, are involved in this process.

PB 1728 | Procoagulant Properties of Microparticles Produced by Monocytes, Granulocytes, Platelets and Endothelial Cells

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Background: Membrane microparticles (MPs) released by activated or apoptotic cells exhibit coagulation activity since they express phosphatidylserine and some of them – tissue factor (TF).

Aims: We compared procoagulant properties of MPs derived *in vitro* from monocytes, granulocytes, platelets and endothelial cells (ECs).

Methods: MPs were produced by activated monocytes, granulocytes and platelets, isolated from donors' blood, and by activated cultured THP-1 monocytic cells and ECs. MPs were sedimented from the culture media and cell supernatants at 20000 g for 30 min. MPs were counted by flow cytometry and their size was evaluated by dynamic light scattering. Coagulation activity of MPs was examined using modified plasma recalcification assay. TF activity was measured by its ability to activate factor X.

Results: All MPs significantly accelerated plasma coagulation in a recalcification assay with the shortest lag times for MPs from monocytes, intermediate – for MPs from THP-1 cells and ECs, and the longest – for MPs from platelets and granulocytes. Coagulation activity of all MPs was completely inhibited by a phosphatidylserine blocker – lactadherin. Average diameters of MPs ranged within 400-600 nm. The largest MPs were produced by ECs and granulocytes, smaller – by monocytes, and the smallest – by THP-1 cells and platelets. The highest TF activity was detected in MPs from monocytes, lower activity – in MPs from ECs and THP-1 cells, and no activity – in MPs from platelets and granulocytes. Anti-TF blocking antibody extended plasma coagulation lag times for MPs from monocytes, ECs and THP-1 cells and equalized them with those for MPs from platelets and granulocytes.

Conclusions: Significant differences of coagulation activity were revealed between MPs of different cellular origin. Higher activity of MPs from monocytes, THP-1 cells and ECs in comparison with MPs from platelets and granulocytes was mainly determined by the presence of active TF.

PB 1729 | Ventricular Assist Devices without Anticoagulation: An Impossible Dream?

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Background: Heart failure patients who receive a ventricular assist device (VAD) often require anticoagulation to prevent pump thrombosis and thromboembolism. However, 40% of patients also suffer from bleeding complications. The result is a fine balance between hemorrhage and stroke with a tipping point that allows for a minimal margin of error in anticoagulation monitoring.

Aims: The aim of this study was to characterize the thrombogenic potential of materials and coatings for use in a prototype miniaturized VAD.

Methods: Blood bank whole blood units were supplemented with heparin at 0.75U/mL and recalcified to 0.5M. Recalcified blood was incubated with or without material samples and rocked at 1Hz. Platelet-rich plasma, isolated from samples collected at 0, 30, 60, 90 and 120 minutes, was run in a modified thrombogram assay. At 2h, materials were gently rinsed in phosphate buffered saline and assessed for platelet adhesiveness through visual inspection and by lysing adhered platelets in 2% Triton X-100 and assaying the resulting solution for lactate dehydrogenase. The thrombogenicity of VAD prototypes was investigated by implanting healthy ovines with the device for up to 90 days with no anticoagulation, followed by a full necropsy.

Results: Materials approved for use in VAD prototypes displayed a minimum 50% reduction in thrombin generation rate compared to bare titanium. Visual inspection after 2h in recalcified blood showed significantly less thrombus adhesion than bare titanium. LDH assays performed on platelet lysate solutions from these materials showed a minimum reduction of 60% in LDH activity over bare titanium. Healthy ovines implanted with prototype VADs survived 90 days with no anticoagulation, and full necropsies showed no evidence of thromboembolic events.

Conclusions: Strict attention to material properties combined with explicit standardized requirements for pre-clinical device testing offer the potential of a VAD that will not require patients to receive anticoagulation.

PB 1730 | Beta-adrenergic Receptor Blockage Reduces Exercise-induced Alterations in Haemostasis

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Background: Exercise-induced alterations in haemostasis enhances the risk of thrombotic events, such as deep venous thrombosis and pulmonary embolism. The latter is associated with elevated catecholamines during exercise.

Aims: To investigate the potential of inhibiting catecholamine effects with beta-adrenergic receptor blocker propranolol on exercise-induced haemostatic alterations.

Methods: Healthy males (n=6) assessed maximal exercise tests in addition of propranolol (40mg) and without propranolol as control. Performance was measured by maximal power output (MPO) and maximal oxygen uptake (VO2max). Blood was collected before, 1 min and 4 hours after exercise. Coagulation profile was assessed by thrombin generation, levels of coagulation factor VIII (FVIII), thrombin-antithrombin (TAT) and prothrombin fragment 1+2 (F1+2). Platelet function was evaluated with platelet count, platelet factor 4 and P-selecti levels. Data was analyzed by Wilcoxon matched-pairs signed rank test a 2-tailed $p< 0.05$ was considered statistically significant.

Results: Propranolol not significantly affects on MPO (300±45Watt vs 310±60Watt) and VO2 max (3919±820mL/min vs 4051±830mL/min).

Nevertheless, thrombin generation potential was reduced upon propranolol addition, reflected by lower endogenous thrombin potential (ETP), peak height and velocity index directly after exercise (table 1). Compared to pre-exercise, the altered thrombin generation potential in controls persisted at 4 hours after exercise, which was prevented by propranolol (table 2). In addition, coagulation activity markers significantly less elevated in the propranolol group upon exercise (table 1). Furthermore, significantly lower platelet count was found after exercise upon propranolol usage (18±7% vs 27±9%), where platelet activation markers remained unchanged in both groups.

TABLE 1 Differences in thrombin generation potential and coagulation activation markers pre- vs 1 min post exercise. *p<0.05

	Propranolol (Median [IQR])	Controls (Median [IQR])	p-value
Peak Height	+26%[19-45]	+98%[43-125]	0.03*
ETP	+10%[6-14]	+41%[19-57]	0.03*
Velocity Index	+35%[20-94]	+144%[64-231]	0.03*
TAT	+18%[9-40]	+35%[16-133]	0.03*
F1+2	+11%[10-23]	+23%[21-56]	0.03*
FVIII	+17%[13-21]	+127%[95-187]	0.03*

TABLE 2 Differences in thrombin generation potential pre- vs 4 hours post exercise. *p<0.05

	Propranolol (Median[IQR])	Controls (Median[IQR])	p-value
Peak Height	+16%[13-19]	+66%[40-94]	0.03*
ETP	+1%[0-7]	+11%[4-29]	0.03*
Velocity Index	+22%[16-32]	+86%[63-124]	0.03*

Conclusions: Our study shows inhibitory effects of beta-adrenergic blockage on exercise-induced haemostatic alterations. However, potential clinical relevance needs to be elucidated in future studies.

PB 1731 | The Modification of Thrombin Generation Assay Parameters in Patients with Recurrent Pregnancy Loss

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Background: Previous investigations of the role of genetic thrombophilic markers in unexplained pregnancy loss have yielded conflicting results. The thrombin generation test (TGT) is an attractive alternative to better explore the hemostatic pathway.

Aims: This study aimed to characterize the underlying prothrombotic potentiel of patients with unexplained recurrent pregnancy loss (RPL) through thrombin generation assessment.

Methods: Women aged 18-40 years who had experienced three or more unexplained miscarriages < 14 weeks or one loss >14 weeks of pregnancy were prospectively recruited from September to December 2016. Venous blood samples were obtained outside the pregnancy and after a minimum of six weeks following pregnancy loss.

Patients were compared to healthy women with no history of pregnancy loss.

Thrombin generation in platelet poor plasma was analysed using two reagents: PPP REAGENT (optimal concentration of tissue factor 5pm and phospholipids 4 nM) and PRP REAGENT (Without adding phospholipids).

TGT parameters analyzed were: lag time, time to peak (ttPeak), Peak height of thrombin (Peak), area under curve (ETP) and velocity index.

Results: Fifty two women provided data for the study (32 subjects and 20 controls) were included. There was no difference in mean ages (29.7 vs 29.6 years). With PPP reagent, there was no significant difference in lag time (2.33 vs 2.11; p=0.19) and ttPeak (4.31 vs 4.08; p=0.3) between controls and subjects groups. While Peak, ETP and velocity index were less increased in controls than patients: respectively (212.5 vs 265; p=0.009, 827.9 vs 1056.3; p=0.003 and 107.01 vs 140.5; p=0.009).

With PRP reagent, the only significant difference was observed in ETP (943.51 vs 1143.4; p=0.037).

Conclusions: In our study, women with RPL had a relative hypercoagulable states assessed by the TGT. It seems that microparticules play a role in this prothrombotic potential. TGT could be usefull in identifying wowen with risk of RPL.

PB 1732 | Desmopressin Administration in a Splenectomised Hemophilia A Patient Does Not Affect FVIII, VWF and tPA Plasma Levels: An Evidence for an Indirect Effect of DDAVP

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Background: Despite four decades of desmopressin acetate (DDAVP) prescription for the management of bleeding disorders, its mechanism of the effect on FVIII, VWF and tPA plasma level enhancement remains largely elusive. There are strong evidences for VWF and tPA concomitant release from endothelial weible-palade bodies in direct response to DDAVP. However, reported observations of vasopressin 2 receptor (V2R) expression on some endothelial cell types (ECs) does not explain the 2-3 fold increase in VWF and tPA levels upon DDAVP administration.

Aims: It's been proposed that DDAVP might drive its VWF/tPA raising property in response to an intermediate agonist which is released from V2R expressing cells. We hypothesized, based on various evidences that those DDAVP responding cell might reside in the Spleen.
Methods: A splenectomised 27-years old male patient affected with FVIII deficiency and β-thalassemia was recruited. PT, APTT, INR, FVIII:C, FVIII inhibitor, VWF: Ag and tPA: Ag levels were assessed in patient plasma samples, collected prior to, and 1, 2 and 4 hours following intravenous administration of 0.3 µg per Kg body weight of Desmopressin Acetate over 30 minutes. The study was conducted in accordance with the Helsinki Declaration and approved by Zanjan University of Medical Sciences Ethics Committee (ZUMS.REC.1395.68; 31 May 2016).
Results: As shown in table 1, no changes in FVIII:C, VWF and tPA antigen levels was observed in patient plasma before and after DDAVP administration. No FVIII inhibitor was detected in patient plasma.

TABLE 1 Patient's laboratory data measured before and after DDAVP administration at various time points

		After DDAVP Administration		
Laboratory test	Before DDAVP Administration	1 hour	2 hours	4 hours
PT patient	13.9	14.5	14.1	14.9
PT Normal	12.8	12.8	12.8	12.8
APTT patient	56.8	60	60.6	62
APTT Normal	30	30	30	30
APTT mixed	36.1	36.5	36.2	35.7
FVIII:C (U/dl)	2.94	2.58	2.15	1.85
VWF:Ag (U/ml)	1.08	0.91	0.99	1.01
tPA:Ag (µg/ml)	4.31	3.84	4.09	4.06

Conclusions: Although it has been shown that DDAVP does not affect FVIII:C levels in patients with moderate hemophilia A, we were still expected to observe a significant (2 to 3 fold) raise in VWF:Ag and tPA levels if the spleen is not important for an indirect response to DDAVP. *In vitro* studies are ongoing to further clarify molecular details of the proposed mechanism.

PB 1734 | Haemostatic Function after Spontaneous Intracerebral Haemorrhage

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Background: The haemostatic function after acute intracerebral haemorrhage (ICH) is not fully elucidated.
Aims: Firstly, we aimed to describe haemostatic function in ICH patients at admission and compare this with healthy individuals. Secondly, to analyse changes in haemostasis during the first 24 hours. Our hypothesis was that ICH-patients display a systemic activated haemostatic function acutely compared to healthy individuals.
Methods: From 2014 to 2016, this prospective, observational study included 41 non-anticoagulated patients with ICH at Aarhus University Hospital, Denmark. Patients admitted to hospital within 6 hours of symptom onset were enrolled. Blood samples were collected on admission, 6 and 24 hours after symptom onset.
Thromboelastometry (ROTEM®) was performed and thrombin generation was quantified by Calibrated Automated Thrombogram®. Thromboelastometry and thrombin generation data on healthy individuals was obtained from Department of Clinical Biochemistry, Aarhus University Hospital.
Results: On admission, maximum clot firmness (MCF) was significantly increased compared with healthy individuals (EXTEM p< 0.0001; INTEM p< 0.0001; FIBTEM p< 0.0001). Thrombin generation results showed a higher endogenous thrombin potential (p=0.01) and peak thrombin (p< 0.0001) on admission compared with healthy individuals. The thromboelastometry parameters did not change significantly between admission and 24 hours after symptom onset. In contrast, thrombin generation results showed a decrease in endogenous thrombin potential (p< 0.0001) and peak thrombin (p< 0.0001) within 24 hours.
Conclusions: Thromboelastometry and thrombin generation indicated a subtle systemic activation of coagulation in the acute phase of ICH compared with healthy individuals. The total thrombin-generating capacity decreased significantly at 24 hours compared with symptom onset.

PB 1735 | Anticoagulant effects of peptide Pro-Gly-Arg in aging rats

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Background: It is known that aging is accompanied by depression of the anticoagulation system of organism (DAS), resulting in hypercoagulability of blood, which can lead to the thrombosis. It was found that the regulatory peptides glyproline are involved in the activation of the anticoagulation system, preventing the formation of blood clots and have fibrinolytic and anticoagulant effects in the organism.
Aims: To evaluate the anticoagulant effects of the peptide Pro-Gly-Arg (PGR) and its influence on the parameters of blood coagulation in aging rats with DAS.
Methods: Male Wistar rats of the two age groups ("young rats" - 3-month-old, and "old rats" - 12-month- old) were used in this study. PGR-treated old rats received peptide PGR (1 mg/kg on the body weigth) by intranasal way during 3 days. Untreated old rats (control) and young rats (normal) received 0.85% saline. Blood samples were taken 1 h after 3rd administration of drugs. Anticoagulant activity of

blood plasma was estimated by standard clotting methods: APTT, Prothrombin time (PT) and Thrombin time (TT). All experiments were complied with the Declaration of Helsinki.

Results: We confirmed DAS in untreated old rats: there were hypercoagulation and acceleration of clot formation in blood (APTT decreased by 35%, prothrombin time - by 47% and thrombin time - by 16% from young normal rats). PGR-treatment led to a marked enhancement of anticoagulant activity of blood: APTT raise by 26% from untreated old rats. This was accompanied by a more significant increase of prothrombin and thrombin time by 89% and 50% respectively from control levels.

Conclusions: Thus, the peptide PGR participates in the regulation of coagulant processes and restores the functional state of the anticoagulation system in aging animals with DAS. The PGR-treatment impact on all phases of blood coagulation, with more alters the activity of prothrombinase and the final stage of coagulation slowing the rate of conversion of fibrinogen to fibrin.

PB 1736 | A Polydeoxynucleotide Derived Drug Defibrotide Inhibits Nucleosome Generation in Whole Blood. Implications in the Management of VOD

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Background: Defibrotide (Defitelio, Jazz Pharmaceutical, Palo Alto, CA) is a single stranded deoxyribonucleic acid derived antithrombotic and anti-ischemic drug of mammalian tissue origin. VOD represents a catastrophic complication of HSCT and is often fatal due to multi-organ failure. Hepatic cellular apoptosis and cell death in VOD results in the generation of nucleosomes which contribute to the propagation of this disease by enhancing thrombotic and vascular complications.

Aims: The aim is based on the assumption that defibrotide may reduce the generation of nucleosomes and modulate their thrombotic and inflammatory responses.

Methods: Defibrotide was obtained in powdered form (Gentium, Villa Guardia, Italy) and dissolved in saline to obtain working concentrations of 10 mg/mL. A commercially available ELISA PLUS, Cell Death Assay was used to measure plasma nucleosome levels. Blood samples were supplemented with this agent at varying concentrations of 0-100 ug/mL. E. Coli and S. Enterica lipopolysaccharide B were used to trigger the generation of nucleosomes in whole blood for 15 minutes at 37 C, and nucleosome levels were measured using a commercial ELISA method.

Results: Both the E.Coli and S. Enterica lipopolysaccharides produced a concentration-dependent increase in the generation of nucleosomes. At 1 ug/mL, the nucleosome levels were in the range of 40-70 AU/mL (47 ± 11). Defibrotide at a concentration of 100 ug/mL produced almost a complete inhibition of the generation of nucleosomes with an IC50 of 35 ug/mL. The level of nucleosome generated in different normal individuals showed wide variations.

Conclusions: These studies suggest that beside other pharmacological effects, defibrotide may blunt the generation and functionality of nucleosome which contribute to its observed therapeutic effects in HSCT associated VOD. Since defibrotide is a single stranded DNA derived agent it may compete with the DNA component of nucleosome and / or interact with the histone.

PB 1737 | Influence of Polyamidoamine (PAMAM) Dendrimers on the Activity of Key Enzymes of the Coagulation System *in vitro*

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Background: PAMAM dendrimers are often used for drug delivery in various diseases. Recently it is shown that cationic high generation (G7) PAMAM dendrimers cause platelet aggregation, inhibit thrombin generation and act directly on fibrinogen to form dense aggregates of fibrinogen. Mechanism of this coagulopathy is not currently clear. Influence of PAMAM dendrimers on activity of enzymes of the coagulation system is not known.

Aims: Studying the effect of anionic and cationic PAMAM dendrimers on activity of key enzymes of the coagulation system *in vitro*.

Methods: Effect of cationic (G1, G2 and G3) and anionic (G1.5, G2.5 and G3.5) PAMAM dendrimers (0 - 0.4 mM) on the amidolytic activities of thrombin and FXa, thrombin time and prothrombin time was studied.

Results: Activity of thrombin against Z-Ala-Ala-Arg-pNA was significantly increased with increasing concentration and generation of cationic dendrimers, whereas it was inhibited by anionic dendrimers: K_i 1.44, 0.7 and 0.28 mM, respectively, for G1.5, G2.5 and G3.5. The increasing concentration of anionic and cationic dendrimers caused marked decrease in activity of FXa against Z-D-Arg-Gly-Arg-pNA (K_i 1.21 mM for G3.5). Both cationic and anionic dendrimers inhibited the clotting activity of thrombin. For example, with increasing concentration of G3 and G3.5 dendrimers up to 0.4 mM, the clotting time of fibrinogen by thrombin in a buffer was gradually increased, while the clotting time of blood plasma by thrombin began to increase sharply at concentrations of the dendrimers higher than 0.3 mM and this effect was more potent. Cationic and anionic dendrimers elongated prothrombin time to a lesser extent than thrombin time.

Conclusions: Cationic and anionic low generation PAMAM dendrimers reduce clotting activities of thrombin and FXa *in vitro* that is likely mediated through electrostatic interactions between the densely charged surface of dendrimers and opposite charged sites of thrombin, FXa and fibrinogen.

PB 1738 | Repetitive Strenuous Exercise Leads to Exhaustion of Haemostatic System

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Background: Physical exercise is beneficial for health and is thought to decrease the risk of cardiovascular disease. However, strenuous exercise could be a risk for thrombosis. It influences the pro- and anticoagulant processes that increase risk of myocardial infarction, ischemic stroke or venous thrombosis. Previous studies showed that one day of strenuous exercise accelerates thrombin generation, activates platelets and affects fibrinolytic activity.

Aims: To study whether repetitive strenuous exercise increases procoagulant processes.

Methods: We measured thrombin generation, platelet activation, plasmin generation, Factor VIII (FVIII) and Von Willebrand Factor (VWF) levels in plasma for 3 subsequent days before and after 80km cycling in hilly environment. Our study protocol was approved by the local medical ethical board. All 7 individuals gave full informed consent.

Results: After the first day of exercise we observed a significant increase in thrombin generation peak FVIII, VWF, platelet activation and plasmin generation with decreased total amount of formed thrombin. Interestingly all measured parameters except for platelet activation recovered to the pre-exercise levels. After the second and third day of exercise all measured parameters were increased again although less than at the first day. Platelets remained activated during the whole study.

Conclusions: Our results demonstrated that daily strenuous exercise leads to exhaustion and rebalancing of the hemostatic system, especially with respect to the fibrinolytic system and endothelium. Exercise had a major but transient impact on FVIII and VWF levels, which increased after exercise and recovered to base line in the next morning. Platelets showed increased activity after repetitive exercise, which did not recover the next morning. Our findings might help to understand the risk of development of thrombotic event during daily strenuous exercise.

PB 1739 | Absence of Thrombotic Events in the Gastric Signet-ring Cell Adenocarcinoma Patients with High Expression of Heparanase

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Background: Gastric signet ring cell adenocarcinoma (SRCA) is characterized by high fibrosis, rapid progression and high frequency of metastasis to the peritoneum.

Aims: To analyze heparanase (Hep) gene expression in tissue and cell line (KATO-III) of SRCA and to investigate the incidence of venous thromboembolism (VTE) in these patients.

Methods: Hep gene expression was investigated in tumoral and peritumoral tissue from patients with SRCA (n=11) and in different cancer cell lines (n=10) including KATO-III using RT-PCR and qPCR. Hep level was evaluated by ELISA in ascites of patient with SRCA (n=5), non-gastric SRCA (n=3) and colic cancer (n=6). Human phospho-Kinase array was done to observe effect of exogenous Hep in phosphorylation profiles of various kinases of KATO-III. in paralell, we interviewed the database of "Federation Française de Cancerologie Digestive, FFCD" for SRCA and for gastric adenocarcinomas.

Results: High expression of mRNA hep was found in KATO-III, in non-SRCA (n=10) and SRCA (n=11) cancer tissues ($p < 0.001$). Hep level was very high in the ascites of patients with SRCA. Phospho-kinase array showed that only 6/45 kinases were highly phosphorylated in non-treated KATO-III cells. After treatment with active Hep, 2 were upregulated (HSP60 and C-Jun) and 4 (GSK-3α/β, β-catenin, Chk-2 and AMPkal-1) were down regulated. Suramin has no antagonist effect in phosphorylation pathway induced by Hep. Despite the high levels of Hep mRNA in SRCA tumor tissues, no VTE was detected in the patients. In view of the significant increase in Hep levels in ascites and in tumors of SRCA patients, as compared to adenocarcinomas,. The results from FFCD showed that preoperatively, there is no more risk of VTE in patients with SRCA: 3.1% pulmonary embolism, 1% Deep vein thrombosis in controls versus 4.1% and 2.9% in patients with SRCA (n=97) and 3.9% and 3.0% in other adenocarcinomas (n=205).

Conclusions: The increase of Hep expression in SRCA was not correlated to thrombotic events.

PB 1740 | Anti-TFPI Antibody Targeting the Kunitz-2 Domain of Human TFPI Efficiently Recovers FVII Activity Comparing Kunitz 1 Domain Targeted TFPI Antagonist

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Background: Tissue factor pathway inhibitor (TFPI) is a single-chain polypeptide which can reversibly inhibit Factor Xa (FXa). TFPI consists of three tandem linked Kunitz domains (KD). Among the three kunitz domains, KD1 is well known for binding to FVII directly and KD2 is for FXa or FX. MG1113 is a human IgG4 type antibody targeting KD2 of human TFPI.

Aims: The purpose of this study was investigate the difference of molecular mechanisms between TFPI KD1 and KD2 targeted antibody. For the verification we have established several enzymatic assay.

Methods: we checked FVII activity in the presence of anti- KD1 or anti-KD2 antibody for the verification whether the blocking of KD2 can activate the FVII. FVIIa activity was measured in the presence of

anti- KD2 or anti- KD1 antibody. Activated FVII is also checked to see whether it affects FXa or not. Through FXase activity assay, we measured the recovery of FX activity by blocking TFPI. Since TFPI can bind and inhibit TF/FVIIa/FX complex, we also measured how much FX is activated through the complex in the presence of anti-KD2 antibody.

Results: Although FVII directly binds to KD1 of TFPI, blocking KD2 by anti- KD2 antibody was much more efficient to recover FVIIa activity than blocking KD1. Activated FVII is efficiently recovered by anti-KD2 antibody in the condition of presence of FXa. These assays demonstrate that anti- KD1 antibody is less efficacy than anti- KD2 antibody in the same condition. In FXase assay, FXa was generated with increasing concentrations of anti- KD2 antibody. FXa was also increased by anti- KD2 antibody treatment through the formation of TF/FVIIa/FX complex.

Conclusions: KD2 targeting is not for direct blocking to binding epitope of FVII and TFPI. However, It shows more efficient recovery of FVII activity than anti-KD1 antibody. Moreover, FXa helps to recover FVIIa when TFPI is blocked by anti-KD2 antibody. Our results suggest the TFPI KD2 targeted antibody is more effective on the coagulation initiation phase for the clot formation.

PB 1741 | The State of Hemostasis after Nonselective Thrombolysis in Patients with Ischemic Stroke

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Background: Based on the data of international guidelines, using of any antithrombotic drugs contraindicated during first 24 hours after thrombolysis; this is determine high frequency of re-thrombosis (about 25%) in the first 24 hours after successful thrombolysis.

Aims: To examine the state of hemostas in patients with ischemic stroke during 24 hours of system thrombolysis.

Methods: Was study 83 patients (47 men and 36 female) aging 52-72 years with acute cerebrovascular diseases, treated in intensive care. All patient has 10-17 units of NIHSS score. All patients match to therapeutic window, and, according to National Guidelines, receive system thrombolysis. Parameters of hemostas (activated partial thrombin time, prothrombin time, fibrinogen, platelet count, thromboelastography parameters) was monitored before injection of Actilyse®, and during first 24 hours on 3rd, 12th and 24th hours after injection. Data was statistically processed with Newman-Keuls method.

Results: Retrospective analysis shown results, depicted in the table 1. Biochemical parameters of hemostas has not statistically significant changes during 24-hours period. But after thrombolysis thromboelastography shown significant disbalance between coagulation and fibrinolysis, leads to change of structural properties of forming clot. On admission to ICU hemostas state was characterized as trend to the hypocoagulation with normal or slightly activated fibrinolysis on the background of loose clot formation. Such changes represent compensatory reaction of stable hemostas to the ischemic injury. After system thrombolysis in an 3 hours all patients shown unidirectional changes to the hypercoagulation on the background of expressed activation of fibrinolysis and formation of a dense clot. That direction of changes reflect embolism risk depend on tromboelastography.

Conclusions: Thereby it is expedient to conduct further study of using of the antithrombotic agents in early post-thrombolysis period.

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PB 581 | Complement Factor H, Extracellular Nucleosomes, ADAMTS13 and the Regulation of von Willebrand Factor Size in Young Patients with Ischemic Stroke and Myocardial Infarction

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Background: High VWF and low ADAMTS13 levels are associated with an increased risk of arterial thrombosis (ischemic stroke and myocardial infarction). Recent *in vitro* studies indicated that complement factor H (FH) binds to VWF and affects VWF multimer length, either as a reductase or by affecting the proteolysis of VWF by ADAMTS13.

TABLE 1 Correlation between VWF:ratio, FH, ADAMTS13 activity, nucleosomes, CRP, VWF:CBA and VWF:Ag in patients with arterial thrombosis. *: p<0.05, **:p<0.01

	VWF:CBA	VWF:Ag	VWF:Ratio	ADAMTS13 activity	FH	Nucleosomes	CRP
VWF:CBA	-	0.76**	0.29**	-0.08	0.03	0.03	0.06
VWF:Ag	0.76**	-	-0.34**	-0.02	0.12*	-0.02	0.17**
VWF:Ratio	0.29**	-0.34**	-	-0.12*	-0.15**	-0.13*	-0.14*
ADAMTS13 activity	-0.08	-0.02	-0.12*	-	-0.06	-0.004	-0.15**
FH	0.03	0.12*	-0.15**	-0.06	-	0.13*	0.22**
Nucleosomes	0.03	-0.02	-0.13*	-0.004	-0.13*	-	0.11*
CRP	0.06	0.17**	-0.14*	-0.15**	0.22**	0.11*	-

Histones and DNA were also found to bind to VWF, and may by competing with ADAMTS13 potentially also influence VWF size.

Aims: To evaluate the relationship between FH, nucleosomes (DNA+histones), ADAMTS13, and VWF (Ag, CBA and VWF:CBA/Ag ratio) in arterial thrombosis.

Methods: In a case control study of 563 young patients with a first arterial thrombotic event and 463 healthy controls the plasma levels of FH and nucleosomes, VWF antigen (VWF:Ag), VWF collagen binding activity (VWF:CBA), VWF:CBA/Ag ratio, C-reactive protein (CRP) and ADAMTS13 activity were measured using (bio)ELISA, and relations analyzed using Spearman rank correlation and linear regression.

Results: FH and nucleosome levels were similar in cases and controls. ADAMTS13 activity was significantly lower in cases than in controls. The VWF:ratio was negatively correlated with both ADAMTS13 activity, FH, nucleosomes, and CRP in the cases (see table). FH levels were also significantly correlated with CRP, VWF:Ag and nucleosomes in cases, while only CRP was significantly correlated in the controls, in line with potential binding of FH to CRP. Nucleosomes positively correlated with CRP and VWF:Ag, however there was a negative correlation with VWF:ratio.

Conclusions: In young patients with arterial thrombosis, reduced levels of ADAMTS13, circulating FH and nucleosomes relate to increased VWF:ratio and thus VWF multimer size.

PB 582 | The Effect of Short Chained Polyphosphates on Complement Activation in Plasma and Human Whole Blood

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Background: Human platelets contain short chain (SC) (60-100 units) inorganic polyphosphate (polyP) stored in dense granules that is released upon activation. In an experimental system with 2% serum monitoring complement activation by erythrocyte lysis, polyP was shown to inhibit complement activation by blocking the formation of the terminal C5b-9 membrane attack complex (MAC), the final step of the complement cascade. We wanted to investigate whether platelet sized polyP affected complement activation under physiological conditions.

Aims: To investigate whether platelet sized SC-polyP affect complement activation in human blood and platelet free plasma (PFP).

Methods: Blood was collected in lepirudin tubes (50 µg/mL) from healthy fasting individuals (n=10). Blood was incubated with and without 1×10^7 *E. coli*/mL and different concentrations of SC-polyP (0-40 µM) at 37°C for different time intervals (0-60 min) and at physiological calcium concentrations. Complement activation was terminated by adding 10 mM EDTA (final concentration) and monitored by measuring C3bc and the terminal complement complex (TCC) by enzyme-immunoassays. The effect of SC-polyP was also evaluated in platelet free plasma (PFP).

Results: SC-PolyP added alone did not induce complement activation assessed by C3bc and TCC neither in PFP nor in whole blood. *E.coli*-incubation of both PFP and whole blood induced a time-dependent increase in C3bc and TCC ($p< 0.0001$). In PFP, combined incubation with 40 µM SC-PolyP and *E.coli* increased plasma levels of C3bc (52±12%, $p< 0.01$) and TCC (25±11%, $p< 0.05$) compared to *E.coli* incubation alone. In whole blood, combined *E. coli* incubation and 40 µM SC-PolyP and *E.coli* showed no effect on TCC or C3bc.

Conclusions: Short chained polyP facilitated *E.coli*-induced complement activation in plasma, but not in a whole blood at physiological calcium concentrations.

PB 583 | Extracellular Histones Can Inhibit Complement Activation through C4 Binding

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Background: Histones are essential building blocks in forming chromatin, but are extremely toxic once released into the circulation following extensive cellular damage. Extracellular histones can play a role in immune responses in bacterial killing and Toll-like receptor signalling, to induce inflammasome activation and cytokine release. Using histone-conjugated beads, we show histones can bind to complement component 4 (C4) in plasma.

Aims: Since complement activation is important in the innate immune response, the aim of this study was to understand the pathophysiological relevance of extracellular histones in interacting with the complement pathway.

Methods: Histone-C4 binding was confirmed by gel overlay assay and surface plasmon resonance (SPR) determined binding affinities. The physiological relevance was then determined by measuring complement activity in serum using functional assays for each pathway.

Results: H3 [KD = 0.76±0.12 nM] and H4 [KD = 0.91±0.07 nM] had much higher binding affinity to C4 than equimolar concentrations of H1 [KD = 7.26±0.80 nM] and H2B [KD = 9.45±1.43 nM], with weak binding to H2A [KD = 12.67±0.59 nM]. Histones significantly inhibited classical and mannose-binding lectin (MBL) but not the alternative pathway, with H1, H2B and H3 and H4 showing the most significant effects. To demonstrate the specificity of histones, anti-histone single chain variable fragment antibody (ahscFv) and heparin were used, which have been shown to specifically inhibit histone toxicity *in vitro* and *in vivo*. Both ahscFv and heparin significantly rescued histone-induced inactivation of classical and MBL pathways.

Conclusions: Mechanistically, extracellular histones bind to complement component C4. Functionally, histones inhibit the classical and

MBL but not the alternative pathway. Translationally, anti-histone treatment may hold therapeutic promise for recovering complement activation as well as blocking histone-induced toxicity in critically ill patients.

PB 584 | Complement Activation in Patients with Venous Thromboembolism

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Background: The complement system, activated via the classical, alternative and lectin pathways (CP, AP and LP, respectively), interacts with the haemostatic system at various levels. Complement activation (CA) may play a role in the pathogenesis and progression of VTE. We have previously shown raised CA markers in venous thromboembolism (VTE) patients with antiphospholipid syndrome (APS), but the role of CA in non-APS VTE patients is undefined.

Aims: To assess CA in non-APS patients with previous VTE.

Methods: EDTA plasma samples were collected from 41 patients with previous VTE without antiphospholipid antibodies; 34 on warfarin treatment (28 target INR 2.5; 6 INR 3.5), and 7 on rivaroxaban 20 mg/day. CA markers were measured as C3a, C5a, and SC5b-9 (markers for all pathways) and Bb fragment (a specific marker of AP activation) by commercial ELISA (MicroVue kits, Quidel Corp). Results were compared with 56 APS patients (receiving warfarin, target INR 2.5) with previous VTE. Normal ranges (55 normal controls: NC) were: C3a 27-96ng/mL; C5a 0.8-13.0ng/mL; sC5b-960-164ng/mL; Bb 0.7-1.7ug/ml.

Results: C3a, C5a and SC5b-9 were higher in non-APS and APS patients compared to NC ($p< 0.0001$), with the exception of Bb that was raised in APS ($p< 0.0001$) but not non-APS patients. In non-APS patients: C3a, C5a, SC5b-9 and Bb were elevated in 9 (22%), 17 (42%), 16 (39%) and 2 (5%) patients, respectively; C3a, C5a and SC5b-9 were raised in 3 patients; C5a and SC5b-9 in 7; all 4 markers in 1 patient. In comparison, our previous study in 56 APS VTE patients receiving warfarin showed C3a, C5a, SC5b-9 and Bb elevation in: 20 (36%), 19 (34%), 42 (75%) and 14 (25%), respectively.

Conclusions: The data suggest that some non-APS VTE patients have CA despite anticoagulant therapy. CA was less frequent in non-APS VTE than in APS VTE patients and rarely involved the AP of complement, suggesting a different pathophysiology.

PB 585 | C5B9 Deposits on Endothelial Cells for the Evaluation of Complement Function in Thrombotic Microangiopathies of Different Origin and Therapy Monitorization

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare, progressive, life-threatening form of thrombotic microangiopathy (TMA) caused by dysregulation of the alternative pathway of the complement system. Nonetheless, there is wide evidence that complement activation has a role in other TMA.

Aims: A reliable method is needed to evaluate complement activation to monitor eculizumab therapy in aHUS and to explore the indication of this treatment in other TMA.

Methods: Complement activation was assessed by exposing endothelial cells (ECs) to patients' sera or to patients' plasma samples mixed with a control sera pool (1:1). C5b9 deposits on ECs were analyzed through immunofluorescence and expressed as fold increase (mean±SEM) vs. control samples .

Results: Exposure of ECs to aHUS plasma resulted in a significant increase in C5b9 deposits (8±2, n=4, $p< 0.01$), which was prevented in samples from the same patients treated with eculizumab (0.7±0.1). Significant fibrin formation was observed together with C5b9 deposition. The specificity of the reaction was confirmed by its blockade with eculizumab and inhibitors of the intrinsic coagulation pathway. Notably, results obtained using plasma samples were much more remarkable and reproducible than those obtained with sera. C5b9 deposition was also increased using samples from HELLP syndrome patients (at acute phase, 6±2, n=4, $p< 0.01$) and 40 days later (3.2±1, $p< 0.01$), and both autoimmune (2.9±1, n=4, $p< 0.05$) and congenital thrombotic thrombocytopenic purpura (2.1±1, n=4). Complement activation was at control levels when analyzing samples from patients with malignant hypertension (0.8±0.2, n=5) or aHUS patients treated at regular doses of eculizumab (0.5±0.1, n=5) and lower (0.4±0.2, n=2).

Conclusions: This method could be useful to monitor therapy and to explore the indication of eculizumab to treat other TMA presenting complement activation. Further research is needed to clarify the basis for the colocalization of C5b9 and fibrin on ECs surface.

PB 586 | The Complement Lectin Pathway in Post Cardiac Arrest Patients: A Randomized Clinical Study

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Background: Recent studies suggest that the complement system influence injury after out-of-hospital cardiac arrest. Lectin pathway proteins are an important part of the complement system, but the majority of these proteins have not been investigated in cardiac arrest patients.

Aims: To investigate levels of lectin pathway proteins in patients resuscitated after cardiac arrest compared with healthy individuals, and to examine whether the protein levels were influenced by 48 hours compared with 24 hours of targeted temperature management (TTM).

Methods: We conducted a randomized, outcome assessor-blinded study including 82 comatose out-of-hospital cardiac arrest patients. The patients were allocated to 24 hours (n=42) or 48 hours (n=40) of TTM at 33±1 °C. Blood samples were obtained 22, 46 and 70 hours after this temperature was reached. Informed consent was collected and the study was approved by the Central Denmark Region Committees on Health Research Ethics. Data from 82 gender matched healthy blood donors were used for comparison. Levels of the lectin pathway proteins MBL, M-ficolin, H-ficolin, CL-L1, MASP-1, MASP-2, MASP-3 and MAp44 were analyzed using time-resolved immunofluorometric assays (TRIFMA®).

Results: We found significantly higher levels of CL-L1, MASP-1, MASP-2 and MAp44 (p-values< 0.03), and lower M-ficolin levels (p=0.01) in cardiac arrest patients compared with healthy individuals. During 70 hours after cardiac arrest, significantly higher levels of MASP-2 and MASP-3, and lower levels of M-ficolin were demonstrated in the patients treated with 48 hours compared with patients treated with 24 hours of TTM. For the remaining proteins no significant differences were found.

Conclusions: The significantly higher levels of CL-L1, MASP-1 and MASP-2 in cardiac arrest patients indicate that cardiac arrest patients have a more activated lectin pathway compared with healthy individuals. However, the protein levels did not seem to be influenced by the duration of TTM.

PB 587 | The Effect of Platelets and Extracellular Vesicles on Complement Activation in Human Plasma

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Background: Studies have shown platelets play an important role in regulation of complement activation, and platelet-derived extracellular vesicles (EVs) are able to promote complement activation. Whether EVs and platelets affect complement activation in a physiological plasma milieu is not clear.

Aims: To investigate the modifying effect of platelets and EVs on *E.coli*-induced complement activation in a plasma system.

Methods: Blood was collected in lepirudin tubes (50mg/ml) from healthy individuals. Blood was then centrifuged twice (3000*x*g for 10 min and 13500*x*g for 2 min) for preparation of platelet-free plasma (PFP). A part of PFP was centrifuged at 20000*x*g for 30 min to prepare EV-depleted plasma (EVDP). Platelet-rich plasma (PRP) was prepared by centrifugation at 140*x*g for 10 min. EVDP, PFP, PRP were incubated with *E.coli* (1 x 10^7/ml) at 37°C to examine complement activation for different time intervals (0-60 min). The reaction was stopped by adding EDTA (10mM). PRP was centrifuged at 3000*x*g for 10 minutes and all plasma samples were frozen at -80°C until analysis. Levels of complement activation products C3bc and TCC were measured using ELISA.

Results: *E.coli*-incubation showed a time-dependent increase (p< 0.01) in both complement activation products from 0 min to 60 min in all plasmas (C3bc: 24±6-131±7.9 CAU/ml in EVDP; 28±8.1-114±7.0 CAU/ml in PFP; 7.1±4.3-58±14.3 CAU/ml in PRP; TCC:1.9±0.1-23±1.5 CAU/ml in EVDP; 2.0±0.4-27±3.5 CAU/ml in PFP; 0.3±0.1-7.6±2.8 CAU/ml in PRP). Plasma levels of C3bc were 13.2±0.5% (p< 0.01) lower in PFP than in EVDP after 60 min incubation, while TCC levels in PFP did not differ significantly from EVDP, suggesting that EVs may limit initiation of complement activation. PRP had 55.8±10.3% (p< 0.01) and 64.9±15.4% (p< 0.01) lower C3bc and TCC levels, respectively, compared to EVDP after 60 min incubation.

Conclusions: *E.coli*-induced complement activation in plasma was lower in PRP than in EVDP suggesting a lower degree of complement activation in the presence of platelets and EVs.

PB 588 | Fibrinogen and Clot Structure is Affected by Eculizumab in Patients with Paroxysmal Nocturnal Haemoglobinuria

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Background: Paroxysmal nocturnal haemoglobinuria (PNH) is a haematopoietic stem cell disorder. It leads to complement-driven intravascular haemolysis and severe thrombosis. Treatment with eculizumab which inhibits complement factor C5 reduces thromboembolic events in PNH, indicating that complement activation plays a role in the pathophysiology of thrombosis in this disease. Patients with other thrombotic disorders form denser fibrin clots that incur greater resistance to fibrinolysis. It is unknown if changes to clot architecture contribute to thrombosis in PNH.

Aims: Characterise fibrin clot structure in patients with PNH and determine the effect of eculizumab on clot structure.

Methods: 35 patients from the PNH National Service were recruited. Plasma samples were obtained and ex vivo fibrin clot structure was analysed by permeation, turbidity and confocal microscopy. Granulocyte clone size was measured by flow cytometry and fibrinogen (FGN) levels by Clauss.

Results: Granulocyte clone size correlated with FGN (r= -0.35, p=0.048), maximum absorbency (r= -0.34, p=0.046) and clot density (r= -0.36, p=0.04). 14/20 patients with large clone sizes were on eculizumab. Analysis of patients not on eculizumab showed a trend towards clone size correlating positively with fibrinogen levels (r=0.42, p=ns). Patients on eculizumab had lower fibrinogen levels (3.1±0.2 v 3.6±0.1g/L, P=0.02), maximum absorbance (0.31±0.1 v 0.40±0.1, p=0.028) and fiber density/100µm (18.5±4.4 v 23.9±4.2, p=0.006), indicative of a less dense clot.

Conclusions: Patients on eculizumab showed reduced FGN, fiber thickness and fiber density. Our data indicate that the beneficial effects of eculizumab in PNH is partly due to reduced complement activation and attenuated inflammatory response, leading to lower FGN levels and a less thrombotic fibrin clot structure.

PB 589 | Hemocompatibility Analysis of Silk Nanoparticles Leads to Different Results under Static and Flow Conditions

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Background: Nanomedicine often administers nanoparticles into the blood stream for diagnostic or therapeutic purposes. The high surface and curvature of these nanosized particles provides them different properties than the corresponding bulk materials. Depending on the type, pro- or anticoagulant properties of nanoparticles are described. Nanoparticles frequently tend to aggregate from nano- to microsized clusters, what may alter their interaction with the blood cascade systems and ultimately their hemocompatibility.

Aims: Test the effect of nanoparticle clustering on their hemocompatibility.

Methods: Silk nanoparticles and reference silica particles of about 100 nm each were incubated in human whole blood for two hours. The incubation was either under static conditions or under flow conditions in a Chandler loop set-up. The shear condition in the Chandler loop incubation keeps the nanoparticles better suspended than the static incubation. After the incubation, parameters of hemostasis and inflammation were determined.

Results: Coagulation activation, measured as prothrombin F1+2 fragment after silk nanoparticle incubation was generally low when compared to silica. For both materials, there was a trend for higher activation during static condition (Fig. 1A). The silk nanoparticles induced very high complement activation (C5a) under static condition, but it was almost completely suppressed under flow, where particles are dispersed (Fig. 1B). PEGylation of the particles did not sufficiently suppress aggregation and complement activation (Fig. 1B, C). Curvature of the single particles prevented the proper assembly of the complement complexes, whereas the bigger particle clusters allowed the complement complex formation. Due to the larger enzyme complexes, the complement system is more sensitive to this effect than the coagulation system.

Conclusions: Nanoparticle aggregation, as it happens under static test conditions, may cause higher activation of enzyme cascade systems than under physiological flow conditions.

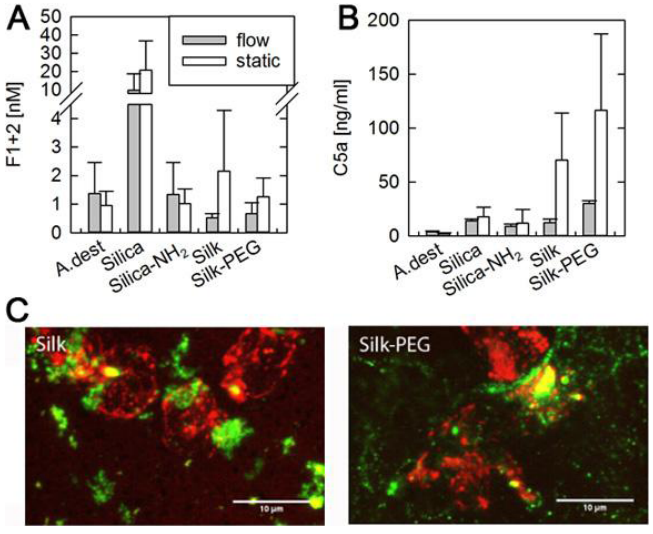

FIGURE 1 Incubation of nanoparticles with whole blood under static or under flow conditions

PB 590 | Character of Haemostatic System in Preterm Labor in Women with Thrombophylia

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Background: It is known that the haemostatic system during physiological pregnancy adaptive response is to increase the coagulation potential, primarily by increasing the concentration of clotting factors and platelet functional activity. These factors create additional conditions for the development of thrombosis on the background of already existing genetic thrombophylia.

Aims: To study the haemostatic system in women with preterm labor and trombophylia.

Methods: Haemostatic system was studied in 64 pregnant women with complicated obstetric history, and preterm delivery (study group). The control group consisted of 20 conditionally healthy women with uncomplicated pregnancy in gestation, corresponding to the III trimester. Assessment tests were used haemostatic system: determining the concentration of fibrinogen and platelet haemostasis research: determining the number of platelets in the peripheral blood, the study

of the functional activity of platelets under the influence of various stimulants of aggregation.

Results: The patients of the main group during gestation in the plasma link haemostasis observed significant increase in levels of fibrinogen, which is the time of inspection was higher than 1.2 times the control (respectively 3.8 ± 0.2 g / l and 3.1 ± 0.1 g / liter, P < 0.01). Platelet counts in the peripheral blood of these patients was significantly higher than in the control group (235,8 ± 6,6 · against · 203.6 ± 10.8 109 / L; Δ% = + 13,6; p < 0.05), which is obviously due to the activation of platelet haemostasis. Platelet aggregation in patients of the main group almost has not changed, practically no different from the control (respectively 97,1 ± 2,0 and 105,3 ± 3,2%, Δ% = - 7,8; P> 0.05).

Conclusions: The haemostatic system in pregnant women with thrombophylia characterized by the activation of platelet haemostasis by increasing the number of platelets, increased coagulation potential by increasing of fibrinogen.

PB 591 | Role of Protease-activated Receptor-1 in Poly I:C Induction of Tissue Factor Expression in Endothelial Cells and in the Activation of Coagulation in Mice

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Background: Viral infections induce tissue factor (TF) expression in monocytes (Mo) and endothelial cells (ECs) and this in turn activates the coagulation cascade as part of the host defense system. However, excessive activation of the coagulation cascade with some viral infections, such as Ebola, can lead to disseminated intravascular coagulation (DIC). A previous study showed that the TLR3 ligand polyinosinic:polycytidylic acid (poly I:C), which is a mimetic of dsRNA, induced TF expression in ECs but not Mo in vitro and activated coagulation in mice.

Aims: .To evaluate the role of PAR-1 in the expression of poly I:C induction of TF expression in ECs in vitro, and in the activation of coagulation in mice after administration of poly I:C.

Methods: In this study, we used poly I:C to mimic a viral infection in ECs in vitro and in vivo. ECs (EA.hy 926 cell line) were stimulated with poly IC with or without PAR-1 agonist peptide, thrombin and we measured TF mRNA and activity. Furthermore, we measured levels of plasma thrombin-antithrombin (TAT) complexes, as a marker of activation of coagulation, in WT and PAR-$1^{-/-}$ mice after injection of poly I:C.

Results: We found that PAR-1 activation by agonist peptide or thrombin enhanced poly I:C induced TF expression in ECs. The PAR-1 inhibitor vorapaxar abolished the enhanced expression. Poly I:C treatment increased levels of TAT in the plasma of WT and $PAR1^{-/-}$ mice, but levels were significantly lower in $PAR1^{-/-}$ mice. To identify the cellular sources of TF that activate coagulation after administration of poly I:C, we are determining the effect of deleting TF in ECs and hematopoietic cells ($TF^{fl/fl}$ Tie-2Cre), myeloid cells ($TF^{fl/fl}$ LysMCre) or ECs ($TF^{fl/fl}$ VE-cadherin) on TAT levels.

Conclusions: Our in vitro and in vivo studies indicate that activation of PAR-1 enhances poly I:C induction of TF expression in ECs and activation of coagulation in mice. Understanding how TF activates the coagulation cascade during viral infections may lead to novel strategies to prevent DIC.

PB 592 | Role of Helicobacter Pylori Eradication Therapy in Platelet Recovery in Chronic Immune Thrombocytopenic Purpura

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Background: Idiopathic thrombocytopenic purpura (ITP) is a bleeding disorder, in which the immune system destroys nativeplatelets. In this condition an auto-antibody is generated against a platelet antigen. ITP affects women more often than men and is more common in children than adults.

Aims: To assess the effect of Helicobacter pylori eradication therapy (HPET) on platelet count in Helicobacter pylori associated chronic immune thrombocytopenic purpura (chronic ITP) in adult.

Methods: It is an interventional prospective study conducted at Liaquat University of Medical and Health Sciences, Jamshoro from 2014 to 2015.A set of 85 patients diagnosed with chronic ITP were included in the study via convenient sampling. Patients with platelets count < 100x 10^9/L for >3 months were selected. They were posed to first line investigations which comprised Complete blood count (CBC) and peripheral blood smear examination followed by second line tests including bone marrow examination and Helicobacter pylori stool specific antigen (HpSA - EIA).Standard H. pylori eradication therapy was offered and the patients were assessed at regular intervals for 6 months.

Results: Of the 85 study patients, 32(37.6%) were male and 53(62.3%) were female.Mean ages of H.pylori positive and negative subjects were 43.89± 7.06 and 44.75± 7.91 years,respectively. Bone marrow examination confirmed the diagnosis and excluded other related BM disorders. H.pylori stool antigen (HpSA) was detected in 34 (40%) patients and hence regarded as H. pylori positive; the rest were negative. - Treatment with Eradication therapy significantly improved the mean platelet counts from 48.56 ± 21.7 x10^9/l to 94.2 ± 26.8 x10^9/l.

Conclusions: We concluded that the anti- H. pylori eradication therapy improves blood platelet counts in chronic immune thrombocytopenia.

PB 593 | Intravascular Parasitic Worms and Hemostasis

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Background: Schistosomes are intravascular parasitic worms (blood flukes) that currently infect over 200 million people globally. The adult parasites, although representing large foreign obstructions in host blood vessels, do not appear to provoke thrombus formation around them *in vivo*.

Aims: Using thromboelastography (TEG), we compared the clotting capability of blood that had been exposed to schistosomes versus blood that had not been exposed to the worms.

Methods: We found that the presence of the worms yielded blood that clotted more slowly and yielded relatively poor, though stable, thrombi. This TEG profile suggests that the worms can act as local anti-coagulants.

Results: Among the proteins identified in the surface membranes of the intravascular worms are a collection of enzymes that may impede coagulation. Using RNA interference to suppress the expression of *Schistosoma mansoni* genes encoding selected surface enzymes, we have shown that one tegumental enzyme, ATPdiphosphohydrolase1 (SmATPDase1), can cleave the pro-thrombotic molecule ADP. Recombinant SmATPDase1 likewise cleaves ADP. By effectively removing exogenous ADP, we hypothesize that SmATPDase1 helps to inhibit platelet activation and aggregation around the worms within the vasculature. Other schistosome surface proteins that may be important in regulating blood clot formation around the worms include housekeeping enzymes such as enolase (SmEno). While best known as a glycolytic enzyme, SmEno can additionally bind the plasma zymogen plasminogen and promote its activation (by tissue plasminogen activator (tPA)) to generate plasmin - a serine protease that cleaves fibrin and dissolves blood clots. Live parasites likewise mediate this effect.

Conclusions: Thus, acquiring host proteases by binding them to e.g. surface enolase may represent another mechanism that schistosomes employ to limit blood clot formation around them and so promote their survival.

PB 594 | Comparative Functional Studies of Thrombin and Staphylocoagulase in Terms of Clotting Kinetics and Properties of the Resultant Fibrin

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Background: *S. aureus* secretes staphylocoagulase (SC), a virulence factor associated with abscess formation, staphylococcal agglutination and sepsis. SC binds to and activates prothrombin (ProT); the SC-ProT complex converts fibrinogen (Fgn) to fibrin (Fn), circumventing the physiological coagulation cascade. Fn deposition typically represents an innate host immune defence, which is in contrast to the proposed role of SC in pathogenesis.

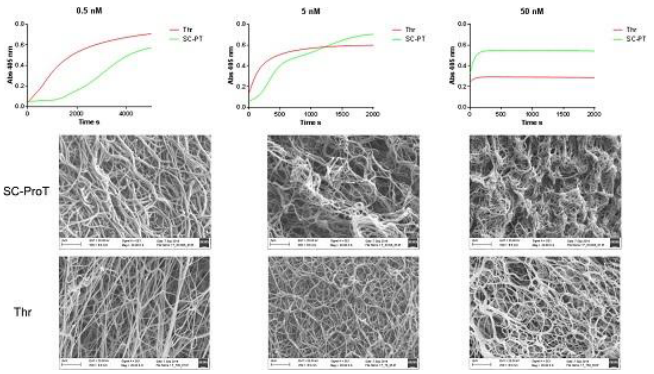

FIGURE 1 Turbidimetric clotting curves and fibrin product SEM for SC-ProT and Thr

Aims: To investigate the enzyme kinetics of SC-ProT, and the physical properties of the Fn product, compared to Thrombin (Thr).

Methods: Recombinant SC expressed in *E. coli*. Kinetic chromogenic assays (S-2238) and turbidimetric clotting assays (Fgn and human plasma) over a range of Thr and SC-ProT concentrations (0.5-50 nM). Fn structure assessed by scanning electron microscopy SEM (fibre diameter), pressure-driven permeation (gel porosity) and oscillation rheology (viscoelastic properties).

Results: Against S-2238 the specific activity of SC-ProT was equivalent to Thr. Against Fgn, based on 50% clotting times, SC-ProT potency was 3-fold less than Thr. The Fn product of SCG-ProT has thicker fibres compared to equimolar Thr, with higher concentrations producing thinner fibres and less porous clots.

Concentration dependence of fibre size is preserved in both Fgn and plasma, and the difference in permeability persists at higher enzyme concentrations, although Thr forms less permeable clots from plasma than from pure Fgn. Compared to Thr, the Fn product of SCG-ProT forms softer clots that are easier to deform, less viscous and less resistant to mechanical forces.

Conclusions: The marked differences in the kinetics of Fn polymerisation by Thr and SC-ProT, and the different structural and physical properties of the Fn products, could play an important role in the severity of S. aureus infections. Thr Fn forms a protective barrier to infection and resists shear, SC-ProT Fn may serve as a deformable shell to enhance microbial replication and dissemination.

PB 595 | Liver Fibrosis and Thrombophilia: Is There a Real Connection? Preliminary Results among HIV/HCV Co-infection

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Background: Thrombin is intimately related to liver fibrogenesis both by a direct effect on hepatic stellate cells and through fibrosis

TABLE 1 Structural characteristics of the fibrin product of SC-ProT and Thr

		Thrombin			SC-ProT		
		5nM	50nM	100nM	5nM	50nM	100nM
	Plasma clot	6.91 (2.00)	4.14 (1.36)	3.52 (0.48)	5.91 (3.98)	5.30 (2.91)	6.04 (1.80)
Permeability coefficient		0.5nM	5nM	50nM	0.5nM	5nM	50nM
(Ks, 10^{-9} cm^2), Mean (SD)	Fibrin clot	40.8 (12.7)	27.8 (14.9)	18.5 (5.05)	49.8 (4.95)	39.7 (11.0)	33.7 (13.8)
		SC-ProT (5nM)	Thr (5nM)	SC-ProT (12nM)	Thr (12nM)		
Viscoelastic properties (Oscillation rheometry)	Storage modulus G´, Pa, Mean (SD)	22.15 (4.87)	39.66 (8.44)	31.43 (2.72)	57.17 (13.49)		
	Loss modulus G", Pa, Mean (SD)	3.95 (0.61)	6.86 (1.14)	4.21 (0.30)	9.26 (1.86)		
	Delta, Mean (SD)	10.28 (1.12)	9.91 (0.79)	7.67 (0.70)	9.27 (0.48)		
	Critical shear stress Pa, Mean (SD)	65.36 (12.29)	178.20 (18.01)	103.80 (9.65)	204.20 (31.25)		

associated with ischemic parenchymal extinction. In fact, it has been reported that anticoagulant therapy may attenuate the progression to liver fibrosis (LF). Besides, it is well known that HIV/HCV patients experience a faster progression to LF.

Aims: We aimed to assess if prothrombotic genetic variants are associated with the development of LF in HIV/HCV patients.

Methods: We performed a case-control study. We studied the genotypic distributions of Factor V Leiden (FVL), Prothrombin 20210A (II20210A), Fibrinogen-γ´10034T (FGG10034T) and Factor XI 7872C (FXI7872C) in 24 HIV/HCV patients. Among them, 13 were categorized as „with liver fibrosis" (LF+) and 11 as „without liver fibrosis" (LF-). The latter group only included patients with a clinical history of, at least ten years of HCV infection. The stage of liver fibrosis was assessed by the results of indirect ultrasonic liver elastography. Furthermore, we studied a reference group (n=223) to estimate the genotypic distributions in general population (RG).

Results: Table 1 describes the most relevant findings for the LF+/LF- groups.

TABLE 1 Clinical characteristics of patients with "liver fibrosis" (LF+) and "without liver fibrosis" (LF-).

Variables (Median with quartiles or %)	LF + (n=13)	LF - (n=11)	p
Age (years)	47 (41-53)	43 (41-46)	0.352
Male sex (%)	61.5 (8/13)	54.5 (6/11)	0.729
Body mass index (kg/m2)	28 (24-32)	27 (24-37)	0.990
Duration of HIV infection (years)	11 (4-13)	16 (14-18)	< 0.01
T cells CD4+ counts (cel/ul)	199 (143-346)	397 (210-585)	0.020
T cells CD4+ counts nadir (cel/ul)	115 (70-187)	202 (149-310)	0.050
Alcohol >20g/day (%)	59 (7/13)	64 (7/11)	0.628
Smoking (%)	83 (11/13)	56 (6/11)	0.106

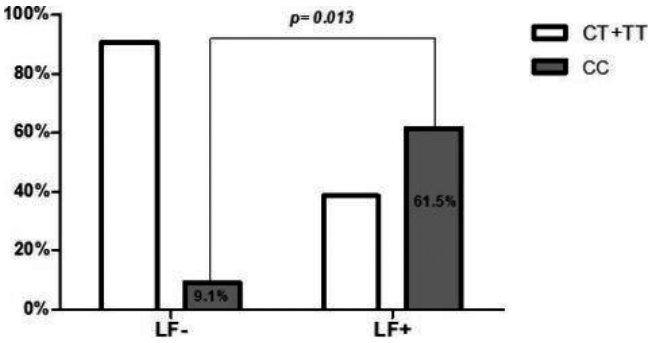

FIGURE 1 Genotype distribution of FXI7872C among patients without (LF-) and with (LF+) liver fibrosis

The distribution of the FXI7872 CC genotype showed significant differences between LF+/LF- (61.5%,8/13 vs 9.1%,1/11, p=0.013) and LF+/RG (61.5%,8/13 vs 32.0%,71/223, p=0.036). (Fig. 1).

No differences were observed in the distributions of FVL, II20210A and FGG10034T.

Conclusions: Our results suggest that the carriage of the FXI7872 CC genotype, that has been associated with an increased expression of FXI and a higher risk of deep venous thrombosis, might also be associated with the development of LF in HIV/HCV patients. This effect might be probably mediated by the presence of a pro-coagulant state. These results are preliminary and the sample size needs to be increased. If further confirmed, this finding would help in the early identification of patients who could benefit from anticoagulant therapy.

PB 596 | Anticoagulants Increase Alveolar Hemorrhage after Influenza A Infection in Mice

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Background: Influenza A virus (IAV) infection is a common respiratory tract infection that causes considerable morbidity and mortality. While viral infections are generally associated with activation of coagulation, alveolar hemorrhage has been reported in patients with influenza pneumonia and in mice infected with IAV. We have recently reported that expression of the procoagulant protein tissue factor (TF) in lung epithelial cells maintains lung hemostasis after IAV infection (Antoniak et al JTH 2016). However, studies on the impact of anticoagulant therapy on the severity of illness and outcomes from influenza are lacking.

Aims: In this study, we investigated the effect of anticoagulants on alveolar hemorrhage after IAV infection of wild-type mice.

Methods: Wild-type mice were anticoagulated with either warfarin via drinking water or the direct thrombin inhibitor dabigatran etexilate (DE) via chow and then infected intranasally with a mouse-adapted IAV (A/Puerto Rico/8/34 H1N1). Activation of coagulation and alveolar hemorrhage were assessed by measuring the levels of thrombin-antithrombin complex (TATc) and hemoglobin in the bronchoalveolar lavage fluid (BALF), respectively. We also measured lung vascular permeability by Evans blue test and viral genomes in the lung, as well as white blood cells counts, inflammatory mediators, and protein in BALF. Furthermore we monitored survival and body weight changes of the mice for 14 days after IAV infection.

Results: Mice receiving either warfarin or DE had decreased activation of coagulation and increased alveolar hemorrhage compared to infected controls after IAV infection. Warfarin but not DE increased vascular permeability and mortality of influenza A-infected mice. Anticoagulation did not affect levels of IAV genomes, white blood cells, inflammatory mediators, or protein in the BALF.

Conclusions: Our results suggest that chronic anticoagulation could contribute to more severe alveolar hemorrhage in the setting of influenza pneumonia.

PB 597 | Comparison of Fibrin Generation Markers in Septic Shock

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Background: Septic shock is associated with an activation of the haemostatic system which markers may have a prognostic value.

Aims: To compare the prognostic value of fibrin generation markers FGM assayed at diagnosis in patients with septic shock, both isolated and integrated into the ISTH DIC score.

Methods: We have recruited a multicentric prospective cohort (SepsiCoag study; *NCT01231672*) to validate the prognostic scores we had previously derivated from retrospective cases (*J Thromb Haemost 2008;6:645-53*). In the present ancillary study, automated assays for FGM, *i.e.* D-dimers (DDi: STA®-Liatest® D-Di), fibrinogen-fibrin degradation products (FDP: STA®-Liatest® FDP) and fibrin monomers (FM: STA®-Liatest® FM) were performed in 780 patients entering ICU with a septic shock diagnosis. Their abilities to predict early death at D7 and late death at D30 were analysed, isolated then integrated into their corresponding ISTH DIC scores.

Results: 18.5% and 38.6% of the included patients were dead at D7 and D30, respectively. DDi and FDP values were highly correlated (0.943) whereas correlations between DDi and FM (0.690) or FDP and FM (0.678) were weaker. Values of DDi, FDP and FM were associated with survival on D7 ($p=0.0061$, 0.0179 and < 0.0001 respectivelly) but only FM values were associated with survival on D30 ($p=0.0001$). For death at D7 (D30), areas under ROC curves were 0.563 (0.525), 0.565 (0.531) and 0.647 (0.617) for DDi, FDP and FM respectively. DIC scores computed with each of the FGM were associated with survival on D7 and D30 but the one based on FM had a better prediction ability.

Conclusions: In patients entering ICU with septic shock, FM, used isolated or integrated into the ISTH DIC score, is the FGM best correlated with clinical prognosis.

PB 598 | Thoracic Manifestations in Behcet's Disease

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Background: Vascular involvement is the leading cause of death in Behcet's disease (BD).

Aims: Pulmonary vasculitis in Behcet's syndrome is unusual but serious. It is related to the well known vascular tropism of the disease.

Methods: Seventy five patients, who fulfilled the criteria of the International Study Group for diagnosis of BD and who were hospitalised in the Internal medecine B department of Charles Nicolle hospital were recruited . We studied the clinical characteristics of patients with pulmonary manifestations.

Results: Three patients had a thoracic event in our study distributed in superior vena cava involvement, pulmonary thrombosis and pulmonary aortic anevrysm. The mean age of diagnosis of the pulmonary event was 35 years. All patients were males. Mean disease duration before the thoracic event was 5,66 years.

An association to another vascular involvement was found in two cases. No ocular neihther neurologic involvement were observed in the three cases. HLA B50 was found in two cases. Corticosteroid treatment was used in 2 cases and immunosuppressive tratment in only one case.

Conclusions: Thoracic involvement of Behcet´s disease is unusual but serious. It may involve the superior vena cava, pulmonary arteries,

aorta and subclavian vessels. Imaging is useful for diagnosis and assess the degree of thoracic involvement. CT scan and MRI are obviously more accurate than angiography.

PB 599 | Macrophages Release Tissue Factor-bearing Microparticles upon Stimulation with *Histophilus somni*

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Background: *Histophilus somni* is a gram negative coccobacillus of cattle diffuse vasculitis and intravascular thrombosis that can lead to multiple organ failure. Macrophages are important cellular mediators of fibrin deposition and removal at sites of inflammation. Recently, it has become evident that macrophages and other cells release microparticles that have an array of biological activities. We sought to determine the role of macrophage-derived microparticles in *H. somni* induced fibrin clot formation *in vitro*.

Aims: Determine whether monocyte-derived macrophages exposed to *H. somni* release microparticles that activate the clotting cascade resulting in thrombus formation.

Methods: Bovine monocyte-derived macrophages were incubated with *H. somni* (at a 1:10 ratio) in RPMI with 10% heat inactivated fetal bovine serum for 6 hr at 37°C with 5% CO_2. Membrane-shed microparticles were isolated from the conditioned media of monocyte-derived macrophages incubated with or without *H. somni*, washed twice with Ca^{2+} and Mg^{2+} free HBSS, and procoagulant activity assessed by a one-step recalcified plasma clotting assay.

Results: We observed greater procoagulant activity for microparticles from *H. somni* stimulated macrophages than from unstimulated control macrophages. Microparticle procoagulant activity was inhibited by addition of an anti-tissue factor antibody. We also observed co-localization of fluorescein-labeled *H. somni* and Annexin V staining as evaluated by confocal microscopy.

Conclusions: These results suggest that exposure to *H. somni* cells causes bovine monocyte-derived macrophages to release microparticles that contain tissue factor. We infer this process might amplify thrombus formation in bovine histophilosis.

PB 1358 | Identification of a Novel Regulator Platelet $P2Y_{12}$ Receptor Function: Tetherin, a Lipid-raft Organizing Protein Negatively Regulates Receptor Activity

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Background: The function of a number of platelet receptors, including the $P2Y_{12}$ (1), is dependent upon their residence in lipid rafts although little is known about the molecular mechanisms regulating their membrane surface compartmentalisation. Tetherin (also known as CD317 or BST2) is a lipid raft-associated transmembrane protein that regulates host response to viral infection by inhibiting viral particle release (2). This integral membrane protein which also plays a role in lipid raft organization (3) is expressed in platelets although its physiological function is unknown.

Aims: To characterize tetherin-dependent regulation of platelet $P2Y_{12}R$ function in tetherin (-/-) mice and establish interaction between these proteins in both cell line models and platelets.

Methods: Platelet ADP receptor activity was assessed in tetherin (-/-) mouse platelets as previously described (4). $P2Y_{12}R$ and tetherin interaction was assessed by confocal, TIRF and FRET-FLIM microscopy and co-immunoprecipitation (Co-IP) approaches in cells expressing human $P2Y_{12}R$ and full length or mutant tetherin constructs.

Results: ADP-stimulated platelet aggregation and $P2Y_{12}R$ activity was enhanced in tetherin (-/-) mouse platelets. In cell lines overexpression of tetherin attenuated $P2Y_{12}R$ signalling and internalization. FRET-FLIM microscopy and Co-IP established that $P2Y_{12}R$ and tetherin interact directly with a glycosyl-phosphatidylinositol anchor at the C-terminus of tetherin required for protein-protein interaction. Intriguingly receptor activation reduced tetherin-receptor interaction.

Conclusions: Tetherin negatively regulates platelet $P2Y_{12}R$ function and represents a potential new target in regulating platelet activity.

PB 1359 | Platelet Endocytosis in Immune Responses to Viral Infection

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Background: Thrombocytopenia correlates with clinical outcomes of viremia and bacteremia. Chronic viral infections significantly increase the risk of acute myocardial infarction (MI). Likely, these infections initiate some level of platelet activation, contributing to increased thrombotic potential. This could be due to the platelets' ability to detect Pathogen and Damage Associated Molecular Patterns (PAMPs and DAMPs). Viral or bacterial DNA/RNA is recognized by Toll-like Receptors (TLR) 7 and 9; an ill-defined process in platelets, since these receptors are intra-platelet and not at the surface. Platelets endocytose virions but the machinery required and its subsequent effects are unclear.

Aims: We sought to understand whether platelet endocytosis is required for their responses to TLR agonists and viral particles.

Methods: Assays on of platelets from transgenic mice.

Results: We examined platelet responses to TLR agonists, loxoribine (for TLR7) and unmethylated CpG oligonucleotides (for TLR9), which activated $I_\kappa B$ kinase (IKK) and PI3K-Akt pathways (*i.e.*, phophorylation of $I_\kappa B$, SNAP-23 and Akt). Each also elicited an attenuated secretory response, which was sufficient to form platelet-leukocyte aggregates (PLAs), as shown by FACS. Inhibitor (Dynasore and NH_4Cl) studies and analysis of platelets from endocytosis-defective mice (VAMP-$3^{-/-}$ and Arf$6^{-/-}$) demonstrated the importance of platelet endocytic trafficking in regulating the responses of these intracellular TLRs. We used HIV-1 pseudovirions to model platelet-viral interactions and showed that platelets endocytose and degrade retroviral particles to release TLR agonists, which initiates platelet activation (IKK and Akt activation), secretion, and PLA formation. HIV-1 pseudovirion uptake and subsequent activation were abolished in VAMP$3^{-/-}$ or Arf$6^{-/-}$ platelets.

Conclusions: Our studies suggest how platelets can act at early stages of pathogen recognition and are not only able to internalize pathogens but are also able to process them to initiate an immune response.

PB 1360 | Pharmacologic Blockade of Platelets Reduces Neuroinflammation in an Animal Model of Multiple Sclerosis

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Background: Multiple sclerosis (MS) is the most frequent inflammatory demyelinating disease of the central nervous system (CNS). While the pathogenesis of MS is still not completely understood, emerging new concepts identified platelets as crucial players in MS pathophysiology that actively assist and amplify inflammatory response. Recent studies showed that platelet depletion reduces disease severity and inflammation in experimental autoimmune encephalomyelitis (EAE), a classical animal model to study MS. Furthermore, platelets occur along with an increased activation status in MS patients.

Aims: In this study, we challenge the relevance of platelet inhibition under pathophysiological conditions *in vivo*.

Methods: 30 or 300 mg/l of the irreversible cyclooxygenase inhibitor acetylsalicylic acid (ASA) were orally administered for 35 days to mice that were subjected to actively-induced EAE.

Results: Here, we demonstrate that pharmacologic blockade of platelets with ASA renders mice less susceptible to autoimmune CNS inflammation. While disease onset was unaltered, oral treatments with high and low-doses of ASA were both associated with a reduced disease maximum and less inflammatory infiltrates within the CNS. At high doses of ASA, amelioration was further accompanied by reduced numbers of interleukin-17A- and interferon-γ-producing T-helper cells as disclosed via intracellular cytokine staining at the disease peak. Thus, ASA at high concentration was related to more pronounced ($P < 0.01$) reduction of disease progression compared to treatment with low doses of ASA ($P < 0.039$), but flow cytometric analysis showed for both no significant difference in the number of infiltrated platelets within the CNS of ASS treated mice compared to control mice.

Conclusions: Indeed, further investigations are needed to reveal the underlying mechanisms of platelet inhibition.

Overall, our findings show that the inhibition of platelet activation is beneficial in animal model of MS and thus indicate a novel therapeutic strategy to combat MS.

PB 1361 | Exploring Pro-thrombotic HMGB-1 Release from Platelets

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Background: Released HMGB-1 acts as damage-associated molecular pattern. HMGB-1 can be passively released from necrotic cells or actively secreted. Platelet-derived HMGB-1 can act as a pro-inflammatory signal, that exacerbates thrombosis. However, the mechanism of HMGB-1 release from platelets is unclear.In this study, we investigated HMGB-1 surface exposure in activated platelets.

Aims: To understand agonist driven HMGB-1 plasma membrane exposure.

Methods: Washed platelets were stimulated with ADP, U46619, thrombin, cross-linked collagen-related peptide (CRP-XL), thrombin-plus-CRP-XL, or the calcium ionophore, A23187. Platelets were co-stained against HMGB-1, CD62P (a marker of a-granule secretion) and CD41, and analysed by flow cytometry.

Results: Platelet stimulation triggered HMGB-1 surface exposure, a-granule secretion and microparticle release in an activator-dependent manner.

The relatively weak activators, ADP or U46619, did not trigger a detectable increase in HMGB-1 surface exposure. Under these conditions, ADP and U46619 triggered weak a-granule secretion or little micro particle release.

Thrombin, Thrombin-plus-CRP-XL and A23187 all induced a greater increase in HMGB-1 surface exposure, while CRP-XL did not. However, all of these activators triggered increased maximal surface CD62P, indicating that HMGB-1 release does not correlate with a-granule secretion.

Intriguingly, under stimulation conditions that led to substantial microparticle release, HMGB-1 appeared to be restricted to the platelets and excluded from the microparticle surface. This contrasts with CD62P, which was found on surface of platelets and micro particles.

Conclusions: In summary, strong platelet activation leads to increased HMGB-1 surface expression. However, the mechanism of this does not directly correlate with platelet a-granule secretion. The apparent exclusion of HMGB-1 from micro particles suggests that its surface exposure is regulated in a complex manner.

PB 1362 | Platelets Enhance Distinct Dynamics of Effector Cell Responses of Naive and Effector Memory CD4$^+$ T cells

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Background: Platelets exert a bi-phasic regulation on effector cell responses of the total CD4$^+$ T cells, but little is known on their impact on naïve and effector memory CD4$^+$ T cells.

Aims: To investigate how platelets differentially regulate effector cell responses of naïve and effector memory CD4$^+$ T cells.

Methods: Human naïve and effector memory CD4$^+$ T cells were challenged with CD3/CD28 MAbs, and cultured without or with platelets (ratio at 1:250) during 7 days. CD4$^+$ T effector cell responses were assessed by Th1, Th2, Th17 and Treg cell phenotyping on day 0, 1, 3, 5, and 7.

Results: T cell receptor (TCR)-challenge evoked marked CD4$^+$ T cell activation and proliferation, seen as increases of cell numbers and T cell aggregate formation from day 3 on. Platelet co-culture did not alter cell proliferation but markedly enhanced cell activation on day 5 and 7, as evidenced by increased T cell aggregate sizes of both subsets. In naïve CD4$^+$ T cells, TCR stimulation mildly enhanced Th1 cell activation, which increased Th1 phenotype from 0.3±0.1% at basal to 2,2±0.7% on day 5. Platelet co-culture markedly enhanced the response, and elevated Th1 to 9.3±3.4%. Platelets accelerated TCR-stimulated Treg activation, and increase Treg from 8.3±2.2% to 33.9%±10.9% on day 5. In effector memory CD4$^+$ T cells, TCR stimulation evoked rapid effector cell responses. Thus, Th1 cells were already evelated to 7.9±5.1%, and further enhanced by platelets to 10.7±5.7% on day 1. Treg cells were also elevated quickly, at 4.5±2.3% on day 1 and with marked enhancements by platelets on day 3 and 5. Neutralization of transforming growth factor 1b (TGF1b) diminished platelet-enhanced Treg activation, but potentiated Th1 responses. TCR stimulation induced only mild increases of Th2 and Th17 responses in the present settings.

Conclusions: Platelets enhance delayed effector cell responses of naïve CD4$^+$ T cells, but promote rapid effector cell responses of effector memory CD4$^+$ T cells.

PB 1363 | The Staphylococcal Toxin Panton-Valentine Leukocidin (PVL) Induces Neutrophil Extracellular Trap (NET) Formation that Leads to Platelet Activation

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Background: Expression of the toxin PVL by *Staphylococcus aureus* has been linked to thrombosis in association with osteomyelitis especially in young immunocompetent patients.

Aims: We therefore tested the effect of PVL on human platelets and neutrophils.

Methods: The effect of recombinant PVL on platelet activation and microparticle formation in the presence or absence of autologous neutrophils was measured by flow cytometry. PVL induced lysis of neutrophils was assessed by propidium iodide. ELISA detected release of neutrophil myeloperoxidase and defensins. NET formation was analyzed by a fluorogenic assay using the fluorescent dye Syto13 binding to nucleic acids, a sandwich ELISA against histone-DNA complexes and by microscopy. The influence of known inhibitors of alpha defensin and of FDP-lysine carrying proteins induced platelet activation was studied.

Results: The toxin PVL strongly induced platelet activation, but only in the presence of human neutrophils**.** Labelled PVL *subunit* S (LukS) bound to the neutrophil surface, but not to platelets. Complete PVL induced neutrophil lysis and NET formation as well as the release of alpha defensins and myeloperoxidase, known mediators of platelet activation. Neutrophil NETs were decorated with alpha defensin 1-3 and stained for FDP-lysine. PVL-induced platelet activation in the presence of neutrophils was inhibited by known defensin inhibitors as well as by the antioxidants resveratrol and glutathione (GSH). PVL actions were blocked by anti-PVL-antibodies in the plasma of some adult blood donors.

Conclusions: Our data provide one possible explanation how thrombosis and osteomyelitis are connected in PVL-*S. aureus* infections and why especially young osteomyelitis patients with a presumably low antibody titer against PVL suffer more from this complication than adults. As the mechanism described might be a more general one, other bacterial toxins are under further investigation.

PB 1364 | Platelets Play a Protective Role in the Host Defense against *Burkholderia pseudomallei* (Melioidosis)

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Background: Melioidosis, caused by the gram-negative bacterium *Burkholderia pseudomallei*, is an important cause of community-acquired pneumonia and sepsis in Southeast Asia with a mortality of up to 40%. So far nothing is known about the role of platelets in this disease.

Aims: To assess the role platelets in the host response during *B. pseudomallei* infection.

Methods: Platelet counts were determined in 34 patients with culture-proven melioidosis and 52 controls. Mice treated with a low or high dose platelet depleting antibody or IgG control were infected via the airways with *B. pseudomallei* and sacrificed at several time-points. In addition, we investigated the role of GPIb, the Von Willebrand factor receptor. IL4-R/Ibα mice (which lack mouse GPIbα without the associated macrothrombocytopenia) and WT mice were infected with *B. pseudomallei*.

Results: Clinical melioidosis was associated with significant thrombocytopenia. Low platelet counts strongly associated with mortality. Murine melioidosis was also characterized by thrombocytopenia. Platelet depletion towards a level of either < 5% or < 1% of normal resulted in increased bacterial counts at the primary site of infection with increased dissemination towards the liver. Platelet depletion also increased inflammatory responses, but pulmonary neutrophils influx was reduced. Platelets are important for the formation of neutrophil extracellular traps (NETs). In the lungs of infected mice, NET formation however did not differ between groups. Mice lacking GPIbα showed decreased platelet-neutrophil complex formation, but also lower platelet counts. GPIbα deficient mice showed increased bacterial growth in the lung, but not to a similar extent as platelet depleted mice.

Conclusions: Admission thrombocytopenia is associated with enhanced mortality in melioidosis patients. During experimental melioidosis, platelets play a protective role in the host defense, possibly in part via GPIb and neutrophil interactions.

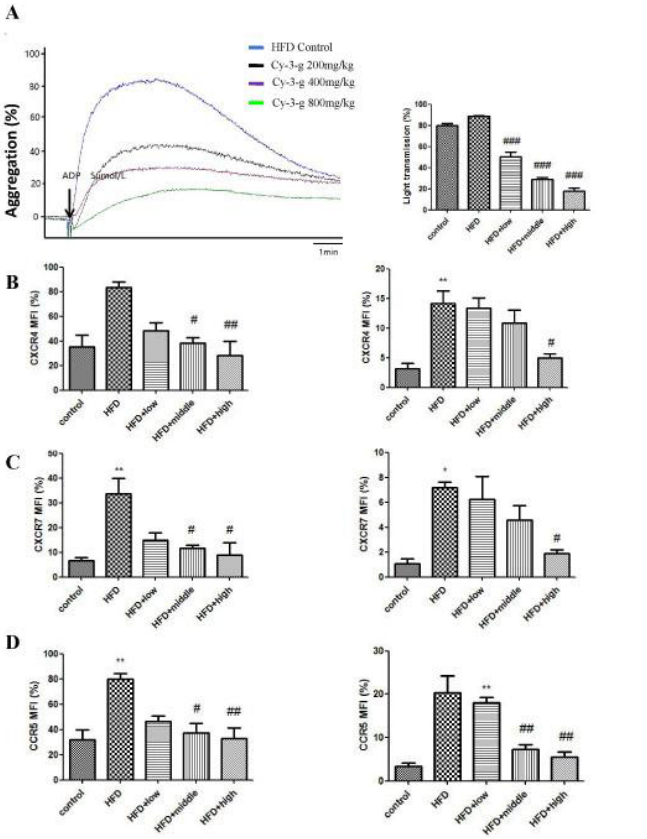

Figure 1 The effects of Cy-3-g on platelet aggregation and chemokine receptors in ApoE-/- mice. Platelet aggregation of PRP stimulated by 5 μmol/L ADP (A). The surface expressions of CXCR4, CXCR7, CCR5 on leukocytes and platelets via flow cytometry (B, C and D). Values are mean ± SD, n = 5 per group. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, as compared to the HFD group. * $P<0.05$, ** $P<0.01$, as compared to the control.

FIGURE 1 The effects of Cy-3-g on platelet aggregation and chemokine receptors in ApoE-/- mice

PB 1365 | Plant Food Cyanidin-3-glucoside Attenuates Atherosclerosis via Inhibiting Platelet-derived Chemokines and Chemokine Receptors in ApoE-/- Mice

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Background: Platelet-derived chemokines play crucial roles in atherosclerosis (AS). Plant food anthocyanin, the main bioactive component of which is cyanidin-3-glucoside (Cy-3-g), possesses anti-platelet effects and cardiovascular protection. Our previous study has shown the inhibitory effects of anthocyanins on platelet chemokines in hypercholesterolemic individuals, however, the effects of Cy-3-g on platelet chemokines in AS need to be clarified.

TABLE 1 Effects of Cy-3-g on plasma levels of platelet chemokine in ApoE-/- mice

	Control	HFD	HFD+ 200mg/kg Cy-3-g	HFD+ 400mg/kg Cy-3-g	HFD+ 800mg/kg Cy-3-g	P value
CXCL4 (ug/mL)	3.109 ± 0.645	9.651 ± 5.516	5.644 ± 3.822	3.219 ± 0.866	2.636 ± 0.253	0.002
CXCL7 (pg/mL)	486.758 ± 71.777	1370.800 ± 122.398	1206.467 ± 135.415	892.383 ±39.183	712.342 ± 102.630	<0.001
CCL5 (pg/mL)	6.262 ± 0.498	14.42 8± 6.957	9.920 ± 3.006	7.630 ± 2.392	5.170 ± 1.211	0.001
CXCL5 (pg/mL)	291.111 ± 53.527	4530.370 ± 1666.098	1460.926 ± 623.991	760.834 ±310.050	239.722 ± 194.655	<0.001
CXCL12 (pg/mL)	2.734 ± 0.050	3.573 ± 0.283	3.104 ± 0.058	3.010 ± 0.050	2.882 ± 0.054	<0.001
CCL2 (pg/mL)	477.444 ± 187.540	3489.279 ± 1780.539	2056.729 ± 1418.911	1042.118 ± 308.976	624.113 ± 258.365	0.001

The data are expressed as the mean ± SD (n=7-9). HFD: high fat diet. All P values were calculated by Student´s t-test.

Aims: To study the inhibitory effects of Cy-3-g on platelet chemokines and chemokine receptors in ApoE-/- mice.

Methods: ApoE-/- mice (6 week old, male) were fed a high-fat diet (HFD; 21% fat, 0.15% cholesterol) that mixed with different doses of Cy-3-g (0, 200, 400 and 800 mg/kg) for 16 weeks. The control were fed with a normal diet. Arteries were separated to determine plaque histology by Oil-Red-O dying. Blood routine were tested by Multispecies Hematology Systems. Platelet aggregation was observed by aggregometer. The levels of serum lipid and platelet chemokines were detected by test or ELISA kits. The expressions of chemokine receptors CXCR4, CXCR7 and CCR5 were analyzed via flow cytometry.

Results: Compared with HFD group, the plasma levels of TG, T-CHO, LDL were inhibited by 800 mg/kg Cy-3-g, while HDL increased markedly. The plasma levels of CXCL4, CXCL5, CXCL7, CXCL12, CCL2, CCL5 were significantly reduced (all $P < 0.002$, Tab. 1). Platelet aggregation (all Cy-3-g groups) and WBC counts (400 and 800 mg/kg groups) were inhibited. The expressions of CXCR4, CXCR7, CCR5 on leukocytes, CCR5 on platelets were attenuated in 400 and 800 mg/kg groups, and obvious inhibition of CXCR4, CXCR7 on platelets in 800 mg/kg group (Fig. 1).

Conclusions: Cy-3-g regulates platelet activities via inhibiting platelet chemokines and chemokine receptors in ApoE-/- mice. Dietary intake of Cy-3-g may have protective effects against the development of AS, and further studies are required to investigate the underlying mechanisms.

PB 1366 | Platelets Promote Immunosuppression and Colorectal Tumor Formation: Inhibition by Clopidogrel

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Background: Clinical and preclinical data strongly suggest that antiplatelet drugs may mitigate colorectal cancer development and improve outcome.

Aims: To investigate the mechanisms of clopidogrel-mediated inhibition of colitis-associated carcinogenesis in mice.

Methods: Colorectal cancer was induced in mice upon treatment with azoxymethane/dextran sulphate sodium (AOM/DSS) for 65 days. Clopidogrel (30 mg/kg/day) was administered continuously via the drinking water. Inflammatory score, dysplasia and adenocarcinoma formation were analysed. Changes in blood cell counts, platelet activation and reactivity were monitored throughout the protocol. Colon infiltration by myeloid and T cells were analysed by immunohistochemistry. Phenotype and immunosuppressive activity of splenic myeloid cells were analysed by RT-qPCR and mixed lymphocyte reaction assays.

Results: Clopidogrel efficiently inhibited cancer-elicited platelet activation and limited diarrhea without inducing intestinal bleeding. It significantly reduced dysplasia and the numbers of adenocarcinoma. It prevented the accumulation of immunosuppressive myeloid cells in tumors, while the numbers of anti-tumor cytotoxic T cells were augmented in dysplasic areas. Interestingly, in blood, clopidogrel reduced the appearance of tumor-induced low-density neutrophils, and it decreased the levels of platelet-neutrophil aggregates. Myeloid cells isolated from spleen of clopidogrel-treated mice failed to inhibit T cell proliferation *ex vivo*, and displayed reduced mRNA expression of immunosuppressive markers, TGF-β, C/EBP β and arginase-1. Platelets isolated from tumor-bearing mice promoted immunosuppression by immature myeloid cells, and were able to annihilate the inhibition of their activity by clopidogrel.

Conclusions: The antiplatelet drug clopidogrel may inhibit colitis-induced carcinogenesis by affecting platelet-dependent regulation of immunosuppressive myeloid cell function, thereby improving antitumor immunity.

PB 1367 | Interplay between Platelets, Neutrophils and Coagulation in Bleeding in a Mouse Model of Inflammatory Bowel Disease

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Background: Inflammatory bowel diseases (IBD) are associated with a persistently elevated risk of thrombosis. Because IBD patients are also at high risk of major gastrointestinal bleeding, thromboprophylaxis management of these conditions remains a challenge. Current recommendations include the use of heparins. There is no data on direct oral anticoagulants. A better understanding of the mechanisms underlying inflammatory bleeding could help to develop tailored antithrombotic approaches. In mouse models of inflammation, it has been reported that platelets and neutrophils are involved in the maintainance and in the loss of vascular integrity.

Aims: To investigate the respective contribution of platelets, platelet receptors, neutrophils, and coagulation to intestinal bleeding in IBD

Methods: We used a mouse model of IBD induced by oral administration of dextran sodium sulfate for 7 days. The effects of platelet or neutrophil-depleting antibodies, clopidogrel (30mg/kg/day), GPVI depletion, P2X1 deficiency, and of rivaroxaban (10mg/kg/day) were compared on the following parameters: disease activity index (diarrhea, bleeding, weight loss), differential blood cell counts, and hematocrit. Histopathology and immunohistochemistry analyses were performed.

Results: Platelet depletion, P2X1 deficiency, or treatment with rivaroxaban provoked intestinal bleeding that resulted in hematocrit drop, which did not occur in control colitic mice. This drop in hematocrit was inversely correlated with levels of neutrophil infiltration in colon. Rivaroxaban treatment and P2X1 deficiency both worsened tissue

damage and increased neutrophil-to-lymphocyte ratio in blood. In agreement with a role for neutrophils in bleeding, neutrophil depletion limited blood loss in P2X1-deficient mice. Neither GPVI depletion nor clopidogrel treatment aggravated bleeding.

Conclusions: Neutrophils, platelets, and P2X1 receptors intervene in anticoagulant-elicited bleeding of the inflamed intestine.

PB 1368 | Platelet-leukocyte Interactions after Major Trauma Primarily Involve Platelet-derived Microvesicles and Are More Frequent in Patients with Poor Clinical Outcomes

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Background: Platelets play important roles in both coagulation and inflammation, but their contribution to these processes in trauma patients is poorly understood.

Aims: To characterise platelet-leukocyte interactions in injured patients and to determine whether these are associated with adverse outcomes.

Methods: Patients meeting criteria for the highest tier of trauma team activation were included and had blood drawn within two hours of injury. Healthy volunteers acted as a control group. Platelet interactions with leukocytes in whole blood were scored from imaging flow cytometry by a blinded assessor, and recorded as a proportion of the number of neutrophils, monocytes and T-lymphocytes. Platelet-derived microvesicles (PMV) were defined as CD61-positive material measuring < 1μm on fluorescence imaging.

Results: In trauma patients (n=25) PMV attached to leukocytes were more common than whole platelet-leukocyte aggregates among monocytes, neutrophils and T-cells ($p< 0.01$ for each group). Within leukocytes, PMV were more common on monocytes (39±8% of the population) than on neutrophils (23±7%, $p=0.02$) or T-cells (10±3%, $p< 0.001$). The frequency of whole platelet-monocyte aggregates was similar in trauma patients (14±5%) and healthy volunteers (11±3%, $p=0.26$). In contrast, PMV-monocyte aggregates were markedly elevated in the trauma population (39±8%) compared to volunteers (4±3%, $p< 0.001$). Significantly more PMV-monocyte aggregates were found in patients who developed multiple organ dysfunction (MOD) or died within 48 hours (49±10%) than in those who had uncomplicated outcomes (20±9%, $p=0.004$). A similar pattern was observed with PMV-neutrophil aggregates (MOD/death: 33±10% vs no MOD: 13±6%, $p=0.01$), although aggregates with T-cells occurred at a similar frequency irrespective of outcome.

Conclusions: Major injury induces widespread platelet-leukocyte interactions which principally involve PMV and monocytes. The functional consequences of PMV-leukocyte aggregation in trauma patients require further investigation.

PB 1369 | Complete Attenuation of PrP(106-126)-mediated Pathological Responses by Flufenamic Acid in Platelets

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Background: PrP(106-126) is amyloidogenic amino acid sequence released from prion proteins in Prion disease. As PrP is expressed in blood following leakage from brain tissue, PrP(106-126) exerts its biological effects. PrP(106-126) shows a rise in intracellular calcium, $[Ca^{2+}]i$, in platelets which is attributable to influx from extracellular fluid. Calcium mobilization then stimulates calpain that partially cleaves cytoskeleton-associated protein talin and extensive shedding of microparticles. As surface of platelet-derived microparticles are enriched with phosphatidylserine and hence are pro-coagulant, exposure to prion favoures thrombogenic state.

Aims: As TRPC proteins reside in platelets, the present study aims to investigate their involvement in prion-induced pathology and therapeutic effect of flufenamic acid (FFA).

Methods: Platelets were isolated from human blood. Aggregation study, measurement of $[Ca^{2+}]_i$, confocal microscopy, flow cytometry and western blot analysis were done.

Results: Addition of PrP(106-126) (20 μM) to platelet suspension under stirring condition at 37°C in aggregometer elicited a rise in light-transmittance to 75±8%. Platelets preincubated with 1 mM $CaCl_2$ following treatment with PrP(106-126) showed 30-fold rise in $[Ca^{2+}]_i$. Fura-2 loaded cells treated with 10 μM Hyp9 in presence of 1 mM calcium demonstrated 7-fold rise in $[Ca^{2+}]_i$. Remarkably, PrP(106-126)-induced rise in $[Ca^{2+}]_i$ was completely prevented by preincubation of cells with 100 μM FFA, an inhibitor of TRPC isoforms except TRPC6, which was suggestive of involvement of non-TRPC6 isoform in PrP(106-126)-mediated Ca^{2+} entry.

Conclusions: Remarkably, when freshly prepared platelets were preincubated with FFA, PrP(106-126)-induced rise of $[Ca^{2+}]_i$ was almost precluded. Our observation, therefore, was strongly indicative of significant role of different TRPC isoforms (except TRPC6). Hence it is tempting to suggest that FFA, an anti-inflammatory drug, is effective therapy against prion-mediated pathological responses.

PB 1370 | Platelet Functional Activity Is Altered in Multiple Sclerosis

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Background: Multiple sclerosis (MS), an inflammatory autoimmune disease of the central nervous system, is a main cause of disability in

young adults. In the last decade, the role of platelets in MS pathology came into focus of research as platelets get activated by inflammatory compounds and can modulate inflammation themselves. Langer *et. al.* proved the presence of platelets in chronic active MS lesions and could show that blocking platelets GP IIb/IIIa reduces experimental autoimmune encephalomyelitis, the mouse model of MS.

Aims: To understand more about the role of platelets in MS we studied platelet functional activity in patients with different stages of MS.

Methods: In this ongoing study, the response of platelets from so far 46 patients suffering from MS in different stages of disease and healthy controls, to different platelet agonists was analyzed by flow cytometry. Different markers for platelet activation as fibrinogen binding, microparticle formation and platelet-lymphocyte association, as well as granule secretion were measured.

Results: Platelets from patients suffering from MS responded less to collagen, ADP, epinephrine and arachidonic acid in an apparently disease state dependent way than control platelets. The platelets of the four patients with an acute relapse did not respond to epinephrine at all. The response to stimulation with thrombin did not differ between MS patients and healthy controls.

Conclusions: These results indicate an *in vivo* preactivation with several platelet agonists leading to an exhaustion of platelet response to these agonists. As the platelet response to stimulation with thrombin is still normal, it is unlikely that increased thrombin formation causes such a preactivation. Probably reactions linked to inflammatory processes during the progression of disease might be the cause of preactivated platelets in MS. Platelets respond to inflammation and can mediate inflammation wherefore they may be a player in the inflammatory processes underlying the primary pathology of MS.

PB 1371 | Contributions of von Willebrand Factor to the Development of Angiotensin II-induced Vascular Inflammation

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Background: We recently uncovered a coagulation-inflammation circuit that promotes vascular dysfunction through factor XI (FXI)-thrombin amplification loop highlighting the possible utility of FXI-targeted anticoagulant in treating hypertension. The role of von Willebrand Factor (vWF) and its interaction with platelets during adhesion to the vascular endothelium in the proinflammatory context of hypertension is unknown.

Aims: We explored the role vWF in the development of angiotensin II (AngII)-induced vascular dysfunction.

Methods: The role of vWF was explored in mice with human GPIbα replacing the endogenous homologue (hGPIbα) mice) compared to mouse GPIbα and mice infused with AngII (1 mg× $kg^{-1\times}$ d^{-1} for 7 days using osmotic minipumps). hGPIbα mice have a prolonged tail bleeding time, explained by the decreased mouse vWF A1 domain affinity for human as compared to mouse GPIbα.

Results: Interestingly, hGPIbα mice were protected from AngII-induced endothelial dysfunction, vascular inflammation (as assessed by *Ccl2*, *Vcam-1* and *Ly6c* mRNA upregulation) and oxidative stress, but infusing human vWF restored the full response to AngII treatment. To delineate whether a normal vWF-GPIbα interaction also influenced the FXI-thrombin loop, we assessed the endogenous thrombin potential (ETP) in PRP of hGPIbα mice infused or not with hvWF. After AngII infusion the ETP did not increase in untreated hGPIbα mice, but it increased in those pretreated with hvWF to the same extent as control C57BL/6.

Conclusions: We showed here that the presence of vWF as GPIbα ligand is required for localized thrombin generation and is crucial in the development of AngII-induced vascular dysfunction.

PB 1372 | Platelets Are Effectors of Articular Degradation in Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease causing progressive articular destruction. Activated platelets and platelet-derived microparticles (PMPs) have been found in synovial fluid (SF) of RA patients. However, the mechanism by which platelets reach SF, get activated and whether they contribute to joint damage have not been investigated yet. Matrix metalloproteinases (MMPs) are primary drivers of cartilage and bone degradation in RA. Platelets contain and release several MMPs (e.g. MMP-2) and trigger MMP-synthesis by other cells.

Aims: To assess platelet contribution to articular degradation in RA exploring the hypothesis that platelets are actively recruited in the arthritic joint where they induce an increased release of MMP-2.

Methods: Knee joint SF was collected from 47 patients with RA and 49 patients with osteoarthritis (OA): platelet and PMPs count, platelet P-selectin and heterotypic platelet aggregates were measured by flow-cytometry, and MMP-2 levels by zymography. Synovial fibroblasts (FLS) were cocultured with platelets and MMP-2 level in cell-free supernatants was measured. Platelet migration towards RA SF was assessed using the *in vitro* transwell assay. The role of platelet MMP-2 in joint degradation was tested in a mouse model of collagen-antibody induced arthritis.

Results: SF from RA patients contains significantly more activated platelets and MMP-2 compared with SF from OA. RA SF exerts a strong significant chemotactic and activating effect on platelets. Platelet-FLS coculture induces platelet activation. Activated platelet-released MMP-2 acts on FLS proteinase-activated receptor 1 (PAR1) inducing MMP-2 release by FLS. Selective depletion of platelet MMP-2 strikingly reduces cartilage degradation and articular platelet recruitment in murine arthritis.

Conclusions: Platelets are effectors of joint destruction in RA by enhancing MMP-2 generation in the synovial space. The blockade of platelet penetration and/or activation in SF may represent a new therapeutic target in RA.

PB 1373 | Procoagulant Extracellular Vesicles Impair Trophoblast Function by a Thrombo-inflammatory Pathway

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Background: Procoagulant extracellular vesicles (EVs) and platelet activation have been associated with pregnancy complications. We have recently identified a thrombo-inflammatory pathway inducing a pre-eclampsia (PE) like phenotype. EVs induce platelet mediated placental inflammasome activation. Whether this thrombo-inflammatory pathway alters trophoblast differentiation and function remains unknown. **Aims:** To identify whether the EV induced thrombo-inflammatory pathway modulates trophoblast proliferation, differentiation, and invasion. **Methods:** We injected C57BL/6 mice with endothelial or platelet-derived EVs to study the role of *EVs in vivo*. We exposed human and mouse trophoblast cells to EVs and platelets to study their role *in vitro*. Trophoblast proliferation was studied using Ki-67 immunostaining and BrdU incorporation assays. RT-PCR for trophoblast differentiation marker genes (PL-II, Tpbpa, Gcm1) was done to study trophoblast differentiation. Matrigel based tube formation assays were used to assess trophoblast invasion. **Results:** Injection of EVs into pregnant mice results in platelet activation and accumulation of activated platelets in the placenta. EV treatment impaired trophoblast differentiation and proliferation both *in vitro* and *in vivo*. These effects were platelet dependent as platelet depletion and genetic (NFE2-/-, Gαa-/-) or pharmaceutical (Apyrase) platelet inhibition abolished these effects. EV treatment resulted in impaired trophoblast tube formation indicating impaired trophoblast invasion. Furthermore, EVs induced inflammasome activation in trophoblast cells, and NLRP3 or caspase-1 deficiency or IL-1 receptor antagonist (Anakinra) abolished these effects of EVs.

Conclusions: These results demonstrate that EV-mediated platelet activation impairs trophoblast function by reducing trophoblast proliferation, differentiation and invasion through activation of the inflammasome. These results support the pathophysiological relevance of enhanced maternal platelet activation at the feto-maternal interface.

PB 1374 | Procoagulant Platelets as a Biomarker in Suspected Sepsis - A Pilot Study

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Background: Sepsis is associated with significant morbidity and mortality. Platelets play a pivotal role in sepsis pathogenesis; however, the procoagulant platelet (PP) subset has not been extensively evaluated.

TABLE 1 Patient demographics

Number of patients, n	12
Age, median (range)	64.5 (46.5-78.0)
Gender (male), n (%)	6 (50)
Sequential Organ Failure Assessment (SOFA), median (range)	1.5 (0.5-3.0)
Sepsis (SOFA>2.0), % (95% CI)	50 (25.4-74.6)
Mortality in ED Sepsis (MEDS), median (range)	7 (2.5-11.5)
Charlson Comorbidity Score (CCS), median (range)	3.5 (2.0-5.0)
Platelet count x 10^9/L, median (range)	245 (204-316)
Hospital length of stay (LOS), days, median (range)	6 (1-12)
Death/intensive care unit (ICU) admission, % (95% CI)	41.7 (19.3-68.1)

TABLE 2 Correlation coefficients (CC). * denotes significant correlation

Variables	MEDS	CCS	Platelet Count	PP - Thrombin (0.5 U/mL)	PP - Thrombin (0.5 U/mL) + Collagen (10 µg/mL)	PP - Thrombin (2 U/mL)	PP - Thrombin (2 U/mL) + Collagen (10 µg/mL)
SOFA, CC (p-value)	-	-	-0.02 (0.94)	-0.08 (0.81)	0.57* (<0.05)	0.52 (0.08)	0.59* (0.04)
MEDS, CC (p-value)	-	-	0.45 (0.14)	0.53 (0.08)	0.58* (<0.05)	0.43 (0.16)	0.57* (<0.05)
Hospital LOS, CC (p-value)	0.7* (0.01)	0.5 (0.09)	-0.06 (0.85)	0.33 (0.3)	0.44 (0.15)	0.37 (0.24)	0.69* (0.01)
Death/ICU admission, CC (p-value)	0.38 (0.23)	0.43 (0.17)	0.05 (0.89)	0.15 (0.64)	0.42 (0.18)	0.58* (<0.05)	0.5 (0.1)
Platelet count, CC (p-value)	-	-	-	0.21 (0.52)	0.04 (0.9)	0.07 (0.84)	0.04 (0.9)

Aims: To explore the utility of PP subset as a biomarker in suspected sepsis.

Methods: We adapted a flow cytometry assay based on novel cell death agent, GSAO (PMID 26474813), to measure PP formation ex vivo in whole blood. Blood from patients presenting to Emergency Dept (ED) with suspected sepsis (inclusion criteria per PMID 27073105) was incubated with low (0.5 U/mL) or high (2 U/mL) dose thrombin ± collagen (10 μg/mL) and assayed by FACS. PP subset was defined as GSAO+/CD62P+ and expressed as % all platelets. Outcome measures included severity scores (SOFA, MEDS, CCS), hospital LOS, and a composite of death/ICU admission. Correlation between PP subset and outcomes was examined by Kendal's Tau method and multiple regression analyses.

Results: Demographics and outcome measures for 12 evaluable patients are summarized in Table 1. Correlation coefficients (CC) for PP against outcomes are summarized in Table 2. Thrombin plus collagen-induced PP subsets showed moderate correlation with sepsis severity scores. MEDS and high-dose thrombin plus collagen response were equally correlated with hospital LOS; however, only the association with MEDS remained significant in multiple regression model (p=0.01). In contrast, high-dose thrombin PP response was superior to MEDS and CCS with respect to death/ICU admission in logistic regression analysis (OR 1.22, p=0.03) with a high area under ROC of 0.86 (95% CI 0.54-0.99, p=0.03).

Conclusions: PP subset in whole blood showed moderate association with sepsis severity scores and LOS in an ED cohort of patients with suspected sepsis. The thrombin-induced PP subset was superior to the more complex MEDS with respect to death/ICU admission outcome. These findings support further evaluation of PP subset as a biomarker and may lead to development of improved sepsis severity scores.

PB 1375 | Contraction of Blood Clots is Promoted by Tissue Factor-expressing Activated Monocytes

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Background: Platelet-driven reduction in blood clot volume (clot contraction or retraction) has been implicated to play a role in hemostasis and thrombosis. Although these processes are often linked with inflammation, the role of inflammatory cells in contraction of blood clots and thrombi has not been investigated.

Aims: The aim of this work was to study the influence of activated monocytes on clot contraction.

Methods: Clotting of whole citrated blood and clot contraction were initiated *in vitro* with the addition of 2 mM $CaCl_2$ and 1 U/ml human thrombin. Quantitative optical tracking was used to follow volume changes in blood clots with time. Monocytes were isolated from blood and activated with phorbol-12-myristate-13-acetate (PMA) to induce tissue factor (TF) expression. Untreated or PMA-treated monocytes were added back into citrated whole blood. To inhibit TF expression by blocking of signaling pathways, isolated monocytes were incubated with PPARα prior to addition of PMA, whereas anti-TF IgG was added following activation of cells with PMA to block the procoagulant activity of TF. TF expression was assessed using flow cytometry.

Results: When a physiological number of isolated human monocytes pre-activated with PMA were added back into whole blood, the extent and rate of clot contraction were increased compared to addition of non-activated cells. Inhibition of TF expression or its inactivation on PMA-treated monocytes reduced the extent and rate of clot contraction back to control levels with non-activated monocytes. The addition of TF to whole blood, which was used to mimic the TF expression on PMA-activated monocytes, caused a dose-dependent increase in the extent and rate of clot contraction; this effect was abrogated in the presence of anti-TF antibodies.

Conclusions: These data suggest that the activated inflammatory cells through expression of tissue factor can directly affect hemostasis and thrombosis by modulating the size of intra- and extravascular clots and thrombi.

PB 1376 | Different Cytokine and Chemokine Patterns in Pediatric Patients with Acute and Chronic Immune Thrombocytopenia (ITP) Compared with Healthy Children

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Background: Primary ITP is an autoimmune disease, mainly caused by autoantibodies against platelet surface receptors, leading to platelet clearance by macrophages. Some studies showed that also autoreactive B cells and $CD4^+$ T-helper (Th) cells, and specifically their cytokines, are associated with ITP. Cytokines and chemokines are known to be important players in the immune response.

Aims: To study the interplay between the immune system and the reduction of platelet count in ITP by screening the different cytokine/chemokine pattern in ITP patients vs controls.

Methods: Informed consent was obtained from patients and controls, and the study was approved by the Hospital Ethics Board. To measure plasma concentrations in chronic and acute ITP patient groups vs healthy controls, we used Luminex technology and analyzed 42 cytokines/chemokines in total.

Results: Significantly increased levels of TNF-α ($p< 0.05$), as well as Eotaxin ($p< 0.05$), IP-10 ($p< 0.05$), MCP-1 ($p< 0.05$) were identified in acute ITP patients compared to chronic ITP patients. The cytokines MDC ($p< 0.05$) and TNF-α ($p< 0.05$) were significantly increased in acute ITP patients as compared to healthy children. There were no significant differences between chronic ITP and healthy children.

Conclusions: Increased plasma levels of the cytokines and chemokines TNF-α, Eotaxin, IP-10, MCP-1 and MDC were observed in ITP patients at initial presentation, suggesting that these cytokines contribute to the pathogenesis of the disease. It is well known these cytokines, TNF- α, IP-10, MCP-1, Eotaxin, are key players in mediating acute inflammatory response by activation of neutrophils and monocytes, at sites of inflammation. Higher plasma levels in acute ITP patients are signs for an acute inflammatory response, consistent with the current model of ITP, in which activated macrophages induce B and T cells to produce anti-platelet autoantibodies.

PB 1377 | Therapeutics of Multiple Ssclerosis, Eexcept of Gglatiramer Acetate, Are Unlikely to Alter Platelet Functional Activity

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Background: Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS). To date MS is remediless but there are numerous therapeutics that help to improve clinical outcome and health-related quality of life. MS patients often show platelet abnormalities like thrombocytopenia along with well-known side effects of subcutaneous applied drugs (e.g. thrombosis and skin lesions) which might indicate a modulating effect of MS therapeutics on platelet functional activity. Starossom *et. al.* showed altered thrombin induced platelet activity after treatment with glatiramer acetate (GA) but not with interferon-beta.

Aims: To elucidate the underlying effects of MS therapeutics we studied the impact of different compounds on ADP and collagen stimulated platelet functional activity.

Methods: The MS therapeutics humanized antibodies alemtuzumab, natalizumab and daclizumab, two interferon beta-1a compunds, glatiramer acetate, and teriflunomide were used. The functional activity of platelets from healthy donors pretreated *in vitro* with MS therapeutics was analyzed by flow cytometry. Additionally the endogenous thrombin potential (eTP) and the lag-time of thrombin formation in plasma prepared from blood preincubated with MS drugs were measured.

Results: Platelets pretreated with GA showed diminished response to stimulation with ADP and collagen. In addition the eTP was reduced in comparison to controls. The lag-time of thrombin formation was increased in GA treated plasma. Responsiveness of platelets pretreated with the other analyzed drugs was normal compared to controls. In addition, eTP and lag-time were unaltered.

Conclusions: The functional activity of platelets was altered by GA but not with interferon-beta or other studied MS therapeutics. These findings indicate that MS compounds except of GA are unlikely to cause differences in platelet functional activity in MS.

PB 1378 | Anticoagulant and Anti-inflammatory Properties of the Novel Antimicrobial Peptide Centrocin 1 from Green Sea Urchin

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Background: Antimicrobial peptides (AMPs) play a pivotal role in the immune system. In addition to their role as bactericidal agents, AMPs are important contributors to the inflammatory response. In the present study we have explored the effect of Centrocin 1, an AMP peptide from the green sea urchin *Strongylocentrotus droebachiensis*, on the expression of LPS-induced tissue factor (TF) and cytokines in monocytes as well as on platelet activation in whole blood.

Aims: To study the biological functions of Centrocin 1 using an ex-vivo whole blood model stimulated with LPS.

Methods: Blood was collected in Fragmin (10 U/ml). Aliquots were immediately stimulated with LPS (5 ng/ml) for 2 hrs at 37 °C with constant agitation at 37 °C in the presence or absence of Centrocin 1. After the incubation period, reactions were terminated by adding EDTA to a final concentration of 5 nM. TF activity was measured in isolated frozen and thawed mononuclear cells, and plasma samples were studied both at the transcriptional and translational level.

Results: Centrocin 1, at concentrations above 2 ug/ml, reduced LPS-induced TF activity of monocytes in whole blood in a dose -dependent manner (max 35 %, $p < 0.05$) and TF mRNA (max 30%, n.s.). Similarly, LPS-induced TNFα secreted to plasma was reduced at peptide concentrations above 0.05 ug/ml (max 34%, $p < 0.05$) at the same time as TNFα mRNA was reduced by peptide concentrations above 2 ug/ml (max 34%, $p < 0.05$).

A combination of flow cytometry and cell counting analysis of LPS-stimulated blood, incubated with and without Centrocin 1, revealed an increase in the number of free platelets compared to the control. However, no aggregates of monocytes and platelets were found to be markedly affected by Centrocin 1.

Conclusions: Centrocin 1 has significant anti-inflammatory as well as anti-coagulant properties. This is demonstrated in an ex-vivo whole blood model system, showing a dose-dependent inhibition of both selected anti-inflammatory markers as well as LPS-induced TF in monocytes.

PB 1379 | Deficiency of Sirtuin 3 Elevates Mitochondrial ROS in Neutrophils and Platelets but Does not Exacerbate NETosis or Venous Thrombosis

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Background: Inflammation is a common denominator in chronic diseases of aging. Yet, how inflammation fuels these diseases remains unknown. Neutrophil extracellular traps (NETs), decondensed chromatin released by neutrophils, have been shown to induce tissue injury and thrombosis.

Aims: To uncover the link between thrombosis, whose occurrence increases with aging, and age-related alterations in neutrophil and platelet function, it is necessary to investigate the role of aging-related genes such as Sirtuin 3 (Sirt3).

Methods: Expression of Sirt3 in neutrophils and platelets was shown by western blotting. Intracellular ROS levels were evaluated by flow cytometry with or without stimulation in the presence of ROS indicators. NET formation was analyzed after stimulation of neutrophils with PMA or ionomycin. Platelet aggregation induced by collagen, ADP and thrombin was analyzed by aggregometry. Venous thrombosis was evaluated by inferior vena cava stenosis for 3 hours.

Results: Both mouse platelets and neutrophils express Sirt3. More mitochondrial ROS were generated in platelets and neutrophils of Sirt3-/- mice compared to WT, when stimulated with a low concentration of PMA and a high concentration of thrombin, respectively. There were no differences in NETosis, with or without stimulation. Platelet aggregation was mildly augmented in Sirt3-/- mice compared to WT mice, when stimulated with a low concentration of collagen. No differences were observed in venous thrombus formation after inferior vena cava stenosis between WT and Sirt3-/- mice. However, we observed a significantly elevated neutrophil count and reduced platelet count in Sirt3-/- mice during thrombosis.

Conclusions: We propose that Sirt3 does not considerably impact NET formation, platelet function or venous thrombosis in healthy young mice. However, Sirt3 modulates the host response to thrombosis.

PB 1380 | Neutrophil Extracellular Traps (NETs) in Septic and Burn Patients

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Background: NET formation (NETosis) is a mechanism of host defense against microbes associated with tissue damage. Besides pathogens, NETs are triggered by mediators of sterile inflammation and activated platelets.

Aims: We determined the presence of NETs in septic and burn patients and correlated them with organ damage, patient evolution, platelet activation and pro-inflammatory cytokines.

Methods: 25 septic and 23 burn patients were studied at 1 and 4 days post admission (dpa) to the intensive care unit and 30 healthy donors as control. Plasmatic nucleosomes and Human Neutrophil Elastase (HNE)-DNA complexes were measured as indirect and direct NETosis indicators, respectively (ELISA). Ex vivo NETosis was studied by DNA release (fluorometry); platelet P-selectin and toll like receptor 4 (TLR4) by cytometry and IL6 and TNFα by ELISA.

Results: Plasmatic nucleosomes and HNE-DNA complexes were elevated in septic and burn patients at 1 dpa remaining high at 4 dpa and a positive correlation between them was found in septic but not in burn patients. Nucleosomes from septics correlated with SOFA score

TABLE 1 Results table

	Circulating NET indicators		Ex vivo DNA release (μg/ml)			Platelets		Cytokines	
	Nucleosomes (μg/ml)	HNE-DNA complexes (OD)	Neutrophils	Neutrophils + platelets + LPS	Neutrophils + TNFα	P-selectin expression (MFI)	TLR4 expression (MFI)	TNFα (pg/ml)	IL6 (pg/ml)
Control	0.01±(0.00; 0.11)	0.01±(0.00; 0.01)	0.22±(0.11; 0.29)	0.36±(0.24; 0.71)†	0.52±(0.33; 0.64)†	4±(3;5)	5±(4;5)	18±(1;51)	0±(0;1)
Septic 1 dpa	0.19±(0.00; 0.38)*	0.02±(0.00; 0.08)*	0.46±(0.24; 0.80)*	0.39±(0.23; 0.79)	0.51±(0.24; 1.3)	5±(4;8)*	7±(4;13)*	109±(0;291)*	291±(61;447)*
Septic 4 dpa	0.17±(0.00; 0.38)	0.06±(0.01; 0.23)	0.48±(0.25; 0.71)	0.40±(0.19; 0.57)	0.20±(0.12; 0.47)	5±(4;8)	8±(4;17)	72±(22;236)	42±(13;167)#
Burn 1 dpa	0.10±(0.04; 0.32)*	0.02±(0.01; 0.12)*	0.27±(0.16; 0.65)	0.24±(0.16; 0.53)	0.31±(0.22; 0.48)	5±(4;7)*	4±(4;5)	90±(24;150)*	58±(15;156)*
Burn 4 dpa	0.17±(0.06; 0.35)	0.01±(0.00; 0.01)	0.29±(0.18; 0.66)	0.21±(0.15; 0.56)	0.15±(0.10; 0.35)	5±(4;8)	8±(5;14)#	89±(21;172)	46±(24;327)

Results are expressed as median±(IQR). *p<0,05 vs. Control, #p<0,05 vs. Patients 1dpa, †p<0.05 vs. Unstimulated neutrophils (Kruskal-Wallis). OD: optical density, MFI: mean fluorescence intensity.

Neutrophils (5X10e5/ml) were incubated alone, with LPS-stimulated platelets or TNFα (20ng/ml) for 1 or 3 hs, respectively in a humidified incubator at 37°C with CO2 (5%). DNA in the supernatant was quantified by fluorometry using SYBR Gold. Patients 4dpa were compared with 1dpa.

at 1 dpa (p< 0.05) and were significantly associated with mortality within 30 dpa (p< 0.05, logistic reg.).

Spontaneous DNA release was elevated in neutrophils from septics and while their stimulation with platelets+LPS or TNFα triggered DNA release in the control group, neutrophils from septic or burn did not respond.

While P-selectin exposure was increased in both patients groups through the study, higher platelet TLR4 expression was found in septics at 1 dpa.

TNFα levels were elevated in septic and burn patients. IL6 levels were high in both groups at 1dpa and decreased in septics by 4dpa. Neither platelet activation markers nor cytokine levels correlated with nucleosomes or HNE-DNA.

Conclusions: Nucleosomes could be an organ damage indicator and predictor of mortality in septic but not in burn patients. Platelet activation and pro-inflammatory cytokines appear not be key mediators of NETosis in these groups of patients.

PB 1382 | Platelets Release Pathogenic Serotonin in Response to Immune Complexes

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Background: There is a growing appreciation for the contribution of platelets to immunity; however, our current knowledge mostly relies on platelet functions associated with vascular injury and the prevention of bleeding.

Aims: While circulating immune complexes (ICs) contribute to both chronic and acute inflammation in a variety of clinical conditions, their impact on platelet responses is unclear.

Methods: We scrutinized platelet responses to systemic ICs in the absence of tissue and endothelial wall injury in mice. We used heat-aggregated IgG as surrogate to ICs, and injected LPS in LPS-immunized mice as an active model of IC-mediated response. Response to ICs was assessed by examining the mouse phenotype, and by intravital investigations of the vasculature using two-photon microscopy. To decipher the mechanism underlying platelet response in vivo, we included candidate transgenic mice, depleting antibodies, and pharmacological approaches to our study.

Results: Injection of ICs in mice resulted in systemic shock, characterized by temperature loss, severe lethargy, and blood vessel vasodilatation. Systemic shock by circulatory ICs in mice critically required platelet activation through a mechanism implicating expression of FcγRIIA by platelets. While diverse components from both alpha- and dense granules were released on IC activation, ICs-driven shock was dominantly dependent on release of serotonin from platelet dense granules secondary to platelet outside-in signaling by αIIbβ3 and its ligand fibrinogen. Intriguingly, although thrombi were evident in lungs during shock, their contribution to shock was not significant.

Conclusions: Our observations demonstrate that platelets are instrumental in the inflammatory response implicating ICs. We propose that the key cellular and molecular players downstream of IC-mediated activation, revealed in this study, may be conserved during chronic and acute systemic inflammation, implicating ICs in conditions such as rheumatic diseases, septic shock and viremia.

PB 1383 | Influence of Intravenous Immunoglobulin and Methylprednisolone on Cytokines Secreted by T Lymphocyte in Children ITP

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Background: About 20-30% ITP progress into chronic stage in children. Studies proved auto-antibodies against glycoproteins existed. However, not all the patients had positive results. Current studies paid more attention to T lymphocyte abnormalities, including TH1/TH2 imbalance. Studies shown that patients suffering from polarized Th1 or Th2 response and cytokine deregulation. Due to children ITP often have severe onset of thrombocytopenia, we prescribed IVIG at the first day of hospitalization to prevent hemorrhage, as well as MP. For this reason, T lymphocyte function evaluation might be interrupted by treatment. In this study, we explored the influence of IVIG and MP on cytokines in children ITP.

Aims: Our study was designed to explore the influence of IVIG and MP on cytokines secreted by T lymphocyte in children ITP.

Methods: We enrolled children ITP at the onset of their disease from our department between December 2011 and March 2013. We followed them until 12months, choosing the patients whose ultimate duration less than 3 months and no recurrence. Cytokines measurement by cytometric bead array included IL-2, IL-4, IL-6, IL-10, TNF, IFN and IL-17. We divided patients into 3 groups according to the treatment they received before testing cytokines level, then we compared T cell cytokines' level among these groups.

Results: We enrolled 38 boys and 24 girls, age range 2-178 months, median 33 months). We found IL-2 level decreased in both group treated with MP for 1 day (N=29, P=0.042) and group in which patients treated with IVIG for 1 day (N=18, P=0.048) compared with group without any treatment (N=15).

Conclusions: As for children ITP, although pathways of MP and IVIG in treating thrombocytopenia were different, they both could decrease IL-2 in children ITP ultimately. As a representative cytokine of TH1

cell, IL-2 has been demonstrated to be important in lymphocyte activation and mobilization. So we considered that MP and IVIG can reduce the T cell activation and production of auto-antibodies by decrease IL-2.

PB 2248 | Clopidogrel Does Not Protect Mice from Sepsis or Septic Shock

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Background: Thrombocytopenia frequently occurs in patients with sepsis and is associated with worsened outcome and thrombocytopenic mice have increased signs of sepsis as compared to controls. Data indicate that septic patients under aspirin may have a better outcome. However the multiple and contradictory roles of platelets during sepsis remain to be elucidated.

Aims: In the present study, we evaluated the effects of antiplatelet therapy with clopidogrel during sepsis in mice.

Methods: Septic shock or more moderate sepsis were induced by cecal ligation and puncture (CLP) with ligation of 100% of cecal length and double puncture (high grade form of CLP) or ligation of 50% of cecal length and simple puncture (mid-grade form of CLP), respectively. Clopidogrel (50mg/kg) or vehicle was administered *per os* the day before and 2h prior to surgery. Analyses were performed 20h later.

Results: In high grade CLP, clopidogrel- and vehicle-treated mice displayed a similar 30% decrease in mean arterial blood pressure (MAP) indicating systemic hypotension and organ failure characteristic of shock. Plasma concentrations of pro-inflammatory cytokines (TNFα, IL6) and myeloperoxidase (MPO) were similarly increased in clopidogrel- and vehicle-treated mice, indicating comparable increase in systemic inflammation. Thrombin-antithrombin (TAT) complexes reflecting thrombin generation and liver damage evaluated by aspartate aminotransferase (ASAT) levels in plasma were also similarly increased in both groups of mice. In mid-grade CLP, where MAP is not decreased, all parameters were similarly increased in both groups, although their levels were significantly lower than those reached in the high grade CLP.

Conclusions: Clopidogrel does not protect mice from sepsis or sepsis shock indicating that the $P2Y_{12}$ receptor does not contribute to the pathogenesis of sepsis and suggesting that anti-platelet therapy with $P2Y_{12}$ receptor antagonists may not be beneficial in patients with sepsis or septic shock.

PB 2249 | Dysregulation of NET Formation Following Thermal Injury and its Association with Multiple Organ Failure

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Background: Uncontrolled neutrophil extracellular trap (NET) formation is associated with thrombosis & multiple organ failure (MOF), which is the leading cause of delayed mortality following major trauma. DNAse activity, responsible for the breakdown of NETs, is regulated by the actin scavenging system, which consists of gelsolin (GSN) & vitamin D binding protein (VDBP).

Aims:

1. Determine if excessive NET formation is associated with MOF
2. Investigate the ability of neutrophils to produce NETs following thermal injury &
3. Investigate DNAse activity in post-burn serum samples.

Methods: 64 consecutive patients with thermal injuries (≥15% TBSA) were recruited into a prospective cohort study. Blood was taken from day of injury up to 12 months post-injury. Levels of plasma nuclear DNA (ncNDA) were quantified by PCR. DNAse activity was quantified as serum- induced NET degradation *in vitro*. Actin was measured in plasma by SDS-PAGE analysis. Levels of plasma VDBP & GSN were quantified by ELISA. 100 μg/ml GSN was incubated with actin spiked serum to restore DNase activity.

Results: ncDNA was elevated on day 1 & day 7 to month 2 in patients with MOF. Isolated neutrophils from patients generated significantly fewer NETs on day 3 & day 7 vs healthy controls. DNase activity was reduced from day 1-28 post-injury and significantly longer in patients with MOF. Actin was detectable on day 1 & during secondary complications. GSN & VDBP were significantly lower in septic patients, in turn predisposing them to the build-up of actin, low DNase activity & MOF. Incubation of actin spiked serum with GSN restored DNase activity.

Conclusions: Thermal injury induces actin release that inhibits DNase activity predisposing patients to uncontrolled NETosis. Elevated levels of NET markers can further the pathogenesis of MOF & are a potential therapeutic target to reduce delayed mortality. Restoring the actin scavenging system by administration of GSN or VDBP has the potential to restore DNase activity & improve patient outcomes.

PB 2250 | Hageman Factor Regulates Inflammatory Responses in Human Lungs

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Background: Increased procoagulant activity in the lung alveolar compartment and uncontrolled inflammation are hallmarks of acute respiratory distress syndrome (ARDS).

Aims: To investigate whether the contact system of coagulation is activated and may regulate inflammatory responses in ARDS lungs.

Methods: Components of the contact system in bronchoalveolar lavage fluids (BALF) from 54 ARDS patients and 43 controls were characterized by ELISA and western blotting, and their impact on cytokine/chemokine expression in human precision cut lung slices (PCLS) was assessed by a PCR array and ELISA.

Results: Activation of the contact system, associated with high levels of Hageman factor (FXII), plasma kallikrein and bradykinin, in ARDS lungs occurred rapidly after the onset of the disease and virtually normalized within one week from the time of diagnosis. FXII levels in BALF were higher in ARDS non-survivors than survivors and were positively correlated with tumor necrosis factor (TNF)-α concentration. FXII induced the production and release of interleukin (IL)-8, IL-1β, IL-6, leukemia inhibitory factor (LIF), C-X-C motif chemokine ligand (CXCL) 5 and TNF-α in human PCLS in a kallikrein-kinin-independent manner.

Conclusions: Accumulation of FXII in ARDS lungs may contribute to the release of pro-inflammatory mediators and is associated with clinical outcome. FXII inhibition may thus offer a novel and promising therapeutic approach to antagonize overwhelming inflammatory responses in ARDS lungs without interfering with vital hemostasis.

PB 2251 | Differential Impact of Triggers on the Risk of Incident Venous Thromboembolism in Hospitalized Subjects with and without Acute Infection - Results from a Population-based Case-crossover Study

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Background: Hospitalization with acute infections often occurs concomitant with short-term triggers of venous thromboembolism (VTE) such as immobilization and surgery. It is not known to what extent these triggers affect the risk of VTE in patients without and with acute infection.

Aims: To investigate the differential impact of VTE-triggers in hospitalized patients without and with acute infection.

Methods: We conducted a case-crossover study of VTE-patients (n=707) recruited from the general population (The Tromsø study). All hospitalizations and triggers were registered within each subject during the last 90 days before VTE diagnosis and in four preceding 90-day comparison periods. A 90-day washout period between the comparison- and VTE-periods was used to avoid potential carry-over effects. Conditional logistic regression was used to obtain odds ratios (ORs) for each trigger in subjects without and with acute infection in the VTE-period. Each patient served as their own control, thus allowing for within-subject adjustment for fixed covariates.

Results: There were 326 (46%) patients diagnosed with acute infection during the VTE-period. When comparing the VTE-period with comparison periods, traditional triggers had a greater impact on the VTE risk in patients without compared to those with acute infection. Triggers such as immobilization (OR 100.0, 95% CI 44.3-226.0 vs. OR 2.9, 95% CI 1.0-8.2), major surgery (OR 9.2, 95% CI 4.8-17.6 vs. OR 0.4, 95% CI 0.1-0.9), central venous catheters (OR 19.9, 95% CI 2.4-163.5 vs. OR 1.4, 95% CI 0.4-4.5), and transfusions (OR 27.3 95% CI 6.2-119.5 vs. OR 2.2 95% CI 0.7-6.5) had a greater impact in those patients without than in those with acute infections, respectively.

Conclusions: We found that established triggers for VTE had a stronger impact on the VTE-risk in patients without than in those with acute infection in the VTE-period. Our findings suggest that acute infections during hospitalization in itself are a strong risk factor for VTE that outweighs other trigger factors.

PB 2252 | Inhibition of the Major Endothelial Cell Integrin αVβ3, Both Pre- and Post-infection, Prevents Bacterial Binding in 2D Dynamic and 3D ex vivo Models of Sepsis

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Background: Sepsis is a life threatening-fast progressing disease characterised by vascular dysregulation caused by bacterial binding to endothelial cells (EC). *S. aureus* and *E. coli* are the most common causes of sepsis. Previously, we demonstrated that treating EC with an αVβ3 antagonist prior to bacterial infection prevented binding, suggesting this drug may have potential for pre-emptive treatment in patients with high risk of developing sepsis.

Aims: The aim of this study was to explore the effectiveness of an αVβ3 antagonist in preventing or displacing bacterial binding after infection using a 2D and 3D *ex vivo* model of sepsis.

Methods: EC were grown in microfluidic chambers. Fluorescently labelled *E. coli* and *S. aureus* were used to assess binding by real time epifluorescence microscopy in the presence and absence of the αVβ3 antagonist. Further experiments were carried out using 3D cylindrical microvessels (150μm diameter). Barrier permeability was assessed by TR-dextran extravasation.

Results: *S. aureus* and *E. coli* attached to the EC under flow within 15sec. Pretreatment of the EC with an αVβ3 antagonist significantly reduced this binding (94±4% and 77±4% vs control; respectively, $P < 0.05$). Furthermore, addition of *S. aureus* or *E. coli* to the EC for 30sec or 180sec prior to the addition of the αvβ3 antagonist still resulted in

a significant reduction in bacterial binding to the EC in flow chambers (90±3%, P< 0.05; *S. aureus and 87±6%, P< 0.05; E. coli*) and in the 3D vessel (80%, *S. aureus*). Similar inhibition post infection was obtained when whole blood was used (82±7%, P< 0.05). Remarkably, vascular permeability as a result of bacterial binding rose by 70±3% (P< 0.05) within 3min of infection.

Conclusions: Blocking endothelial αVβ3 effectively and promptly reduced bacterial binding to EC under flow, both pre and post infection. These results suggest that blocking the major integrin αVβ3 on EC presents potential as a new strategy for the treatment of established sepsis and as prophylaxis in patients at risk.

PB 2253 | RNA and Histones in Dead Cells Synergistically Provoke FSAP Activation in Serum

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Background: The plasma serine protease Factor VII-activating protease (FSAP) has been implicated in thrombosis and vascular remodeling. Moreover, FSAP is crucially involved in the release of chromatin from dead cells. FSAP circulates in an inactive form and is activated upon contact with late apoptotic or necrotic cells. FSAP binds to purified negatively charged molecules, e.g. RNA and DNA, and positively charged molecules, e.g. histones, which have both been implicated in the auto-activation of FSAP albeit through different mechanisms.

Aims: To identify the component(s) from dead cells that mediate FSAP auto-activation in serum.

Methods: Binding of FSAP to late apoptotic Jurkat cells treated with RNase and DNase was studied using (confocal) microscopy and flow cytometry. Live Jurkat cells were subjected to subcellular fractionation and treated with DNase, RNase and proteinase K. FSAP activation in serum was determined by detecting FSAP- α2-antiplasmin complexes in ELISA.

Results: Digestion of RNA in late apoptotic cells markedly reduced the binding of FSAP to these cells, and concurrently the activation of FSAP induced by these cells was also reduced. In contrast, DNA digestion strongly enhanced both the binding and activation of FSAP. Upon cellular fractionation, the cytoplasmic fraction containing most RNA did not induce FSAP activation, whilst the nuclear fractions that predominantly contained histones did induce the activation of FSAP. In serum, the addition of histones induced FSAP activation, whilst activation did not occur upon addition of RNA. However, when RNA was combined with histones this markedly enhanced the activation of endogenous FSAP in serum.

Conclusions: Our results indicate that both RNA and histones are involved in the activation of FSAP by dead cells. RNA does not directly induce the auto-activation of FSAP, but may promote/facilitate auto-activation of FSAP induced by histones.

PB 2254 | Impact of Acute Infection during Hospitalization on the Risk of Incident Venous Thromboembolism - Results from a Population-based Case-crossover Study

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Background: Acute infection is a well-established risk factor for venous thromboembolism (VTE), but little is known about the impact of different infectious foci on the risk of the separate VTE entities, deep vein thrombosis (DVT) and pulmonary embolism (PE).

Aims: To investigate the impact of hospitalization with acute infections, and the most common infectious foci (respiratory- (RTI) and urinary- (UTI) tract infections) on the risk of VTE.

Methods: We conducted a case-crossover study of VTE-patients (n=707, 299 PEs and 408 DVTs) recruited from the general population (The Tromsø study). All hospitalizations and triggers were registered within each subject during the last 90 days before the VTE diagnosis and in four preceding 90-day comparison periods. Acute infection was recorded when described in the medical notes, or if the patient received antibiotic treatment (except for prophylactic purposes). Conditional logistic regression was used to obtain odds ratios (ORs) for infection, RTI and UTI as triggers for VTE, DVT and PE. Each patient served as their own control, thus allowing for within-subject adjustment for fixed covariates such as age, anthropometric measures and chronic co-morbidities.

Results: Acute infection was registered in 326 of the VTE-periods and in 128 of the comparison periods. The OR for having infection in the VTE-period compared to preceding periods was 25.0 (95% CI 18.3-34.2), and after adjustment for cancer and surgery the OR was 19.1 (95% CI 13.7-26.5). The OR for having RTI in the VTE-period was 23.7 (95% CI 14.2-39.5) and the OR for having UTI in the VTE-period was 13.6 (95% CI 9.1-20.4). For DVT, the OR for infection was 20.1 (95% CI 13.8-31.3), for RTI 10.7 (95% CI 5.5-20.8) and for UTI 14.6 (95% CI 8.6-24.9). For PE, the OR for infection was 31.4 (95% CI 19.2-51.2), for RTI 54.6 (95% CI 22.1-135.4) and for UTI 12.3 (95% CI 6.6-22.8).

Conclusions: We found that acute infection is a strong trigger for VTE and that RTIs seem to be an especially important trigger for PE.

PB 2255 | C-reactive Protein and Risk of Venous Thromboembolism - Results from a Population-based Case-crossover Study

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Background: C-reactive protein (CRP) has not been shown to predict future venous thromboembolism (VTE) in prospective studies with long-term follow up. Inflammation is, however, a common feature in several conditions known to increase the risk of VTE, such as cancer and acute infection. The impact of CRP, as a downstream biomarker of pro-inflammatory triggers, on short-term risk of VTE is scarcely investigated.

Aims: To investigate the impact of CRP on the short-term risk of VTE.

Methods: We conducted a case-crossover study of VTE-patients (n=707) recruited from the general population (The Tromsø study). All hospitalizations and corresponding blood tests were registered during the last 90 days before the VTE diagnosis and in four preceding 90-day comparison periods. The highest measured CRP-value within each period was used in the analysis. Subjects admitted less than 3 days before date of VTE where excluded, to avoid reverse causation. Conditional logistic regression was used to obtain odds ratios (ORs) per 25, 50, 75 and 100 mg/L increase in CRP. Each patient served as their own control, thereby allowing for within-subject adjustment for fixed covariates.

Results: CRP was measured in 329 of the comparison periods (mean 57.1 mg/L), and in 286 of the VTE-periods (mean 122.6 mg/L). The OR increased with increasing CRP levels, and was 1.18, (95% CI 1.11-1.27) per 25mg/L, 1.40 (95% CI 1.22-1.60) per 50mg/L, 1.66 (95% CI 1.35-2.03) per 75 mg/L, and 1.96 (95% CI 1.50-2.57) per 100 mg/L increase in CRP, when comparing the VTE-period with all comparison periods. We also compared the CRP-levels in the VTE-period with each comparison-period separately, and there was no trend for change in OR according to time between the risk- and comparison periods.

Conclusions: CRP, as a biomarker of unspecified pro-inflammatory triggers, is associated with increased short-term risk of VTE. This may suggest that acute inflammation, regardless of cause, is an important trigger for VTE.

PB 2256 | DNA and Factor VII-activating Protease Protect against the Cytotoxicity of Histones

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Background: Circulating histones have been implicated as major mediators of inflammatory disease due to their strong cytotoxic effects. Histones form the core of nucleosomes, however it is unclear whether free histones and nucleosomes are equally cytotoxic. Nucleosomes are found in the circulation upon inflammation, however the presence of free histones has never been addressed. Notably, Factor VII-activating protease (FSAP) is activated in serum upon contact with histones and subsequently proteolyzes histones.

Aims: To determine the role of FSAP in the regulation of histone and/or nucleosome induced cytotoxicity.

Methods: Purified histones or nucleosomes were incubated with purified FSAP, or healthy donor, FSAP deficient, or FSAP depleted serum, added to HEK293 cells and LDH release was detected. Moreover, we added benzonase nuclease to nucleosomes to release histones, or added DNA to histones. Histone proteolysis was studied on blot using anti-H3 antibody. Nucleosomes were isolated from serum of *E. coli* challenged baboons and meningococcal disease patients using an immobilized anti-dsDNA antibody, whereafter remaining free histones were precipitated.

Results: FSAP protected against histone-induced cytotoxicity by cleaving free histones also in serum. Histones as part of nucleosome complexes were not cytotoxic, whilst DNA digestion of nucleosomes restored cytotoxicity. Incubation of nucleosomes with FSAP resulted in clipping of the N-terminal tail of histone H3. The specific isolation of either nucleosomes or free histones from serum of *E. coli* challenged baboons and patients with meningococcal sepsis, revealed that histones were present in the form of nucleosomes, whilst free histones were not detected. Notably, in both baboons and patients histone H3 in nucleosomes was clipped, and of similar size as observed with FSAP-treated nucleosomes *in vitro*.

Conclusions: Our results suggest that DNA and FSAP jointly limit histone cytotoxicity, and that free histones do not circulate in appreciable concentrations in sepsis.

PB 2257 | Nucleated Platelet Response to a Single Stranded RNA Viral Product through Pattern Recognition Receptors Revealed by RNA Sequencing

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Background: Thrombocytes express Toll-like receptors (TLRs) and respond to agonists of various origins. These cells are equivalent to enucleated platelets and can serve as a model for platelet response to bacterial or viral infections.

Aims: This study was performed to comprehend the role of thrombocytes stimulated with a TLR7/8 agonist (single stranded viral RNA analog) on gene expression.

Methods: Isolated chicken thrombocytes were treated with thymidine homopolymer phosphorothioate oligonucleotide (Poly(dT)). Highly sensitive RNA-sequencing technologies were used, and Panther derived gene ontology (GO-slim terms) was used for broad classification of biological processes, protein classes and biochemical pathways.

Results: Alignment of sequences with the *Gallus gallus* genome database revealed thrombocytes expressed 14,326 gene transcripts. After 1 hr stimulation with Poly(dT) 1,013 genes were up-regulated and 249 genes down-regulated with ≥ 1-fold change relative to unexposed

thrombocytes. Analysis of GO functional categories demonstrated that there were 13, 25 and 93 categories up-regulated for biological processes, protein classes and biochemical pathways, respectively; while 13, 22 and 53 categories that were respectively down-regulated for biological processes, protein classes and biochemical pathways. In addition to gene transcripts for TLR, nucleotide binding oligomerization domain-like receptor and retinoic acid-inducible gene 1-like receptor signaling pathways; gene transcripts for cytokines, chemokines, colony stimulating factors, and co-stimulatory markers were also detected.

Conclusions: Thrombocytes express many gene transcripts associated with anti-viral response through PRR signaling pathways. The information generated with this cellular model could be of enormous significance for both veterinary and human medical applications related to early platelet response during viral infections and rapid test diagnostics to intervene with onset of septicemia.

PB 2258 | MiR146a-5p Deficiency Exacerbates Neutrophil Extracellular Trap Formation

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Background: Neutrophils extracellular traps (NETs) are released to prevent sepsis but they are also involved in several immune and inflammatory diseases. Indeed, NET perpetuation contributes to tissue damage and prothrombotic state although the underlying mechanism of NET formation is not fully understood. MiR146a-5p is an important brake of immune and inflammatory diseases through blocking TLR/NFkB pathway and it is involved in higher risk of cardiovascular events.

Aims: To evaluate the role of miR-146a-5p in NETs formation *in vivo* and *in vitro*.

Methods: For evaluating NET release *in vivo*, blood samples were collected after endotoxemic induction (LPS 0111/B4, 1mg/Kg, 24h) in wild-type (WT) (N=10) and miR-146$^{-/-}$ (N=10) mice. *In vitro* NET evaluation was done in bone marrow isolated neutrophils from WT (N=8) or miR146$^{-/-}$ (N=8) mice, after stimulation with PMA (20 nM, 2h). Cells were permeabilized and stained for citrullinated Histone 3 (citH3). In parallel, stimulated neutrophils were incubated with MNase to free NETs from neutrophils. Circulating or released DNA in the supernatants and blood plasma samples were quantified using SYTOX green assays. Neutrophil elastase activity (NE) in plasma was measured by ELISA. Soluble citH3 was further detected by western blot (WB) and quantified by densitometric analysis.

Results: After LPS treatment, miR-146$^{-/-}$ displayed higher levels of cfDNA (1606±310 vs 457±74 ng/mL, p< 0.001), NE (2458±57 vs 1525±61 ng/mL, p< 0.001), and citH3 (WB densitometric area: 1 *vs* 1.55, p< 0.05) than WT mice. Moreover, PMA-treated neutrophils from miR-146$^{-/-}$ mice showed higher cfDNA and NET levels (% ratio citH3 positive cells) than from WT littermates (3671±337 *vs.* 2841± 284 ng/mL, p< 0.05 and 5.7± 0.3 *vs.* 3.7± 0.4 %, p< 0.01, respectively).

Conclusions: miR-146a-5p modulates NETs formation and can contribute to increase the prothrombotic state. Unraveling the mechanisms involved in this regulation could help to control pathological NETs response.

ISCIII y FEDER (P14/00253), Fundación Séneca 19873/GERM/15

PB 2259 | Inflammation Augments NFκB Dependent Production of TF in Preadipocytes

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Background: Observational studies have shown a strong and consistent association between obesity and risk of venous thromboembolism. The underlying mechanism is unknown, but it is feasible to assume that low-grade chronic inflammation in obesity may promote a prothrombotic phenotype of the adipose tissue with systemic impact.

Aims: To investigate whether inflammation, induced by tumour necrosis factor (TNF), influences tissue factor (TF) production in preadipocytes.

Methods: The SGBS cells are human cells derived from a patient with Simpson-Golabi-Behmel syndrome. Those cells retain characteristics of human preadipocytes and have the ability to differentiate into adipocyte-like cells. SGBS were cultivated according to the recommendations by the manufacturer (DMEM/F12 supplemented with 10% FCS, panthotenat, biotin and penicillin / streptomycin). Cells were treated with TNF, and / or JSH-23. F3 gene expression was assessed by RT-PCR. TF antigen levels were assessed by IMUBIND TF ELISA. TF activity was measured using a validated FXa generation assay.

Results: SGBS preadipocytes constitutively expressed high levels of tissue factor under resting conditions. TNF stimulation led to a time-dependent increase in both TF mRNA and TF antigen levels which peaked after 24 hrs, implicating an autoregulatory mechanism of TF upregulation. The levels of TF mRNA were 2,6 higher than time matched vehicle controls(p< 0,001). The increased expression of TF mRNA was accompanied by a significant increase in TF antigen levels vs control (12,4 vs 7,1 ng TF/ mg protein, p< 0,01). Importantly, TF activity levels were also significantly elevated (46% increase vs vehicle, p< 0,01). The observed effects appear to be dependent on the activation of NFkB transcription factor, as application of an inhibitor of NFκB, JSH-23, completely reversed the actions of TNF on TF production in preadipocytes.

Conclusions: Inflammation can directly affect the thrombogenicity of adipose tissue by increasing the production of TF in human preadipocytes.

PB 2260 | Evaluation of the Impact of Hypofibrinolysis Induced by Tranexemic Acid in Murine Experimental Sepsis

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Background: Coagulation activation is an integral part of the immune response to infections, and is thought to contribute to innate immunity by both containing the spread of pathogen foci, and by generating antimicrobial peptides from cleaved coagulation proteins. Although PAI-1-induced hypofibrinolysis has been long recognized as a hallmark of sepsis, little is known about the role of the firbinolytic system in the pathogenesis of this condition. Of note, the cooption of fibrinolysys pathways as virulence factors of several pathogens suggests that fibrinolysis is a relevant element in the pathogenesis of sepsis.

Aims: Using the cecal ligation and puncture (CLP) model associated with the inhibitor of fibrinolysis, tranexamic acid (TnxAc), we investigated the impact of hypofibrinolysis on murine experimental sepsis.

Methods: C57Bl/6J mice were treated with tranexemic acid or vehicle from two days before CLP induction until the end of the study. Animals were evaluated for clinical and laboratory parameters of sepsis severity.

Results: TnxAc-induced hypofibrinolysis was confirmed by a prolongation of the euglobulin lysis time and an increase in TAT levels beyond those observed in sepsis alone. TnxAc 100mg/kg did not change 7-day survival or a clinical sepsis score, while a higher dose (600mg/kg) was associated with increased mortality. At 24h post-sepsis induction, no differences were observed in biochemical or histological parameters of sepsis severity in TnxAc-treated animals. TnxAc was associated with a lower pathogen burden in liver and blood, and lower levels of IL-6 and MCP-1. At 24h post-sepsis, histological and laboratory markers of disseminated intravascular coagulation were not changed by TnxAc treatment

Conclusions: TnxAc induced a hypofibrinolytic state in mice submitted to the CLP model, which was associated with a lower pathogen burden, but a similar clinical course in murine sepsis. At supra-pharmacologic dose, TnxAc an increased mortality in the CLP model.

PB 2261 | Elevated Levels of Endogenous Activated Protein C Predict Mortality in Septic Shock

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Background: Activation of the coagulation cascade in septic shock is a common occurrence that may lead to life threatening events such as disseminated intravascular coagulation. Due to its anticoagulant and anti-apoptotic properties, activated protein C (APC) is believed to play a major role in the course of septic shock.

Aims: To assess the prognostic value of plasma APC measurements in patients with septic shock.

Methods: 48 consecutive patients presenting with septic shock were included in the study. Blood samples were drawn on admission (day 1) and on day 2, day 4 and day 7. Plasma APC levels were measured using an oligonucleotide (aptamer)-based enzyme-capture assay (OECA). Further analysis included the measurement of plasma levels of protein C, thrombin-antithrombin-complexes (TAT), prothrombin fragment 1.2 (F1.2), and D-Dimer by routine laboratory assays.

Results: APC levels were markedly elevated in 45 of the 48 (93.8%) patients of whom 19 (39.6%) died of septic shock. Non-survivors had significantly higher plasma APC levels on admittance (0.95 vs. 0.44 ng/ml, $p < 0.005$) and significantly higher individual peak APC levels (1.37 vs. 0.58 ng/ml, $p = 0.008$) measured during the course of sepsis. Also levels of TAT were higher on admission (19.4 vs. 10.1 ng/ml, $p = 0.01$) and when comparing individual peak levels (25.5 vs. 16.5 ng/ml, $p = 0.04$) during the course of sepsis in non-survivors to survivors. However, ROC curve analysis revealed the highest AUC (0.76) for APC levels on admission, consistent with a satisfactory discriminative diagnostic capacity. APC levels above a cutoff of 0.74 ng/ml during the course of sepsis were associated with increased mortality within 30 days (OR 7.8, 95% CI 2.8 - 22.0, $p < 0.001$).

Conclusions: Levels of APC are increased in patients with septic shock. Peak APC levels above a cutoff of 0.74 ng/ml measured during the course of sepsis were found to be associated with an increased 30-day mortality.

PB 2263 | In vitro Disruption of Endothelial Barrier Integrity by Serum from Patients with Septic Shock

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Background: Endothelial barrier (EB) breakdown is a hallmark of sepsis, and pathways that participate in the regulation of EB integrity are emerging as promising targets in septic shock. However, little data is available about EB breakdown in human sepsis.

Aims: Here we investigated the effect of serum from patients with septic shock on EB function.

Methods: Serum was obtained from patients with septic shock (n=18) and healthy volunteers (n=11). EB function was assessed by electric

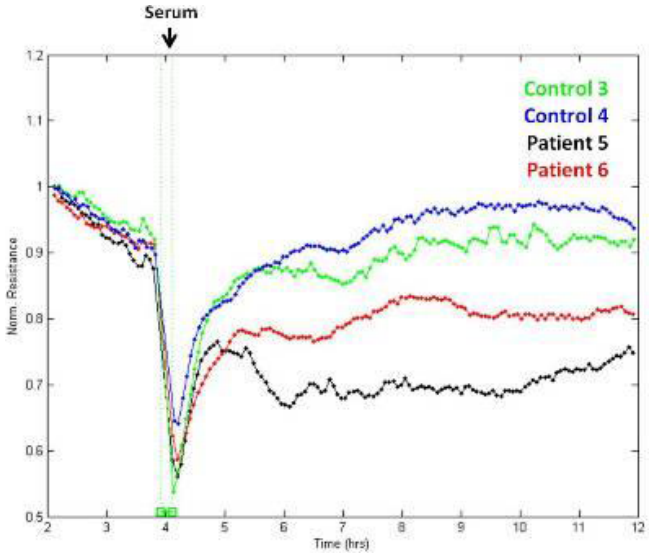

FIGURE 1 Representative figure shows the effect of serum from two patients and two healthy individuals in endothelial barrier resistance.

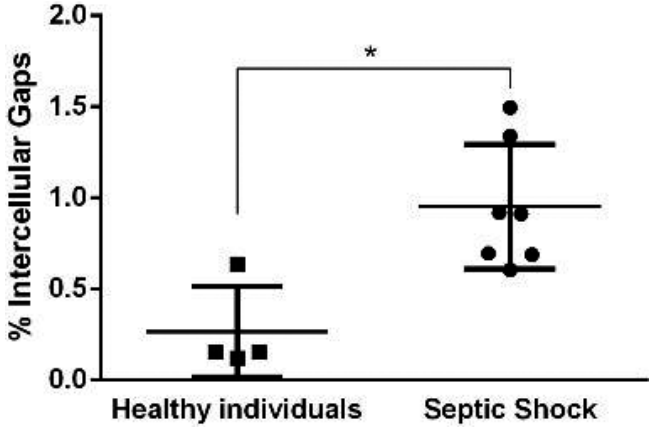

FIGURE 2 Extension of intercellular gaps was significantly higher in cells incubated with serum from septic shock patients (*P = 0.01)

cell-substrate impedance sensing, which measures the transendothelial electrical resistance (TEER) of endothelial cell monolayers, and by immunofluorescence-based studies of cell-cell junctions. Serum levels of VEGF-A and angiopoietin 2 (Ang-2) were also evaluated, to investigate the mechanisms of EB breakdown.

Results: Serum from patients with septic shock caused a stronger decrease in TEER than serum from healthy volunteers after 4h (21.52 ± 12.71 vs. 10.93 ± 10.69; P= 0.03) and 6h (22.08 ± 13.24 vs. 12.32 ± 9.86; P= 0.04) of incubation with HUVEC monolayers (Fig 1).

These functional alterations were associated with an increase in the extension of intercellular gaps (0.265 ± 0.247% vs. 0.950 ± 0.342%; P=0.01) (Fig 2).

EB disruption could not be directly attributed to VEGF-A or Ang-2 in serum, since no correlation was observed between TEER and these proteins despite a marked increase of Ang-2 levels in serum from patients with septic shock (14,391 ± 4,192 vs. 2,594 vs. 788; P< 0.0001).

Conclusions: Our study provides direct evidence that serum from patients with septic shock is capable to induce functional and morphological changes associated with EB disruption, but that additional mechanisms other than VEGF-A and Ang-2 are associated with these changes. As EB regulation is increasingly regarded as a therapeutic target in sepsis, additional studies are warranted to identify the mechanisms associated with these findings.

PB 2264 | The Potential Roles of IL-33 and TGF-β1 in the Pathogenesis of Stevens-Johnson Syndrome/ Toxic Epidermal Necrolysis

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Background: Stevens Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are rare, life-threatening and often drug-induced severe cutaneous adverse reactions with long-term ocular and other organ sequelae utilizing significant healthcare resources taking into account World Health Organization's introduction of the concept of a disability-adjusted life year (DALY). Given the increased morbidity and mortality due to multi-organ failure and sepsis, its pathogenesis still remains elusive and not completely understood. While the roles of IL-33 and TGF-β1 have been well defined for a variety of pro-inflammatory and apoptotic epidermal diseases, their roles in the apoptotic pathway of SJS/TEN remain unexplored. Based on this background we hypothesize that IL-33 and TGF-β1 play crucial roles in the pathogenesis of this syndrome.

Aims: To determine the potential roles of IL-33 and TGF-β1 in the apoptotic pathway of SJS/TEN.

Methods: Under an IRB approved protocol, collected and archived unstained slides of skin from patients with biopsy confirmed SJS/TEN were used for this study. Immunohistochemical analysis was performed using IL-33 and TGF-β1 antibodies followed by imaging on a DeltaVision microscope. ELISA analysis was used to determine the levels of IL-33 and TGF-β1 expression in citrated plasma samples. Statistical analysis was performed using linear regression analysis, ANOVA, and Turkey's *post-hoc* tests on STA-TA software.

Results: ELISA analysis of SJS/TEN patient plasma samples showed no marked elevation of TGF-β1 or IL-33 compared to normal human plasma (p= 0.41 and 0.26 respectively). Immunofluorescent microscopy of SJS/TEN skin biopsy samples revealed elevated levels of both TGF-β1and IL-33 in the epithelium compared to lichen planus skin biopsy samples. The results of this study may enhance our understanding of the pathogenesis of SJS/TEN and lead to the development of new treatment modalities for this disease.

Conclusions: TGF-β1and IL-33 play a crucial role in the apoptotic pathway of SJS/TEN.

PB 2265 | Assessment of the Concentration and the Functional Activity of Plasma Inter-alpha Trypsin Inhibitor in Patients with Inflammatory Diseases

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Background: Inter-alpha trypsin inhibitor (ITI) is a plasma proteoglycan, composed of 1 or 2 various heavy chains (HCs) linked through a chondroitin sulfate to the light chain, bikunin. Bikunin is the protease inhibitor chain and HCs can be transferred to hyaluronic acid (HA) in presence of TSG-6 to form HC-HA complexes. Such circulating complexes were found increased in acute inflammatory state. If the exact role of ITI is still unclear, anti-inflammatory properties of ITI have been described.

Aims: We compared the concentrations of circulating ITI components and their activity by measuring the transfer capacity of HC to HA from ITI present in plasmas of normal donors vs patients with auto-immune disease (AID).

Methods: Plasma ITI levels were quantified by using sandwich ELISA tests for the bikunin and HCs. TSG-6 level was measured using a commercial kit. The HCs to HA transfer was assessed using an ELISA method developed in the lab.

Results: The plasma amounts of ITI, as determined by bikunin measurement, in patients with AID (SLE, Crohn's or Cystic fibrosis) were slightly higher than that of normal donors (mean ± SD: 286 ± 43.6 µg/ml, n=33 vs 235 ± 36.7 µg/ml, n= 42). In normal plasma, the level of TSG-6 measured to 6.9 ± 4.1 ng/ml whereas in plasma from SLE, Crohn's, and cystic fibrosis the level of TSG-6 was significantly increased to 11.7 ± 12.1 ng/ml (n=20), 10.37 ± 4.37 ng/ml (n=10), and 15.6 ± 3.8 ng/ml (n=3), respectively. Using control and AID plasmas, binding of HCs to HA was found very faint. The addition of TSG-6 in plasma induced a large increase in level of HCs to HA transfer but without difference between control and AID plasmas.

Conclusions: No difference, except a slight increased amount of ITI in plasma of patients with AID, in ITI composition and HCs to HA binding capacity was observed in plasma of normal or patients with AID. However, due to the extreme sensitivity of the transfer activity of ITI to TSG-6, the situation might be different in tissues where TSG-6 is locally secreted.

PB 2266 | 2',3-dihydroxy-5-methoxybiphenyl Suppresses fMLP-induced Superoxide Anion Production and Cathepsin G Release by Targeting the β-subunit of G-protein in Human Neutrophils

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Background: Neutrophils are a major cellular component of innate immune response and play a crucial role in the elimination of invading microorganisms by producing reactive oxygen species.

Aims: This study investigates the effect and the underlying mechanism of 2',3-dihydroxy-5-methoxybiphenyl (RIR-2), a lignan extracted from the roots of *Rhaphiolepis indica* (L.) Lindl. *ex* Ker var. *tashiroi* Hayata *ex* Matsum. & Hayata (Rosaceae), on fMLP-induced respiratory burst in human neutrophils.

Methods: Signaling pathways regulated by RIR2 on neutrophils were evaluated by an interaction between β-subunit of G-protein and downstream signaling induced by fMLP, Src family kinases activities and by immunoblotting analysis of the downstream targets of G-protein.

Results: RIR-2 inhibited fMLP-induced superoxide anion production (IC_{50}=2.57±0.22 µM), cathepsin G release (IC_{50}=18.72±3.76 µM) in a concentration dependent manner. Further, RIR2 specifically suppresses fMLP-induced Src family kinases phosphorylation, -Raf/ERK phosphorylation, by inhibiting an interaction between β subunit of G-protein. However, RIR-2 was not found to inhibition of Src kinases activities. Consequently, RIR2 attenuated the downstream targets of Src kinase, such as Tec translocation from the cytosol to the inner leaflet of the plasma membrane, phosphorylation of AKT, P38, PLCγ2, PKC and membrane localization of $p47^{phox}$ and $P40^{phox}$, furthermore, RIR-2 attenuated fMLP-induced intracellular calcium mobilization. RIR2 was not a competitive or allosteric antagonist of fMLP. On the other hand, PMA-induced phosphorylation of Src, AKT, P38, PKC and membrane localization of $p47^{phox}$ and $P40^{phox}$ remained unaffected.

Conclusions: In conclusion, RIR2 specifically modulates fMLP-mediated neutrophil superoxide anion production and cathepsin G release by inhibiting an interaction between β subunit of G-protein and which subsequently interferes with the activation of intracellular calcium, PLCγ2, AKT, p38, PKC, ERK, $p47^{phox}$ and $p40^{phox}$.

PB 2267 | A Comparison of Scoring Systems and Surrogate Markers of Death in a Cecal Ligation and Puncture Sepsis Model

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Background: Despite increasing standards for conducting ethical, humane research involving animals, death is still considered the only valid endpoint in murine sepsis studies. Recently, the Mouse Clinical Assessment Score for Sepsis (M-CASS), Murine Sepsis Score (MSS), and Mouse Grimace Scale (MGS) were developed as potential surrogate endpoint tools for assessing disease severity in models of sepsis.

Aims: To assess the validity of the M-CASS, MSS, and MGS for evaluating disease severity and predicting death in a resuscitated cecal ligation and puncture (CLP) model of sepsis.

Methods: Healthy C57BL/6 mice were subjected to CLP, a surgical procedure involving two punctures of the ligated cecum to induce sepsis, or a control, non-septic sham surgery (no ligation or puncture). Mice were resuscitated using Ringer´s lactate and continuously monitored every 4 hours post-surgery for potential surrogate markers of death until endpoint or 24 hours post-operatively. M-CASS, MSS, and MGS scores were assigned every 4 hours and the utility of the systems was determined.

Results: Around 50% of septic mice expired within 16 hours post-CLP and 100% expired within 20 hours post-CLP. M-CASS, MSS, and MGS increased in a time-dependent manner in septic mice and all scores were 0 in non-septic mice over 18 hours. The following components of the scoring systems were considered to be informative indicators of disease severity: responsiveness of mice to external stimuli, posture, facial grimace markers, and fur condition. Temperature was a reliable, objective marker to assess disease progression. Vocalizations, chest sounds, cyanosis, and restlessness/tremor did not correlate with disease progression in our CLP model.

Conclusions: The M-CASS, MSS, and MGS are effective tools for assessing disease severity in the CLP model. Efforts should be made towards using these assessment tools as well as other relevant surrogate markers as valid endpoints rather than mortality.

PB 2269 | High Altitude Venous Thromboembolism

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Background: Ascent to high altitude (HA) is often complicated by acute, chronic mountain sickness, HA pulmonary & cerebral edema, which are well known entities. However, HA venous thromboembolism (VTE) is relatively unknown & is yet evolving in terms of knowledge, pathophysiology & management. Most literature are retrospective studies from the high mountainous regions of the Himalayas, where given the topography, access to advanced medical care is challenging. Singapore, a tropical island in the South China sea is an unusual location for patients to be seen with HA VTE.

Aims: We study of the presentation, management of 3 patients who presented with breathlessness after recent moderate altitude mountaineering.

Methods: This is a retrospective study of patients presenting with VTE with no apparent risk factor but high altitude mountaineering.

Results: All patients were young females, with no previous personal/family history of VTE. Neither did patients have any traditional risk factors associated with VTE. They presented with breathlessness of 1-4 weeks duration and were investigated for VTE as common causes were negative and had an elevated d-dimer. All had pulmonary embolism, with/ without DVT. They were all treated with anticoagulation for 6 months and all had resolution of the VTE.

Considering the idiopathic or lesser known entity of HA VTE, all had a thrombophilia screen performed after anticoagulation was discontinued, which was negative. At an average of 6 months follow up, none developed a recurrence of VTE.

Conclusions: A high index of suspicion is required for patients presenting with breathlessness after ascent to a high altitude to differentiate aetiologies. After diagnosing VTE, work up for underlying thrombophilia is warranted to exclude co-existent conditions. Duration of anticoagulation is recommended for a minimum of 6 months followed by a discussion about the risks Vs benefits of continued anticoagulation and lifestyle changes.

PB 2270 | Venous Involvement in Behcet's Disease: A Retrospective Analysis of 75 Cases

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Background: Vascular involvement is the leading cause of death in Behcet's disease (BD)

Aims: The vascular involvement in Behcet´s disease (BD) varies from 7, 7 to 43% depending on ethnicity. Venous lesions are the most frequent vascular manifestation in BD. In this study, we looked at the pattern and outcome of venous events in BD.

Methods: Seventy five patients, who fulfilled the criteria of the International Study Group for diagnosis of BD, were recruited. We studied the characteristics of patients with thrombotic venous events. Clinical data parameters were recorded, including age at onset and extra-vascular manifestations of the disease.

Results: Twenty six from 75 patients had vascular event. Twenty three of these patients had a venous event. The mean age of the patients at the first venous event was 32 years. There were 22 males and 4 females. The first venous event occurred before BD diagnosis in one case and in the same time of onset of the disease in 2 cases. In the other cases, venous event occurred in patients followed for BD and the mean disease duration was 5, 82years. The mean number of recurrence of venous events was 1, 46. Deep vein thrombosis was the most frequent single vascular event (76, 92%). The most frequent localizations were in legs (23 cases).Four patients had cerebral vein thrombosis.A pulmonary venousinvolvement, a Budd-Chiari syndrome,an inferior and superior vena cava syndrome and arms thrombosis were found in only one case each one. An arterial event was associated in 2 cases. An association with ocular manifestations was observed in 26, 9% patients and neurologic manifestations in 11, 53%.

Conclusions: Although there is no agreement on the frequency rate of the vascular lesions in the littérature, most the vascular lesions in the litterature, most of the reported series indicate that the venous lesions are by far, more common than the arterial lesions.

In conclusion, the frequency of vascular complications of BD in our patients is similar to those reported around the world.

PB 2271 | Arterial Involvment in Behcet's Disease: A Retrospective Study of 75 Cases

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[1]*Charles Nicolle Hospital, Internal Medicine B, Tunis, Tunisia,* [2]*Hôpital Régional El Yasminat, Ben Arous, Internal medicine, Tunis, Tunisia*

Background: Vascular involvement is the leading cause of death in Behcet's disease (BD).

Aims: Vascular involvement is the leading cause of death in Behcet's disease (BD). The vasculitis may involve large, medium and small vessels of both the arterial and venous circulation. We aimed to explore in this study the arterial involvement in Behcet's disease.

Methods: Seventy five patients, who fulfilled the criteria of the International Study Group for diagnosis of BD were recruited. We studied the clinical characteristics of patients with arterial manifestations. Clinical data parameters were recorded, including age at onset, the vascular and extra-vascular manifestations of the disease.

Results: We identified 26 patients with vascular involvement among the 75 patients (34, 6%). Four patients had arterial involvement (15% of all vascular manifestations). The mean age at the diagnosis of the arterial event was 52, 5 years. All patients were males. Mean disease duration before the arterial event was 22 years . The arterial events were divided in arterial thrombosis and anevrysm found in two cases each. The arterial events were pulmonary artery thrombosis,pulmonary artery anevrysm, femoral artery thrombosis and abdomianl aortic anevrysm in one case each one. An association with a venous event was found with two patients. An association with ocular manifestations was observed in 40%. No neurologic manifestation was observed. HLA B 5 was found in 40% cases. Cortisteroid treatment was used in 40% cases and immunosuppressive treatment in only one case.

Conclusions: As previously reported, we found a male predominance in BD patients with arterial involvement. We found a preferential location on the aorta, the femoral, and pulmonary arteries. Saadoun et al. reported in their cohort a prevalence of 40% of deaths with thoracic aorta and pulmonary arteries. Aortitis is infrequent in BD and was not found in our cohort.

Arterial involvement represented 15% of vascular manifestations in our cohort of BD.

PB 2272 | Self Extracellular RNA and Exogenous Danger Signals Act Synergistically to Induce Cytokine Production in Macrophages

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Background: Self-extracellular RNA (eRNA) released from stressed or injured cells during ischemia-reperfusion or other pathologies has been shown to act as an alarmin by inducing proinflammatory responses.

Aims: Studies were performed to investigate whether self-eRNA can sensitize macrophages to amplify their inflammatory reactions towards damaged- or pathogen-associated patterns (DAMP, PAMP) like toll-like receptor (TLR)-agonists.

Methods: Mouse macrophages were differentiated from bone marrow-derived stem cells by mouse colony stimulating factor and characterized by CD68-, F4/80- and CD11b-staining using FACS analysis. Highly purified RNA was harvested from NIH3T3 cells and used as eRNA. Cytokines were quantified by ELISA.

Results: In macrophages, various doses (10 pg/ml - 1 µg/ml) of isolated agonists of TLR2 (Pam2CSK4), TLR3 (PolyIC), TLR4 (LPS), or TLR7 (R848) induced the release of TNF-a in a concentration-dependent manner. In the presence of eRNA, a considerable shift to the left of the dose-response curve of Pam2CSK4 (Pam) was observed. At low Pam-concentrations, eRNA synergistically enhanced the cytokine liberation dramatically. The synergistic activation of TLR2 by eRNA was confirmed by using other TLR2-agonists; this effect was much weaker for TLR4-, but not existent for TLR3-, or TLR7-agonists. The synergistic action of Pam-eRNA was prevented by RNase1, whereby Pam alone or together with eRNA was blocked by antibodies against TLR2 or by an inhibitor of the NFkB-signaling pathway. Since inhibition of p38MAP-kinase by SB203580 or of the ERK-kinase by PD98059 significantly reduced the combined eRNA-Pam response, several pathways feed the TLR2-NFkB signaling route.

Conclusions: eRNA considerably enhances the sensitivity of macrophages towards DAMPs/PAMPs, particularly involving TLR2-NFkB-signaling mechanisms. Thus, the presence of self-eRNA sensitizes innate immune responses and may thereby contribute to the onset or progression of vascular disease.

PB 2273 | Neutrophil Extracellular Trap (NET) Formation in Patients with Behcet's Disease

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Background: Behçet´s disease (BD) is a systemic inflammatory disorder characterized by recurrent episodes of oral and genital ulcers, uveitis as well as thrombosis. The etiology of BD is poorly understood but activated neutrophils have been proposed to contribute to the disease. However, evidence supporting a role for primed neutrophils in BD-associated thrombotic risk is scant. To respond to inflammatory

insults, neutrophils release web-like structures, known as neutrophil extracellular traps (NETs), which are prothrombotic.

Aims: To evaluate the role of NETs in the thrombotic complications in BD disease.

Methods: Blood samples were collected from BD patients and healthy controls (HC). Cell free DNA (CfDNA), myeloperoxidase (MPO) and DNA complexes were measured in plasma using Picogreen assay and ELISA. NETosis was assessed in purified neutrophils from BD patients and HC, and analyzed by microscopy after immunostaining of MPO and DNA.

Results: BD patients (n=73) had elevated plasma CfDNA levels compared to HC (n=15) (928.7 ± 36.3 vs 711.3 ± 28.4 ng/ml, respectively p< 0.001). MPO-DNA complexes were significantly increased in BD plasma (n=73) compared to HC plasma (n=15) (0.383 ± 0.10 vs 0.065 ± 0.01 OD, respectively, p< 0.001). Purified neutrophils from BD patients exhibited spontaneous NETosis compared to HC (31.7 ± 4.4% vs 5.4 ± 1.9% respectively, p=0,004). Consistently, PMA-activated neutrophils from BD patients have increased NET formation compared to neutrophils from HC (41.8 ± 2.7% vs 18 ± 3.8% respectively, p=0.002). Thrombin generation in BD plasma was significantly increased and positively correlated with MPO-DNA complexes (r^2= 0.9, p=0.002). Importantly, DNAse treatment significantly decreased thrombin generation in BD plasma but had no effect in HC plasma suggesting a procoagulant role of NETs.

Conclusions: Our data show that circulating NETs are elevated in BD patients. Targeting NETs may represent a potential therapeutic target for the reduction or prevention of BD-associated thrombotic risk.

PB 2274 | A Basal-state Monocyte Gene Transcription Profile Is Associated with Circulating Levels of Th1 Cells: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Background: Differentiation of CD4+ T helper cells into distinct effector subtypes, including T helper type 1 (Th1) cells, is an incompletely understood process involving antigen presentation, co-stimulation, and transcriptional regulation pathways. Monocyte signaling is known to play a role in T-cell polarization, but details are poorly understood.

Aims: We aimed to identify a gene transcriptomic profile expressed by monocytes associated with Th1 cell polarization.

Methods: 238 white, African-American, and Hispanic MESA participants (aged 54-93 years) had measures of circulating Th1 cells and monocyte mRNA expression. Th1 cells were measured in blood by flow cytometry as CD4+interferon-gamma+ lymphocytes. Monocytes were isolated using magnetic separation with anti-CD14 antibody. Genome-wide monocyte mRNA expression (*n*=10,898 genes) was determined by HumanHT-12 v4 Expression BeadChip in 14,619 transcripts. Cross-sectional associations between monocyte transcripts and Th1 cell level was estimated by linear regression, adjusted for age, sex, race/ethnicity, expression chip, contamination with non-targeted cells, and the first 4 ancestry principal components (false discovery rate = p< 0.05).

Results: 6 genes expressed by circulating monocytes were associated with Th1 cells. Granzymes H ($p=6x10^{-5}$) and K ($p=3x10^{-3}$) and CD8α ($p=2x10^{-4}$) were associated with higher Th1 cell levels; lymphoid enhancer binding factor-1 (*LEF1*) ($p=2x10^{-3}$), myelin and lymphocyte protein (*MAL*) ($p=7x10^{-3}$), and C-C chemokine receptor 7 (*CCR7*) ($p=1x10^{-2}$) were associated with lower Th1 levels. Ingenuity pathway analysis identified the Communication Between Innate and Adaptive Immunity Pathway as enriched among these genes, supporting their potential role in Th1 cell polarization.

Conclusions: Our results suggest a basal gene transcription profile expressed by monocytes influences Th1 cell polarization. If confirmed, findings may have implications in modulating Th1 cell-mediated inflammatory diseases.

PB 2275 | Proinflammatory Polarized Macrophages Have Reduced Microvesicles and Coagulatory Function

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Background: Microvesicles are small particles deriving directly from the cellular membrane of the cell by an active budding process involved in cell to cell communication and activation of the coagulation cascade. Macrophages can be polarized differentially according to their surrounding environment leading to different function and behavior of macrophages.

Aims: To identify changes in macrophage coagulatory function and microvesicle production under different polarization conditions.

Methods: Primary human macrophages were polarized towards a pro-inflammatory state (M1) using LPS and IFN-gamma, alternatively polarized macrophages (M2) were generated using IL4 and IL13.

Results: Unpolarized macrophages constantly produce microvesicles. Upon M1 polarization however, microvesicle production is significantly reduced whereas M2 polarization leads to increased shedding of microvesicles. Microvesicle production in all polarization conditions is independent from Ca^{2+} signaling including activation of Caspase-3. In addition, inhibition of Rho did not change cellular microvesicle production regardless of polarization condition. Unpolarized and M2 macrophages had 24% and 22% microvesicles positive for phosphatidylserine respectively, M1 polarized macrophages displayed only

13% positive microvesicles. However, tissue factor activity in the microvesicle fraction was highest in M2 macrophages followed by M1 macrophages and unpolarized macrophages. This was also confirmed on the protein level.

Conclusions: Our data indicate that macrophages are a constant source of microvesicles. Alternative polarization induces microvesicle production together with an increase in tissue factor and tissue factor bearing microvesicles. Proinflammatory activation leads to opposite behavior culminating in reduced tissue factor acitivity together with reduced microvesicle shedding. This suggests that alternative activation of macrophages leads to a procoagulatory macrophage phenotype.

PB 2276 | Modulation of Macrophage Functions by Thrombin

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Background: Besides its role in blood coagulation, thrombin is known to act as a cell signaling molecule via activation of protease-activated receptors (PARs), thereby connecting this coagulation protease to immune/inflammatory responses. Previous studies demonstrated that thrombin activates adhesion and promotes release of interleukin 6, monocyte chemotactic protein or tissue factor pathway inhibitor 2 in macrophages. However, gene expression changes and cytokine/chemokine profiles of thrombin-stimulated macrophages have not been systematically studied so far.

Aims: The aims of this work were to analyze gene expression and cytokine secretion profiles in macrophages following thrombin treatment and to investigate whether thrombin induces polarization changes in macrophages.

Methods: Bone marrow-derived macrophages were generated from bone marrow cells of C57BL/6J mice cultured in DMEM containing macrophage colony stimulating factor (M-CSF). On day 7 in culture the cells were replated in DMEM media (without M-CSF) for one day, followed by stimulation with thrombin. Subsequently, real-time PCR was performed using specific primers and cytokines in pooled cell culture supernatants were assessed using a cytokine array Kit.

Results: Several proinflammatory cytokines (e.g. IL1α, TNFα) and chemokines (MIP1α, MIP2, IP10) were differentially expressed in macrophages in response to thrombin. Moreover, high thrombin concentration promoted differentiation of macrophages towards the M1-phenotype, whereas low concentrations induced cell differentiation into the M2 phenotype. In a concentration-dependent manner, thrombin also influenced the expression of PARs in macrophages. Thrombin-stimulated macrophages exhibited pro-coagulant and anti-fibrinolytic properties by upregulation of tissue factor and plasminogen activator inhibitor-1 mRNA levels.

Conclusions: Our results provide new experimental evidence on the existent link between coagulation and innate immunity

PB 2277 | Interleukin-6 in the Development of Venous Thrombosis

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Background: Interleukin-6 (IL-6) is of crucial relevance in inflammatory processes, especially in the recruitment of myelomonocytic cells. IL-6 was described to accelerate thrombus development.

Aims: The aim of our study was to analyse the role of IL-6 in the development of deep vein thrombosis (DVT).

Methods: Mice overexpressing IL-6 in myelomonocytic cells (LysM-IL-6$^{ind/+}$, controls: IL-6$^{ind/+}$) and IL-6 deficient mice (IL-6KO, controls: C57BL/6 mice) were analysed for the development of DVT after one week of ligation of the inferior vena cava (IVC ligation stenosis model). DVT development was analysed by ultrasound. We performed histological as well as FACS analysis and rtPCR measurements of the cellular infiltrate in thrombi and venous wall. Thrombin generation and coagulation factors were analysed in citrated blood as well as cytokine levels.

Results: We found elevated levels of IL-6 in thrombus and venous wall in C57BL/6 mice after IVC ligation compared to sham-treated mice. Interestingly LysM-IL-6$^{ind/+}$ mice had no thrombus development after IVC ligation. Overexpression of IL-6 in myelomonocytic cells resulted in a bleeding phenotype with prolonged aPTT levels (LysM-IL-6$^{ind/+}$: 79s, IL-6$^{ind/+}$: 29s) and a slightly but significantly increased INR (LysM-IL-6$^{ind/+}$: 0,69, IL-6$^{ind/+}$: 0,65) partially due to mildly reduced levels of clotting factor II (LysM-IL-6$^{ind/+}$: 101%, IL-6$^{ind/+}$: 124%), and moderately reduced levels of clotting factor V (LysM-IL-6$^{ind/+}$: 522%, IL-6$^{ind/+}$: 957%), and IX (LysM-IL-6$^{ind/+}$: 93%, IL-6$^{ind/+}$: 145%). There was an increase in vonWillebrand Factor in LysM-IL-6$^{ind/+}$ and elevated levels of fibrinogen and antithrombin compared to IL-6$^{ind/+}$ mice. In parallel, IL-6 KO mice had a normal DVT development after IVC ligation compared to control mice.

Conclusions: Overexpression of IL-6 in LysM+ cells prevents DVT formation in IVC-ligated mice. Further analyses will follow on the bleeding phenotype of the LysM-IL-6$^{ind/+}$ mice and the influence of downstream IL-6 signaling on coagulation.

PB 2278 | The First Year after Diagnosis of Anti-Neutrophil Cytoplasmic Antibody (ANCA)-Associated Vasculitis is Critical for Development of Venous Thromboembolism (VTE) in These Patients

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Background: Patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) entail an increased incidence of venous thromboembolism (VTE) compared to the general population.
Aims: To investigate the influence of the ANCA-specificity, disease duration and classical VTE risk factors on the development of VTE in AAV-patients.
Methods: The occurrence of VTE was evaluated in the retrospective cohort study of AAV-patients at the Karolinska University Hospital, Stockholm, Sweden. 187 ANCA-positive patients with granulomatosis with polyangitis (GPA) or microscopic polyangitis (MPA) diagnosed between 2005 and 2014 were included. We assessed haemostasis in a pilot study using two global haemostatic methods: endogenous thrombin potential (ETP) and overall fibrinolytic potential (OFP) in AAV-patients with active flare (n=19), sex- and age matched AAV-patients during inactive period of the disease (n=15) and healthy controls (n=15).
Results: 28 VTEs occurred in 24 patients over a total follow-up time of 1020 person-years, rendering a total incidence rate of 2.74 cases of VTE per 100 person-years. A majority of the VTEs occurred in close temporal proximity to the AAV-diagnosis, with more than half of the patients having a VTE within the first year after the AAV-diagnosis. ANCA-specificity was not significantly associated with VTE development, nor were AAV-diagnosis (GPA/MPA), sex or renal involvement. High age ($p < 0.01$) and previous VTE
($p < 0.05$) were significantly more common in the VTE group.
Conclusions: The main finding of this study is the striking prevalence of VTE in AAV-patients within the first year after the AAV-diagnosis. High age at AAV-diagnosis and previous VTE should be taken into account when estimating VTE-risk. Prothrombotic condition is present in active AAV-patients, where ETP and OFP may be useful markers for identifying patients at high risk.

PB 2279 | Protein Arginine Deiminase-4 Does Not Influence Antibacterial, Inflammatory or Procoagulant Responses during Gram-negative Pneumonia Derived Sepsis

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Background: Neutrophil extracellular traps (NETs) can capture and kill bacteria. NET formation is regulated by Protein arginine deiminase 4 (PAD4). NETs can aid in host defense in skin and liver infection; their role during pneumonia is less well known.
Aims: We aimed to investigate the contribution of PAD4 to antibacterial, inflammatory and procoagulant responses during pneumonia derived sepsis.
Methods: Intensive Care patients with either pneumosepsis or non-infectious critical illness without lung pathology were subjected to a non-directed bronchoalveolar lavage. Lavage fluid was assessed for formation of NETs. Isolated neutrophils were stimulated with phorbol myristate acetate (PMA), Klebsiella pneumoniae, Pseudomonas aeruginosa or Lipopolysaccharide (LPS) and NET formation was assessed. PAD4 deficient and wild-type mice were infected with Klebsiella or Pseudomonas via the airways.
Results: Patients with pneumosepsis, but not patients with non-infectious disease, had NETs in their airways. Klebsiella, Pseudomonas and LPS induced NET formation by human neutrophils, albeit to a lesser extent than PMA. PAD4 deficient mice had reduced NET formation in the lung, but had similar levels of bacterial growth during infection with Klebsiella or Pseudomonas. Cell free DNA levels correlated to some extent with NET formation, whereas nucleosomes levels were not different between PAD4 deficient and wild-type mice.. PAD4 deficiency reduced platelet activation but did not prevent sepsis induced thrombocytopenia or alter activation of coagulation or distant organ damage.
Conclusions: These results suggest that NETs are present in the airways during human and murine pneumosepsis and that this may be a direct effect of causative pathogens. However, PAD4 mediated NET formation does not play a role in antibacterial defense during gram-negative pneumonia, suggesting that other immune functions are more dominant during this type of lung infection.

PB 2280 | NETs, FSAP and their Complicity in the Context of Immunity and Thrombosis

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Background: Neutrophils can release neutrophil extracellular traps (NETs), which are a web-like meshwork composed of nucleosomes, and decorated with neutrophil granules components and anti-microbial proteins. They are involved in a broad variety of patho-physiological processes, including atherosclerosis, thrombosis, autoimmune and

infectious diseases. Factor VII Activating Protease (FSAP) is a plasma circulating serine protease, considered to be involved in haemostasis, inflammation and vascular remodeling. Key activators of zymogen FSAP *in vivo* are histones and nucleosomes which are also the main components of NETs.

Aims: We have investigated the bidirectional interactions between FSAP and NETs.

Methods: Human neutrophils were stimulated with phorbol 12-myristate 13-acetate (PMA) and various NET-related responses were measured.

Results: FSAP showed binding to NETs and promoted NET formation in response to PMA. FSAP-bound NETs were protected against degradation by *S. aureus* nuclease even though the microbicidial activity of NETs was not influenced by FSAP. Surprisingly, NETs did not activate zymogen FSAP even though they consist of known activators. However, upon DNAse treatment of NETs, there was a robust activation of purified pro-FSAP and plasma FSAP. FSAP in turn degraded histones and neutralized their cytotoxicity towards endothelial cells. We found no evidence of nucleosome releasing activity of FSAP.

Conclusions: FSAP modulates the activity of NETs by boosting their formation, binding and protecting them from degradation. On the other side, NETs bind to FSAP but do not activate it except when histones are released by DNAse. Presumably, the DNA-histone complex formation neutralizes both components with respect to FSAP activation. In this system we did not find any evidence for the nucleosome release activity of FSAP that has been described in a number of earlier reports. This study gives new insights on interactions involved in the regulation of NET functions in vascular biology.

PB 2281 | Immunology Studies on Recombinant Factor VIII Fc Fusion Protein

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Background: The main complication of prophylactic replacement therapy with factor in hemophilia A is the formation of inhibitors (neutralizing anti-factor VIII antibodies) in ~30% of severe hemophilia A patients. Further understanding of how the immune system responds to FVIII is an ongoing effort in hemophilia research. The extended half-life recombinant factor VIII Fc fusion protein (rFVIIIFc) is an effective and safe therapy to prevent and control bleeding episodes. The Fc region of this molecule is not only responsible for increasing rFVIII half-life, but also seems to promote antigen-specific tolerance, as shown in a preclinical animal model (Krishnamoorthy et al., Cell Immunol 2016), and suggested in immune tolerance induction case reports (Malec et al., Haemophilia 2016; Ragni et al., Haemophilia 2016).

Aims: The goal of this study is to characterize the interactions of the Fc portion of rFVIIIFc with the immune system.

Methods: Peripheral blood-derived human and bone marrow-derived mouse antigen presenting cells (APCs) were used to investigate the effects of rFVIII on Fc receptor (FcR) binding, signaling and cytokine production, as well as subsequent interactions and effects on T cells *in vitro*.

Results: rFVIIIFc engages FcRs on APCs, as indicated by the loss of cell surface receptor levels monitored by flow cytometry. Further, phosphorylation signals immediately downstream of the receptors were observed. These signals do not induce pro-inflammatory cytokines as seen with immune complexes, but instead induce cytokines, such as TGFβ and IL-10, and other molecules which are involved in dampening immune responses. rFVIIIFc-treated APCs co-cultured with naïve T cells influence differentiation towards a regulatory T cell phenotype.

Conclusions: The Fc portion of rFVIIIFc appears to influence the phenotype and function of antigen presenting cells.

PB 2282 | Luminex© Bead-based Assay for Early Detection of GPIIbIIIa Allo-immunization in Glanzman Thrombasthenia

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Background: Glanzmann Thrombasthenia (GT) is a severe hemorrhagic disorder due to a quantitative or qualitative defect of platelet integrin αIIbβ3 (GPIIbIIIa). In case of GPIIbIIIa deficiency, the patients are likely to develop allo-antibodies against GPIIbIIIa after platelet transfusion. To date, MAIPA (monoclonal antibody-specific immobilization of platelet antigen) test remains the gold standard method to identify antibodies in all contexts of platelet immunization.

Aims: Recently a new Luminex© bead-based platelet antibody detection assay (PAKLx©) has been designed for detection of anti HPA-1, -2, -3, -4, -5, GPIV and HLA class I allo-antibodies. We evaluated this new tool in comparison with MAIPA for detection of GPIIbIIIa allo-antibodies and follow-up of GT patients.

Methods: 52 samples from 44 GT patients (diagnosis in Strasbourg and Paris) were tested in MAIPA and PAKLx assays.

Results: MAIPA and PAKLx results were concordant in 38 cases (73%), with 24 positive and 14 negative samples, whether patients were presenting a type 1, 2 or variant Glanzmann disease. In two patients (3 samples), MAIPA result was at the threshold or positive, whereas the Luminex was negative. In 11 samples (21%), anti-GPIIbIIIa was only identified by Luminex (MFI 1000-3000) with a negative MAIPA (OD < 0.2). This group included 3 adult patients with positive MAIPA in their childhood, including one pregnant woman. For this patient, the detection of anti-GPIIbIIIa with PAKLx only (MFI around 1500), has been followed by a strongly positive result in both MAIPA (OD>1) and PAKLx (MFI> 5500) early after effective platelet transfusion for delivery.

Conclusions: Our results suggest that the PAKLx is more sensitive than the MAIPA to detect GPIIbIIIa antibodies in GT. It may be of special interest for early detection of GPIIbIIIa allo-immunization in GT during pregnancy, and help in the management of patients in obstetrical, surgical and bleeding contexts. Further studies are necessary to confront PAKLx results to platelet transfusion efficiency.

PB 2283 | Tranexamic Acid Blocks Fibrinolysis but Not Overall Plasmin Generation and Modulates the Immune Response in Patients Undergoing Coronary Artery Surgery. A Sub-study of the "Aspirin and Tranexamic Acid for Coronary Artery Surgery" (ATACAS) Trial

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Background: Cardiac surgery is associated with increased fibrinolysis resulting in enhanced bleeding, and immunosuppression making patients susceptible to infection. We reported that plasmin inhibits the ability of dendritic cells (DCs) to mediate an allogeneic response. Hence, anti-fibrinolytic agents (tranexamic acid; TXA) used to reduce blood loss, might also modulate the immune response.
Aims: To evaluate the temporal effects of TXA on plasmin generation, fibrinolysis and the immune response after cardiac surgery.
Methods: Blood was taken from 41 cardiac surgery patients randomised to TXA in the ATACAS trial. Plasma obtained prior to surgery, post-operatively, 24 and 72 h was tested for D-dimer, plasmin-anti-plasmin complex (PAP) and by clot lysis assays (CLA). Flow cytometry was performed for functional markers of myeloid and lymphoid cell populations. Multiplex ELISAs were used to detect cytokine levels. The effects of TXA (1 g, oral) was also determined in 10 healthy volunteers.
Results: TXA completely inhibited fibrinolysis (reduced D-dimer and CLA) in all patients (n=22) at the post-OP and 24h time points. However, PAP levels were increased at the same time points. As TXA inhibits lysine-dependent plasmin generation, these data indicate that lysine-independent mechanisms exist for plasmin formation in the cardiac surgery setting. Flow cytometry showed that TXA significantly increased expression of the activation marker CD83 on both classical monocytes and DCs, suggesting enhanced immune competence. Baseline levels of pro-inflammatory cytokines TNF-α and IL-6 were significantly reduced by TXA in healthy volunteers, indicating a role for lysine-dependent plasmin formation for controlling cytokine expression.
Conclusions: TXA effectively blocks fibrinolysis and modulates the immune system in healthy volunteers and cardiac surgery patients. Plasmin formation still occurs via lysine-independent mechanisms and the consequences of this finding remain to be determined.

PB 2284 | Impact of Systemic Inflammation in Lung Transplant Outcome

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Background: Primary graft dysfunction (PGD) is the leading cause of early post-lung transplantation (LTx) morbidity and mortality. Although the diagnosis of PGD has improved, its etiology remains controversial. It is hypothesized that pro-inflammatory mediators released post-LTx modulate the innate and adaptive immune responses and predispose the patient to chronic lung allograft rejection. There have been limited studies to date.
Aims: To investigate a battery of cytokines in the same patient to well characterize the inflammatory status early post-LTx, and to determine if patient subgroups who respond differently to the donor organ, as shown by the etiologies of the end-stage lung disease, have a different host immune status.
Methods: Individuals undergoing LTx for cystic fibrosis (CF) or idiopathic pulmonary fibrosis (IPF) were enrolled, excluding patients >65 yrs or undergoing re-transplantation. Serum collected pre-operatively, 1-2, 24 and 72 hrs, 7, 30 and 90 days following LTx was analyzed for inflammatory cytokines using the Randox bio-chip array. ISHLT-defined PGD grade at 24 and 72 hrs, rejection episodes, pulmonary infection and development of bronchiolitis obliterans syndrome were documented.
Results: At 72 hrs post-transplant, 2/10 CF patients and 4/12 IPF patients had PGD score ≥2. Mean serum levels of IL-6, IL-8, IL-10, VEGF, TNFα (100-500 vs < 100 pg/mL) and MCP-1 (500-800 vs 300-400 pg/mL) were notably higher in CF patients compared to those with IPF. Difference between CF and IPF patients was most striking for PGD scores ≥2. In CF patients, IL-6, IL-8, IL-10, TNFα and MCP-1 increased with increasing PGD score (2-3 vs 0-1). Higher VEGF levels were observed in CF patients with lower PGD scores.
Conclusions: Cytokine activation in the early post-transplant period is different among subsets of LTx patients. If validated, identifying the specific early cytokine response of the host can guide individualized treatment to reduce PGD and late graft failure.

PB 2285 | Protease Activated Receptor-2 (PAR-2) Serves as a Dominant Receptor for Factor VII Activating Protease (FSAP)

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Background: Protease Activated Receptors (PARs) mediate signaling for many factors from the hemostasis system and are important drug targets in vascular biology. The mechanism of action of FSAP on cells

is largely unknown but some preliminary studies suggest that PARs may be involved. To date, there are no methodical studies investigating the interaction between FSAP and PARs.

Aims: To investigate the role of PARs in mediating the cellular effects of FSAP.

Methods: HEK293 and A549 cells were transfected with PAR-1 and PAR-2 constructs with an N-terminal soluble alkaline phosphatase (SEAP)-tag. FSAP-mediated secretion of SEAP as well as ERK1/2 activation using Western blotting was determined. Point mutations were introduced in the N-terminal tethered ligand part of PAR-1 and -2 using site-directed mutagenesis to determine the exact cleavage sites involved.

Results: The cleavage of PAR-2 was much more extensive than that of PAR-1. Even though PAR-1 was a poor receptor for FSAP, ERK1/2 signaling was observed when SEAP-PAR1 was expressed in cells. In PAR-2 over-expressing cells a robust FSAP- mediated ERK1/2 activation was observed. Recombinant serine protease domains (SPD) of wild type (WT) FSAP, but not the Marburg I isoform of FSAP, could cleave and signal through PAR-2. Arg31, Arg36, Lys34 and Lys41 in the N-terminal of PAR-2 were mutated to Gly to further identify the exact cleavage sites. PAR-2*K34G* completely abolished, PAR-2*R36G* partially abolished and the others exhibited no differences in the FSAP mediated cleavage and ERK1/2 signaling compared to WT PAR-2.

Conclusions: FSAP cleaves PAR-1 and PAR-2 and induces ERK1/2 signaling in cells. PAR-2 serves as a dominant receptor for FSAP and K34 and R36 in PAR-2 are important for the FSAP mediated cleavage and cell signaling. PAR-3 and PAR-4 are currently being tested. This molecular characterization of the actions of FSAP via PARs will help to define the broad spectrum of the actions of FSAP beyond hemostasis.

PB 2286 | Protective and Detrimental Effects of Anticoagulation in a Mouse Model of Multiple Sclerosis

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Background: Multiple sclerosis is an autoimmune disease of the central nervous system. Blood-brain barrier damage, activation of coagulation and extravascular fibrin deposition have been demonstrated in a mouse model of disease (experimental autoimmune encephalomyelitis - EAE). Inhibition of thrombin or fibrin-microglia interactions reduces severity of EAE.

Aims: Determine the mechanism by which tissue factor (TF)-dependent activation of coagulation contributes to the EAE progression.

Methods: To induce EAE, 10 weeks old C57Bl/6J female mice (n=15-25 per each group) were immunize with MOG35-55 peptide. A clinical score reflecting the degree of paralysis was recorded daily for 30 days. Spinal cords were collected for histological evaluation of myelin loss and inflammatory cells infiltration. mRNA expression of chemokine/cytokines and markers of immune cells were also analyzed. Genetic and pharmacologic approaches were used to inhibit the TF-thrombin pathway.

Results: Clinical score, neuronal damage and macrophages and T cells infiltration into the spinal cord were significantly reduced in EAE mice expressing low levels of TF (1% of wild type) as compared to EAE mice with normal levels of TF. CCL2, CCL20, IL-17A, TNFA mRNA expression were lower in EAE low TF mice. Treatment with the anti-TF antibody (20mg/kg, ip, every 3 days) or the thrombin inhibitor dabigatran (60 or 120mg/kg, daily, oral gavage) provide similar protection in EAE mice. However, all anticoagulant approaches resulted in bleeding complications in 15-20% of EAE mice. In contrast, astrocyte-specific deletion of TF or total deficiency of PAR-2, reduced EAE progression without bleeding complications. PAR-1 deficiency provided only a modest protection whereas PAR-4 deficiency had a detrimental effect in EAE mice.

Conclusions: Our study suggests that various components of the TF-initiated coagulation response differentially regulates central nervous system hemostasis and autoimmune inflammation during EAE.

PB 2287 | Crosstalk between Epidermal Growth Factor Receptor (EGFR) and TF/FVIIa/PAR2 Pathway Mediates Chemotherapy Resistance in Cervical Cancer

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Background: Cervical cancer (CC) is the fourth most common cancer in women worldwide. Cisplatin-based chemotherapy has been established as the standard treatment for advanced disease. However, these patients have survival rates lower than 50% and novel therapeutic strategies are needed. EGFR is an oncogene overexpressed in CC. In addition, it was reported that thrombin transactivates EGFR in other models.

Aims: We have employed CC cell lines and malignant tissues to evaluate the role of EGFR, tissue factor (TF), factor VIIa (FVIIa) and protease activated receptor-2 (PAR2) in resistance to cisplatin.

Methods: The expression of EGFR and TF was evaluated by immunohistochemistry in malignant tissues biopsied from 11 patients. In CASKI and C33A cell lines, the expression of EGFR, TF and PAR2 was analyzed by qRT-PCR and Western Blot. SLIGKL-NH2 peptide and FVIIa were used as PAR2 agonists, while cetuximab was used as EGFR inhibitor. The activation of EGFR-ERK signaling pathway was monitored by Western Blot. The sensitivity to cisplatin was evaluated by cell death assays.

Results: The more aggressive cell line, CASKI, showed high expression levels of EGFR, TF and PAR2 when compared to C33A. PAR2 agonists activated ERK and EGFR, and induced expression of protumoral genes through an EGFR-dependent mechanism. PAR2 agonist peptide protected CASKI cells against apoptosis induced by cisplatin, while cetuximab reversed this phenomenon. Treatment of CASKI cells

with cetuximab also decreased TF expression levels, suggesting that EGFR activation upregulates TF. Lastly, analysis of TF and EGFR expression in malignant tissues showed that EGFR is expressed in all tumors, while TF is expressed in 8/11 tumors (73%).

Conclusions: Our results suggest that EGFR is an effector of TF/FVIIa/PAR2 pathway in CC cells, promoting chemoresistance. EGFR also upregulates TF expression, revealing the presence of a positive feedback loop. EGFR, TF and PAR2 emerge as novel targets for CC treatment.

PB 2288 | Monocyte Activation by Thrombin Leads to an Upregulation of Molecules Involved in Fibrinolysis

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Background: Thrombin, a serine protease, plays a crucial role in hemostasis and inflammation. Next to its ability to activate fibrinogen, it also functions as a signal molecule via the activation of protease-activated receptors PAR1,3 and 4 on a variety of cell types including monocytes. Monocytes can be divided into three subsets: classical monocytes (CM, CD14++,CD16-), intermediate monocytes (IM, CD14++,CD16+) and non-classical monocytes (NCM, CD14+,CD16+).

Aims: The aim of this project was to evaluate how thrombin acts on monocytes and monocyte subsets in vivo and in vitro.

Methods: LPS treated and untreated whole blood samples were stained with antibodies against CD14, CD16 and PAR1, 3 and 4 and were analysed via flow cytometry. Moreover, whole blood samples were incubated with brefeldin A and were then stimulated with LPS for 4h. Subsequently, samples were stained for flow cytometry. In addition, monocytes were stimulated in vitro with TRAP-6 or additionally pre-treated with LPS and RNA was isolated for qPCRs. Furthermore, monocytes were isolated from blood samples of volunteers who received a dose of vorapaxar prior to LPS infusion and RNA was isolated for qPCRs.

Results: CD16 positive monocytes, namely IMs and NCMs have significantly more PAR1 compared to CMs. IMs also express significantly more PAR3 and NCM more than CMs. All three subsets show a similar expression pattern for PAR4. When monocytes are activated with LPS they respond with an upregulation of PARs on their surface in vitro and in vivo and experiments with brefeldin A showed that this might be because of an intracellular storage pool. When activated with LPS and stimulated with TRAP-6, monocytes react with an increased expression of PAI-1, uPA and TFPI. When PAR1 activation is blocked by vorapaxar in vivo, PAI-1 and TFPI are significantly down regulated.

Conclusions: In conclusion, based on our data, thrombin is not just involved in coagulation and inflammation but also activates fibrinolytic genes in monocytes.

PB 2289 | Activated Protein C (APC) via PAR1 Protects Neurons from Death in the Model of Neuroinflammation in vitro

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Background: The hemostasis system is involved in important processes: inflammation, tissue repair, clot formation, and others. Serine proteases, thrombin and activated protein C (APC) play an important role in regulation of the inflammatory process. These proteases via the activation of specific protease-activated receptors (PAR) demonstrate the multidirectional effect due to biased agonism. Activation of PAR1 may be realized by peptides - analogs „tethered ligand". At contrast to thrombin isn't clear what types of peptides can demonstrate the protective effect on cells like APC.

Aims: The aim of this study is to investigate the influence of APC and synthetic peptide, liberated by APC from PAR1 on neurons in the model of neuroinflammation induced by the toxic effects of endotoxin-activated mast cells.

Methods: The effects of APC (10 nM) and peptide NPNDKYEPF-amide (AP9, 10 μM) on survival of neurons were estimated at toxicity induced by the lipopolysaccharide (LPS)-activated mast cells. Morphological assay of neuronal death by Syto-13 (vital dye), Hoechst 33342 (apoptosis) and EthD (necrosis).

Results: Using a model of the neuroinflammation we shown that APC and AP9 protect neurons from death induced by LPS-activated mast cells. The pretreatment activated mast cells with APC(10nM) or AP9 (10μM) decreased the number of apoptotic neurons to 1,9 and 1,8 fold, respectively. Receptor mechanism of APC and AP9 action was identified by the blocking of PAR1 on mast cells or neurons by anti-PAR1 antibodies or PAR1-inhibitor SCH 79797. We shown that the blockage of PAR1 abolished protective effects of APC and AP9 and increased apoptotic cells at 1,4 and 2,8 fold, respectively. Thus, PAR1 was required for the protective effect of the APC and AP9.

Conclusions: Thus, at the first time we have demonstrated the neuroprotective effects of APC and AP9 in the neuroinflammation. The protective effects of APC and AP9 on mast cells and neurons are the same and realized through PAR1. Thus, the new peptide AP9 is a functional analog of APC.

PB 2290 | Role of Protease-activated Receptor 4 in Regulating Platelet-leukocyte Interactions in Whole Blood

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Background: Activated platelets bind leukocytes such as neutrophils and monocytes via expression of P-selectin on the platelet surface, which binds to leukocyte PSGL-1. Platelet aggregation to monocytes or neutrophils may be a more sensitive marker of disease states such as atherothrombosis than platelet-platelet aggregation. The thrombin receptor PAR-4 is an emerging target in thrombosis, although the effect of PAR-4 inhibition on platelet-leukocyte interactions is unknown.
Aims: This project aims to determine the effect of PAR-4 inhibition on platelet P-selectin expression, dense granule release, and platelet binding to neutrophils and monocytes.
Methods: Novel small molecule PAR-4 inhibitors were used to determine the role of PAR-4 in platelet activation, dense granule secretion, and platelet-leukocyte binding. Platelet dense granule secretion was assessed by luminescent ATP detection assay. Flow cytometric assays in washed platelets or whole blood were used to determine platelet P-selectin expression and platelet-leukocyte interactions using markers for platelets, neutrophils, and monocytes.
Results: Inhibition of PAR-4 impaired P-selectin expression following activation by PAR-4 agonist peptide but not by PAR-1 agonist peptide or collagen-related peptide (CRP), demonstrating inhibitor specificity. Platelet P-selectin expression induced by low but not high concentrations of thrombin was impaired by PAR-4 inhibitors. Platelet binding to neutrophils or monocytes was also impaired by PAR-4 inhibitors following activation by PAR-4 agonist peptide but not by PAR-1 agonist peptide or CRP. PAR-4 inhibitors demonstrated a similar inhibition of platelet dense granule secretion following stimulation with PAR-4 agonist peptide or low concentration thrombin but not PAR-1 agonist peptide or CRP.
Conclusions: Our results demonstrate inhibition of PAR-4 via small molecule inhibitors impairs platelet P-selectin expression, dense granule release, and platelet-neutrophil and platelet-monocyte interactions.

PB 2291 | Activation of PAR2 by Factor Xa Increases the Permeability across Cultured Endothelial Cell Monolayers

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Background: It has been shown recently that activated factor Xa (fXa) exerts direct influences on a wide variety of cells through activation of protease activated receptor 2 (PAR2). In this study, we have explored the influence of PAR2 activation on the permeability of human artery endothelial cells, and compared this to the outcome from incubation with VEGF.
Aims: To examine the influence of activation of PAR2 on the permeability of endothelial cell monolayer.
Methods: Human coronary artery endothelial cells (6×10^4) were seeded out within Transwells® with permeable membrane (pore size 3 μm) and placed in 24-well plates and cultured in endothelial growth medium for 4 days to attain impermeable monolayers. The cells were then adapted to serum-free medium for 2 h prior to assaying. To assess the change in the permeability across the monolayer, blue dextran MW 2×10^6 (1 μg/ml final concentration) was added to the upper chamber. The cells were then incubated with PAR2-agonist peptide (AP) (SLIGKV-NH; 20 μM), fXa (10 nM), VEGF (25 ng/ml) or used without activation. The activator reagents were added to the upper chambers and incubated at 37°C for up to 60 min. The seepage of dextran blue across the monolayers was then monitored by measuring the absorption of the medium in the lower chambers at 640 nm.
Results: The permeability of endothelial cell monolayer progressively increased on incubation with PAR2-AP reaching 36% and 45% at 15 and 30 min, respectively. Furthermore, incubation of the endothelial cell monolayer with fXa resulted in a delayed increase in permeability, reaching < 2% and 85% at 15 and 30 min, respectively. Finally, incubation of cells with VEGF induced a rapid but transient increase in permeability reaching 82% and 60% at 15 and 30 min, respectively.
Conclusions: In conclusion the activation of PAR2 on endothelial cells either through fXa activity or using a synthetic activator peptide promotes endothelial cell permeability which is comparable to that observed by using VEGF.

PB 2292 | The Influences of Diabetes and Inflammation on the Effects of Thrombin and Activated Protein C on Mast Cell Secretion

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Background: According to World Health Organization, over 422 million people have diabetes. This disease has many complications such as retinopathy, nephropathy, diabetic foot ulcers, etc. Most of them are accompanied with inflammation. Mast cells (MC) are important participants of inflammatory response. It is known serine proteases of hemostasis via protease-activated receptors (PAR) regulate the activity of mast cells. But it isn´t clear what role of thrombin (Th) and activated protein C (APC) play in the regulation of mast cell functions at diabetes.
Aims: The aim of this work is to study the influence of diabetes on the Th and APC-regulated secretion of MC at inflammation.

Methods: The experiment was performed on the male Wistar rats (250-300g). Diabetes was induced by a single injection of streptozotocin (STZ, 60 mg/kg). Inflammation was modulated by 4% thioglycolate. MC were obtained from rats peritoneal cavity. The level of MC´s secretion was estimated by the analyze of released β-hexosaminidase from cells.
Results: The single administration of STZ leads to the development of diabetes after week and the increase of the plasma glucose levels and the decrease of the body weight compared to control group rats confirms this. At present study we found inflammation leads to increase a spontaneous secretion of MC at 2-fold. At the same time the diabetes doesn´t influence on a spontaneous secretion of MC. The effects of Th (50nM) and APC (10nM) on MC secretion are different at inflammation and diabetes. The proteases induce the raise of MC secretion at inflammation, but at diabetes Th and APC lead to lower the secretion of MC compared to control rats. The analyze of the proteases effects on MC at inflammation and diabetes at the same time shows that the MC´s responses are similar to their response at diabetes alone, while the spontaneous secretion is similar to their reactions at inflammation.
Conclusions: Thus, the impact of diabetes on the effects of PAR1-agonists is more pronounced with compare to inflammation.

DIAGNOSTICS AND OMICS

PB 399 | Failure of Wells Score and D-dimer to Safely Exclude Diagnosis of Deep Vein Thrombosis: A Single Centre Experience of over 3500 Patients

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Background: In England diagnosis of deep vein thrombosis (DVT) is guided by the National Institute of Health and Care Excellence (NICE) recommendations that include a 2-level Wells score and D-dimer testing. Patients with Wells score < 2 (DVT unlikely) and negative D-dimer (< 500 μg/L) may be discharged without compression ultrasonography (CUS). DVT diagnosis at the Royal London Hospital includes additional consideration of risk factors before CUS is omitted. The resulting additional CUS scans enabled a comparison of this diagnostic pathway with that of NICE.
Aims: To estimate the failure rate for proximal DVT diagnosis had NICE guidance been followed using Wells score < 2 and D-dimer < 500 μg/L to omit CUS.
Methods: Data obtained from referrals to the DVT diagnostic service included patient demographics, DVT risk factors, Wells score and D-dimer. Data were analysed using MS Excel. We simulated a patient pathway to estimate diagnostic failure rate in the Wells unlikely group with negative D-dimer.
Results: 3837 referrals were made from 2010 to 2016. Upper limb DVT referrals or those with incomplete D-dimer or Wells score data (n=276) were excluded. Of 3561 referrals included, 3381 (95%) were scanned one or more times. 360 DVTs were diagnosed on 4282 scans. DVTs were more prevalent in those aged < 50 years compared to those aged >50 years (IR 12.0 v 8.6, p=0.009), with an overall median age of 53 years. In the Wells unlikely group with negative D-dimer (n=1156), 27 DVTs would have been missed had NICE guidance been followed (failure rate 2.3%; 95% CI 1.5-3.4%). In patients aged < 50 years the failure rate was higher (3.6%; 95% CI 2.3-5.3%, p=0.12).
Conclusions: Following NICE guidelines would have reduced the proportion of patients scanned from 95% to 70% but DVTs would have been missed in an unacceptably high number of patients. A possible explanation for this may be the higher prevalence of DVT in the younger patients. We suggest considering specific risk factors in addition to the Wells score before omission of CUS.

PB 400 | Residual Vein Thrombus Does Not Predict for Recurrent Vein Thrombosis in the ASPIRE Study

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Background: In other studies Residual Vein Thrombosis (RVT) as assessed by ultrasound has been associated with recurrent vein thrombosis, other vascular events and mortality.
Aims: We examined whether RVT in the ASPIRE was associated with subsequent clinical events.
Methods: In the ASPIRE study 822 patients who had completed 6-24 months of anticoagulant therapy after a first unprovoked VTE were randomized to aspirin, 100 mg daily, or placebo for up to 4 years. At randomisation all patients with deep vein thrombosis (DVT) undertook an ultrasound assessment for RVT. Sites recorded the presence of RVT in local CRF. Additionally RVT reports were retrieved and reviewed for the presence and extent of RVT by 2 authors (TAB, AMB). The association of RVT with recurrent vein thrombosis was evaluated using Cox regression models.
Results: In the ASPIRE study, DVT was present in 583 of 822 patients (71%) enrolled. Sites recorded RVT data in 580 patients. Ultrasounds reports were available and reviewed in 412 patients with RVT reported in 265 (64%). The presence of RVT was more prevalent in males. There are 72 first recurrent VTE events, in the 412 patients with residual thrombosis data: 32/147 (22%) without RVT and 40/265 (15%) with RVT. There was no association between presence of RVT and time to first recurrent VTE event when adjusted for study treatment (HR = 0.76, 95% CI 0.48-1.21, p=0.25). Analysis of the 580 patients with site-recorded RVT information showed similar results. There was also no association between RVT and major vascular events (HR 0.88, 95% CI 0.56-1.38) or net clinical benefit (HR 0.96, 95% CI 0.62-1.47) after adjustment for treatment.

Conclusions: In patients who had completed anticoagulation therapy after unprovoked VTE the presence and the extent of RVT was not associated with recurrent vein thrombosis or other clinically important events.

PB 401 | Utility of Thromboelastometry Analysis in Patients with Mild Bleedings Disorders

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Background: Viscoelastic methods are regarded as promising concept to overcome the limitations of conventional laboratory assays in patients with haemostatic disorders, particularly in the perioperative setting. Their performance regarding frequently occurring mild bleeding disorders (MBD) such as von Willebrand disease, platelet function disorder, or mild haemophilia is however unknown.

Aims: We conducted a prospective cross-sectional study to investigate the value of thromboelastometry analysis for diagnosis and prognosis of MBD.

Methods: Thromboelastometry analysis (ROTEM®) was conducted in all consecutive patients referred between January 2011 and March 2013 with a suspected bleeding disorder. Diagnostic work-up was done according to current guidelines.

Results: MBD was diagnosed in 111 out of 217 patients (52.1%), median age was 40.1 years, IQR 28.9, 59.2; 67.6% were female. Possible or definite platelet function disorder was diagnosed in 50 patients (42.7%), von Willebrand disease (vWD) in 24 patients (11.1%), mild haemophilia in 4 patients (1.8%), mild factor XI deficiency in 2 patients (0.9%), low von Willebrand factor associated with blood group 0 in 13 patients (6.0%), anticoagulation treatment in 3 patients (1.4%), and a systemic disorder in 15 patients (6.9%). Presence of MBD was not associated with a significant difference in thromboelastometry parameters (CT EXTEM, MCF EXTEM, CT INTEM, MCF INTEM, MCF FIBTEM). In addition, no significant differences were observed with regard to categories of the ISTH bleeding assessment tool. Minor differences - all within the established ROTEM reference ranges - were noted for some MBD: mild haemophilia (MCF EXTEM, MCF INTEM, MCF FIBTEM), definite vWD type 1 (MCF FIBTEM), anticoagulation treatment (CT EXTEM), and systemic disorders (CT EXTEM).

Conclusions: Our data do not support the utility of thromboelastometry analysis for diagnosis, prognosis or management in patients with mild bleeding disorders, particularly in the perioperative setting.

PB 402 | Increased Maximum Lysis of ROTEM in Pregnancy: It Is Not Hyperfibrinolysis

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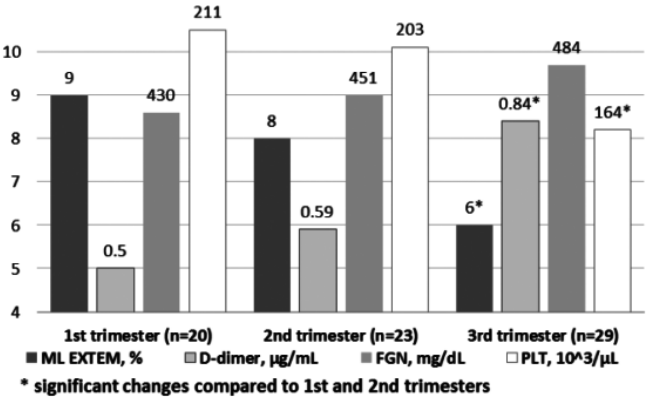

FIGURE 1 Changes in hemostatic tests across trimesters of pregnancy

Background: Thromboelastometry (ROTEM) analysis provides visual tracings of blood coagulation from clot formation, stabilization, until clot dissolution. While Maximum Lysis (ML) values on EXTEM >15% is usually attributed to hyperfibrinolysis, ML < 15% may still correlate with bleeding associated with excessive fibrinolysis.

Aims: The aim of the study was to evaluate ML in ROTEM variation during normal pregnancy and contribution of hemostatic system components in ML values.

Methods: Citrated blood was collected from 72 pregnant women of 32±4 years old and EXTEM, INTEM, and FIBTEM were performed on ROTEM (IL, USA), while routine coagulation tests were performed on STAR Evolution analyzer (Stago, USA) as a part of reference range establishment. Platelet count (PLT) was performed on Sysmex XE-5000 analyzer (Sysmex America, USA) in citrated specimen. All data are presented as mean±SD and data analysis was performed using one way ANOVA and Pearson correlation (SPSS v23, 2016, IBM, USA). P value of < 0.05 was considered as significant.

Results: There were 20 women in 1st (11±3 weeks of gestation), 23 in 2nd (21±5 weeks) and 29 in 3rd trimester (33±3 weeks) of pregnancy; no significant difference in ML EXTEM, fibrinogen (Fgn), PLT, or D-dimer was found between 1st and 2nd trimesters. ML EXTEM and PLT were lower, while Fgn and D-dimer were higher in the 3rd trimester (Figure 1). D-dimer and Fgn showed a negative correlation and PLT showed positive correlation with ML EXTEM or INTEM. ML showed the highest correlation with PLT to Fgn ratio (Table 1) and ML >10% is associated with average PLT/Fgn as 1:2, while ML < 5% with 1:3 ratio. ML FIBTEM was 3±2% in 1st trimester and 0% in 2nd and 3rd trimesters (p< 0.001).

TABLE 1 The correlation of ML with other hemostatic tests

Pearson correlation, p value	D-dimer	FGN	PLT	PLT/FGN ratio
ML EXTEM	-0.20, 0.09	-0.30, 0.001	0.41, <0.001	0.59, <0.001
ML INTEM	-0.29, 0.01	-0.37, 0.001	0.24, 0.04	0.56, <0.001

Conclusions: ML on EXTEM and INTEM in pregnant women does not reflect physiologic activity of fibrinolytic system as was estimated by D-dimer and ML FIBTEM remained 0. It rather reflects platelet clot retraction that is dependent on PLT to Fgn ratio in citrated plasma.

PB 403 | Significant von Willebrand Disease Can Be Missed if a von Willebrand Factor Activity Assay Is Not Performed

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Background: British Committee for Standards in Haematology guidelines for the diagnosis of von Willebrand disease (vWD) state that a diagnosis can be made when the von Willebrand factor (vWF) activity is less than 30iu/dL. On a recent World Federation of Hemophilia (WFH) twinning visit it was observed that some laboratories with restricted budgets may only perform assays for vWD in patients with reduced FVIII activity, and may only test for FVIII if the APTT is prolonged. In patients with prolonged APTT but normal FVIII and FIX, a diagnosis of "FXI or FXII deficiency" is assumed.

Aims: How many significant diagnoses would be missed if the observed regime was applied to samples already tested?

Methods: Results of 397 vWD screens performed over a 12 month period at our UK centre (including 147 samples from 108 patients with known vWD) were audited.

Results: 21 samples were found to have normal APTT (ratio ≤1.2) but reduced FVIII activity (< 50iu/dL), including 2 type 1 vWD, 6 mild vWF reductions, 12 type 2 vWD and one mild haemophilia A carrier.

26 samples had normal APTT and FVIII but low vWF antigen (< 50iu/dL): 2 type 1, 16 mild vWF reduction, 7 type 2 and one post Wilate.

21 samples had normal APTT, FVIII and vWF antigen but reduced vWF activity (< 50iu/dL): 5 mild vWF reduction, 10 type 2, 2 post Wilate and 4 normals.

Without a functional assay, only 10% of type 1 and 31% of type 2 patients would have been correctly identified as having vWD. Furthermore, 14% of type 1 and 9% of type 2 patients would have been misdiagnosed as having FXI or FXII deficiency. No patients with severe type 3 vWD would have been misdiagnosed.

Conclusions: When budgets are restricted, laboratories should use a functional activity assay for vWF such as that described in the Laboratory Manual available from the WFH in preference to a latex immunoassay, and should assay it in patients with a bleeding history regardless of the APTT or FVIII. If budgets are sufficient for a latex immunoassay, an activity assay is prefered to an antigenic one.

PB 404 | Validation of Flow Cytometric Mepacrine Uptake and CD63 Expression in the Diagnosis of Storage Pool Disease

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Background: Storage Pool Disease (SPD) is a bleeding disorder characterized by a reduced number of platelet dense granules, resulting in impaired secondary platelet activation. Diagnosis of SPD depends on the measurement of platelet ADP content, since platelet aggregation is often normal in these patients. However, this test is time consuming, requires a large blood volume and cannot be performed in thrombocytopenia. Flow cytometric analysis of platelet uptake of mepacrine, a fluorescent acridine derivative with affinity for adenine nucleotides, and the dense granule marker CD63 have been suggested as alternatives, but these approaches lack standardization, which precludes their use in a diagnostic setting.

Aims: To compare standardized flow cytometric analysis of platelets mepacrine uptake and CD63 expression with measurement of platelet ADP content during the diagnostic workup for SPD.

Methods: Platelet dense granule content was assessed in 10 patients with known SPD with flow cytometry and TEM, and compared with platelet ADP content. Reference values for flow cytometry were based on the 2.5th percentile of the response in 60 healthy controls.

Results: All SPD patients had a reduced number of dense granules as determined with TEM. 8 out of 10 SPD patients showed decreased mepacrine uptake, whereas 9 out of 10 patients showed reduced CD63 expression after platelet stimulation. In contrast, only 5 out of 10 patients showed reduced P-selectin expression after platelet stimulation. Mepacrine binding was in good agreement with platelet ADP content (κ=0.722) and TEM (κ=0.800). Additionally, CD63 expression showed very good agreement with platelets ADP content (κ=0.851) and TEM (κ=0.900).

Conclusions: Standardized analysis of mepacrine uptake and CD63 expression with flow cytometry can be used to detect SPD in whole blood samples. Both require a minimal amount of blood and are promising tools in the diagnostic workup of SPD.

PB 405 | Post-analytical External Quality Assessment - Interpretation of UK NEQAS (Blood Coagulation) Results

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Background: External Quality Assessment (EQA) is a useful tool to improve and maintain standards of laboratory performance. EQA is usually directed at analytical processes, but evaluation of pre- and post-analytical phases is also required.

Aims: Post analytical processes are based on interpretation of results, and we describe here data from UK NEQAS for Blood Coagulation (BC) exercises in respect of data interpretation for screening tests, factor assays and genetic investigations.

TABLE 1

	Total number of patients, n	PE unlikely Wells score and negative AADD, n	PE prevalence, %	Mean age, y	Efficiency % (95% CI)	Failure rate % (95% CI)
All centers	6,756	2,347	22	56	33 (25-41)	0.89 (0.58-1.4)
D-dimer assays						
- INNOVANCE	1,458	402	18	63	25 (19-31)	0.25 (0.03-1.7)
- VIDAS	2,671	1,044	23	54	35 (23-43)	0.86 (0.44-1.6)
- STA-Liatest	613	189	31	61	30 (27-35)	0.46 (0.03-6.6)
- Tina-quant	2,014	712	25	53	35 (33-38)	1.4 (0.76-2.6)

Methods: Participants interpret INR, heparin dosage (HD) and D-Dimer data on their test result and brief patient details.

Results: For INRs >90% agreement on dosage is achieved in most exercises. In 1 exercise 73% of centres reported a patient adequately dosed and 26% overdosed -interpretation differed amongst centres using different reagents. For HD, a sample with 0.34u/ml anti-Xa activity was considered underdosed by 77% of 618 centres and adequate by 21% (of which 10% reported results below their therapeutic range). For D-Dimer, a sample which 93% of 641 centres reported as VTE unlikely was reported as VTE not excluded by 52% of centres using one reagent. There is >90% agreement in FVIII assay interpretations (normal or abnormal) when levels are normal or low, but for samples with borderline FVIII:C (44 -57u/dl) concurrence fell to 60% in one exercise. Sometimes interpretation of results does not correlate with reported reference ranges.

Historically, diagnostic errors up to 38% of centres were made in thrombophilia screening EQA; exercises in 2016 demonstrated >92% achieving a correct diagnosis. For centres performing genetic investigations for haemophilia, UK NEQAS BC exercises have shown interpretation failure rates average 2% in each survey, double the rate of analytical failures.

Conclusions: Post analytical EQA can highlight differences in reagent sensitivity, in use and application of reference ranges, and in interpretation of laboratory data, and is an important area in which standardisation and improvements are required.

PB 406 | Different D-dimer Assays Have Similar Performance Using the Age-adjusted Threshold for the Diagnosis of Pulmonary Embolism

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Background: Among patients with clinically suspected pulmonary embolism (PE), imaging can safely be withheld based on a "PE unlikely" Wells score and a D-dimer below the age-adjusted D-dimer (AADD) threshold. Several D-dimer assays have been used to support AADD testing, but their performance in ruling out PE is unclear.

Aims: To compare the efficiency and failure rates of different D-dimer assays in ruling out PE using the AADD threshold.

Methods: Individual patient data from six prospective studies in which the diagnostic management of PE was guided by the Wells rule and D-dimer testing were used. The efficiency and failure rate of AADD testing were evaluated overall and for the four assays separately (INNOVANCE, VIDAS, STA-Liatest, and Tina-quant) in a one-stage random effects meta-analysis. Efficiency was defined as the proportion of patients in whom imaging could be withheld based on a "PE unlikely" Wells score and a D-dimer below the age-adjusted threshold, defined as ≤500 µg/L or as the patient's age times 10 µg/L in those >50 years. The failure rate was defined as the proportion of patients with a "PE unlikely" Wells score and negative AADD with confirmed venous thromboembolism (VTE) at baseline or during 3-month follow-up.

Results: Information on the D-dimer assay was available for 6,756 patients. The mean age was 56 years and 22% had PE. The efficiency ranged from 25% (95% CI 19-31%) for INNOVANCE to 35% (95% CI 33-38%) for Tina-quant (Table 1). Failure rates ranged from 0.25% (95% CI 0.03-6.6%) with INNOVANCE to 1.4% (95% CI 0.76-2.6%) for Tina-quant. In a multivariable analysis, adjusting for age, sex, immobilization, previous VTE, and cancer, no significant differences in efficiency between the assays were found (Table 2).

TABLE 2

D-dimer assay	Odds ratio* for efficiency (95% CI)
- INNOVANCE	1 (reference)
- VIDAS	1.1 (0.77-1.4)
- STA-Liatest	1.0 (0.82-1.3)
- Tina-quant	1.2 (0.89-1.6)

* adjusted for age, sex, immobilization, previous VTE, and cancer.

Conclusions: In this indirect comparison, all four evaluated D-dimer assays had a comparable efficiency and safety. Hence, for an AADD strategy all assays can be applied.

PB 407 | Safety of Multidetector Computed Tomography Pulmonary Angiography to Exclude Pulmonary Embolism in Patients with a Likely Pretest Clinical Probability

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Background: In patients with suspected pulmonary embolism (PE) classified as having a likely pre-test clinical probability, the necessity of performing additional testing after a negative multidetector computed tomography pulmonary angiography (CTPA) remains a matter of debate.

Aims: The aim of this study was to assess the safety of excluding PE by CTPA without additional imaging in patients with a likely pre-test probability of PE.

Methods: We retrospectively analysed patients included in two multicentre management outcome studies that assessed diagnostic algorithms for PE diagnosis.

Results: 2522 outpatients with suspected PE were available for analysis. Out of these 2522 patients, 845 had a *likely* clinical probability as assessed by the simplified revised Geneva score. Of all these patients, 314 had the diagnosis of PE excluded by a negative CTPA without additional testing, and were left without anticoagulant treatment and followed-up for three months. Two patients presented with a venous thromboembolic event (VTE) during follow-up. Therefore, the three-month VTE risk in likely probability patients after a negative CTPA was of 2 / 314 (0.6%; 95% CI 0.2%-2.3%).

Conclusions: In outpatients with suspected PE and a likely clinical probability, CTPA seems to be able to safely exclude PE, with a low three-month venous thromboembolic event rate, similar to the VTE rate following the gold standard pulmonary angiography. Additionnal testing may thus not be necessary in these patients.

PB 408 | Familial Multiple Coagulation Factor Deficiencies - Need for Deeper Clinical and Scientific Assessment

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Background: The familial multiple coagulation factor deficiencies (FMCFDs) are a group of rare haemostatic disorders of genetic origin, where deficiency of more than one coagulation factor is diagnosed. They may present significant challenges in laboratory diagnosis, prediction of bleeding risk and in treatment.

Aims: Collecting laboratory and clinical data of FMCFD patients.

Methods: NGS of FMCFD patients.

Results: 67 FMCFDs patients, categorized in three groups, have been diagnosed in our center. The 1-st group covers FMCFD patients arising from co-inheritance of independent coagulation factor deficiencies - FVII/FX(7), FV/FVII(4), FVIII/FG (3), FVII/FVIII(2), FVIII/FXI(2), FX/FV(2), FVIII/FX(1), FVIII/FIX(1), FXI/VW(1), FV/VWF(1), FXI/FXIII (1), FXI/FG(1), FX/FXIII(1), FII/FXI(1), FII/FVII(1), FV/FG(1), PC/PS(1), PC/FG(1), AT/VWF(2). The 2-nd group comprises FMCFDs arising from a single genetic defect, best represented with combined FV/FVIII(21) deficiency. The identified molecular genetic defects lay in either *LMAN1 or MCFD2* genes. Combined VKDCF deficiency is the second representative of the group: deficiency is connected with mutations either in *GGCX or VKORC1* genes. The 3-rd group FMCFDs includes deficiency due to cytogenetic abnormalities as large deletion of the chromosome 13 end, including FVII and FX (5).

Conclusions: As FMCFDs is an extreme rare disorder, collecting and deeper investigation of patients with such defects in the blood coagulation will provide important insights into the pathogenesis of the single coagulation factor deficiency disorders, will improve the clinical management of these patients and will facilitate prediction of inheritance patterns within kindred. All this emphasizes a clear need for co-operation between clinicians and scientists and establishment of FMCFDs as register allowing collection of valuable information on these disorders with respect to prevalence, diagnostic events, clinical manifestations, treatment strategies, as well as disease and treatment-related complications.

PB 409 | Performance of Anti-factor Xa Heparin Assays in External Quality Control Surveys

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Background: The treatment of patients with, both unfractionated heparin (UFH) and low-molecular weight heparin (LMWH), is mostly monitored by the measurement of plasma anti-Factor X_a activity. The heparin assays use various reagents and methodologies.

Aims: The aim of this work is to describe the state-of-the-art of the main commercial methods and to have an overview of their standardization, by using external quality assessment (EQA) results of UFH and LMWH assays, obtained from ProBioQual (PBQ), a French proficiency testing association.

Methods: Participating laboratories (360 in 2016) received, per year, 16 human citrated plasmas lyophilized specimens (8 spiked with UFH;

8 with LMWH) at 4 different concentration levels. Statistical evaluation was performed according to the ISO guideline 13528 by applying robust algorithm A to calculate two consensus values: the mean of all participant's results (AP) or of peer group (PG). The methods imprecision is quantified by the inter-laboratories coefficient of variation (CV), and the inaccuracy is evaluated as median (bias50) and 90th percentile (bias90) bias of laboratory results from consensus value (AP or PG).

Results: The inter-laboratories dispersion is inversely proportional to the UFH level: the AP CV is 25.6% for 0.21 U/mL and 6.3% for 0.69 U/mL. This inverse relationship is less pronounced for LMWH: AP CV decreases from 12% to 6.9% for a range of 0.40 -1.00 U/mL.

The AP bias90 (41.10%) is much higher than the PG bias90 (25%) for 0.20 U/mL UFH. Between 0.39-1.03 U/mL, the AP and PG bias90 decrease, respectively, from 19.45% to 13.33% and from 15.59% to 10.21%. For LMWH, AP bias90 are always higher than the PG bias90, showing large dispersion between the different methods consensus values.

Conclusions: In this work we show that methods imprecision and inaccuracy are the highest for the lowest UFH and LMWH levels. In the EQA surveys, laboratories results should be assessed using, in priority, the PG consensus values.

PB 410 | Ex-vivo Plasma Clot Formation and Lysis as a Universal Measure for Anticoagulation in Patients on Vitamin-K-antagonist, Dabigatran, Rivaroxaban, Apixaban, or Low-molecular-Weight Heparin

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Background: There are specific tests for quantifying anticoagulation for vitamin-K-antagonists (VKA), low-molecular weight heparins (LMWH), and direct oral anticoagulants (DOACs) separately, but there is no consistent test across all.

Aims: To quantify ex-vivo plasma clot formation and lysis with one test, consistently across different anticoagulants.

Methods: We obtained citrate plasma samples from 328 atrial fibrillation (AF) patients on anticoagulation and 24 control AF patients but no anticoagulation. Anticoagulation intensity was measured by INR for VKA patients (all phenprocoumon), anti-Xa for LMWH patients, and by mass spectrometry for DOAC patients. Plasma clot formation and lysis were read on a photometer after addition of an activation mix (tissue factor 2pmol/l and tissue plasminogen activator 333ng/ml). We used linear regression and ANCOVA for correlation analysis.

Results: On treatment INR, anti-Xa levels, and DOAC concentrations are provided in table 1. The lag phase of clot formation was significantly prolonged (p< 0.001) in anticoagulated samples and positively correlated to specific measures of the respective anticoagulant (figure 1). The maximum rate and peak clot turbidity of clot formation were

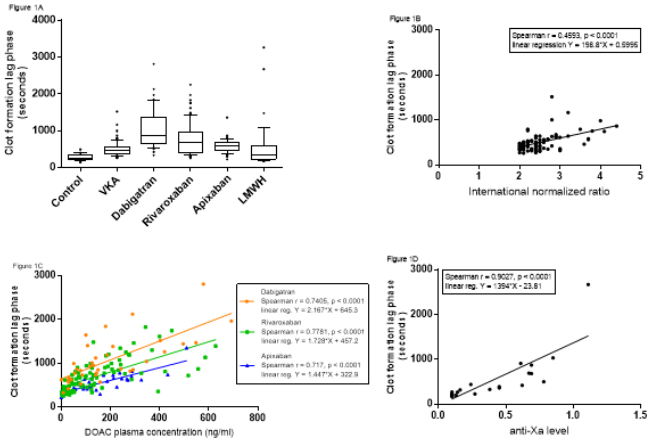

FIGURE (A) Clot formation lag phase comparison. (B) Lag phase versus INR in VKA patients, (C) versus DOAC concentration and (D) versus anti-Xa levels in LMWH

TABLE 1 Characteristics of patients on anticoagulation and controls

Characteristics, median (25th to 75th percentile)	Control (N=24)	Vitamin-K-antagonist (N=94)	Dabigatran (N=40)	Rivaroxaban (N=110)	Apixaban (N=42)	Low-molecular-weight heparin (N=42)
Age (years)	64.5 (59 - 73)	73 (68 - 79)	71.5 (62 - 79.5)	71 (66 - 78)	72 (64 - 75)	70 (63 - 76)
BMI (kg/m2)	27.6 (25.1 - 29.1)	27.8 (24.7 - 31.6)	27.2 (25.7 - 29.3)	27.9 (24.3 - 32.6)	27.2 (22.5 - 29.3)	26.2 (24.0 - 28.9)
CHA2DS2-Vasc	2 (2-4)	4 (3-5)	5 (3-7)	4 (2-6)	4 (3-5)	3 (2-4)
HAS-BLED	1 (1-2)	2 (1-2)	2 (2-3)	2 (1-2)	2 (1-2)	2 (1-3)
INR	1.1 (1.0 - 1.2)	2.4 (2.1 - 2.7)	n.a.	n.a.	n.a.	n.a.
DOAC concentration (ng/ml)	n.a.	n.a.	135.5 (54.3 - 280)	99.55 (33.3 - 205.5)	158.0 (94.6 - 246)	n.a.
Anti-Xa (U/ml)	n.a.	n.a.	n.a.	1.51 (0.49 - 1.51)	1.44 (0.88 - 1.51)	0.38 (0.1 - 0.69)
Time since drug administration (hours)	n.a.	22.0 (4.0 - 25.5)	3.9 (3.0 - 15.33)	9.66 (2.33 - 24.5)	4.75 (2.55 - 13.75)	5.25 (3 - 18)

significantly lower in anticoagulated samples (p=0.002 and p=0.045) and dependent on INR, anti-Xa, and DOAC concentration, respectively. Clot lysis time was not significantly different between controls and anticoagulated samples (p=0.3), but correlated positively with anti-Xa levels in LMWH samples (r=0.77, p< 0.001). At corresponding plasma concentrations, samples on dabigatran had significantly longer lag phases (p< 0.001), slower clot formation (p=0.002), lower peak clot turbidities (p=0.003), and longer clot lysis times (p=0.012) than samples on rivaroxaban and apixaban.

Conclusions: Ex-vivo plasma clot formation and lysis can directly compare between different anticoagulants. At corresponding drug concentrations, dabigatran exerted a stronger inhibition of clot formation than rivaroxaban and apixaban.

PB 411 | Thrombin Generation Response of rFVIIa in Haemophilia A Patient Plasma when Using Different Triggers and Phospholipids

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Background: NovoSeven® (rFVIIa) shows high efficacy in the treatment of bleedings in haemophilia patients with inhibitors. In the standard commercial thrombin generation assay CAT (Calibrated Automated Thrombogram) using platelet poor plasma therapeutic relevant rFVIIa levels is expected to show a poor response [Turecek 2003]. We tested combinations of different triggers and phospholipids (PL) in the CAT assay including PL vesicles containing phosphatidic acid (PA) which has been reported to enhance the rate of FX activation by FVIIa [Tavoosi 2013].

Aims: To investigate the influence of different triggers and phospholipids on the thrombin generation response when monitoring therapeutic relevant rFVIIa levels in plasma from haemophilia A (HA) patients.

Methods: HA plasma (80 µL) spiked with rFVIIa (0-500 nM) was mixed with different combinations of TF/FXIa and commercial PL preparations/in-house prepared PL vesicles containing PS (phosphatidylserine, PC (phospatidylcholine), PE (phosphatidylethanolamine) and/or PA (4-60 µM). PS:PC (20:80), PS:PC:PE (20:40:40), PS:PA:PE (5:25:70), PS:PC:PA:PE (2:40:28:30). Thrombin generation was initiated by addition of a fluorogenic thrombin substrate containing CaCl2 and CAT was determined.

Results: The commercial PPP Reagent Low (1 pM TF + 4 µM PL, Thrombinoscope) resulted in a weak dose-response of rFVIIa. The same was observed for FXIa in combination with a commercial PL reagent

(4 µM PL, Rossix AB). For PS:PC:PE and PS:PC:PA:PE concentrations >20 µM in combination with FXIa the analytical window increased significantly, however with only a slight improvement in sensitivity. High concentrations of soluble TF (sTF) improved both the analytical window and the sensitivity significantly.

Conclusions: The dose-response of rFVIIa in thrombin generation assays is highly dependent on the trigger and the PL source used. With nM concentrations of sTF an assay with a significantly improved analytical window and a sensitivity of 1 nM rFVIIa was obtained.

PB 412 | Performances of the Hydragel 5 von Willebrand Multimers - A New within-Day von Willebrand Factor (VWF) Multimer Screening Method

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Background: Analysis of VWF multimers is essential for the diagnosis and classification of von Willebrand disease (VWD). Multimers analysis is currently non-standardised and laborious. The local in-house method takes 4 days to produce interpretable results. A novel semi-automated within-day VWF multimer test has recently become available.

Aims: The aim of this study was to assess the performance of the semi-automated Hydragel 5 VW multimers system (Sebia) as compared to in-house multimers in VWD diagnosis and classification.

Methods: Multimers analysis of 188 well characterised VWD patients or normal donors were performed in parallel. The in-house method routinely used 1.6% SDS agarose gel electrophoresis, followed by visualisation with alkaline phosphatase-conjugated antibody. Hydragel 5 VWF multimers were performed on the Hydrasys 2 semi-automated system. Patients were grouped according to the ratio of VWF activity/antigen with a ratio of < 0.6 equating to type 2 VWD.

Results: Concordance of 96.8% was demonstrated between methods. A normal multimer pattern was observed using Hydragel in all normal donors (n=31), 28/29 non-VWD patients, 53/54 type 1 VWD, 18/21 2M and all type 2N (n=10). Loss of multimers was observed in all types 2A (n=30), 2B (n=9) and 3 VWD (n=4). Five samples (including 3 type 2M patients) had normal multimer distribution using the in-house method and a slight loss of high molecular weight multimers (HMWM) with the Hydragel 5 VW multimers.

Conclusions: The Hydragel 5 von Willebrand multimers demonstrated excellent agreement with the in-house method for VWD classification and subtyping. The presence of a unique pattern for some type 2M individuals using the Hydragel 5 von Willebrand multimers may be related to the genetic mutation of the patient. Hydragel 5 von Willebrand multimers is suitable for inclusion in a routine screening program for VWD. The method saved on staff time, produced highly reproducible results and allowed for easy interpretation due to in-built densitometry.

PB 413 | The Selective Use of Computer-assisted Strain Gauge Plethysmography as an Additional Screening Test for Suspected DVT Can Decrease the Need for Compression Ultrasonography: A Validation Study

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Background: In 2009 a DVT diagnostic service was set up in a community hospital in Brentwood, Essex, UK. Patients with suspected DVTs were screened with a Wells score (WS) and Point of Care (POC) quantitative d-dimer (DD). Compression Ultrasound (CUS) was carried out on site as per standard protocols. In the first two years >90% of CUS were negative in patients with discordance between the WS and DD. In two previous retrospective studies we showed that Strain Gauge Plethysmography (SGP), used as an additional screening test, had the potential to decrease the need for CUS as a negative SGP was highly predictive of a negative CUS.

Aims: To confirm in a new, prospective study whether the selective use of SGP in patients with discordance between the initial WS and DD screening tests could safely decrease the need for CUS.

Methods: From Jan 2015 to Mar 2016 with a revised protocol we assessed 615 patients presenting to a community clinic with suspected DVTs. All patients were screened with a 2-level WS and a POC DD. Patients with concordant negative results were discharged and those with concordant positive results had proximal CUS. Patients with discordance between the WS and DD had SGP performed. Patients with negative SGP were discharged and had a 90-day follow up to determine the subsequent incidence of VTE. If the SGP was positive a CUS was done.

Results: 237/615 patients (38%) had discordant screening tests. In 153 (65%) there was a valid SGP result of which 114 (74%) were negative. 101/114 were followed up at 90-days and none had developed a VTE. 13 patients could not be contacted but had not been referred back with suspected VTE.

Conclusions: With discordance between the WS and DD a negative SGP can safely exclude a proximal DVT without the need for CUS. In this group the CUS scanning rate was decreased by 48% improving the efficiency and cost effectiveness of the service. Although SGP was either not tolerated or produced an invalid result in ~1/3rd of patients there was still net overall benefit.

PB 414 | Semi-automated Platelet Aggregometry Employing the Sysmex CS-2x00; Preliminary Findings in a Multi-centre Study

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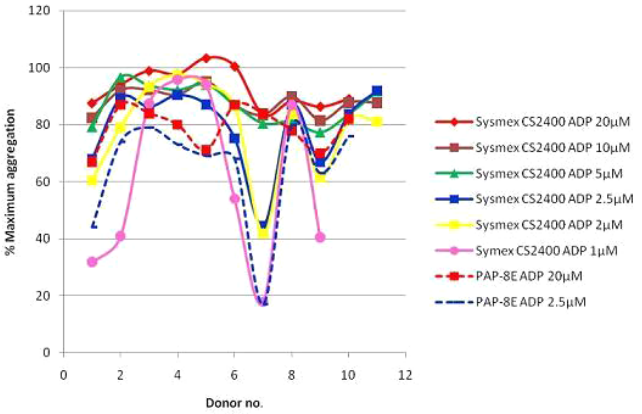

FIGURE 1 % Maximum aggregation in response to a range of ADP concentrations obtained using donor platelet rich plasma.

Background: The labour intensive technique of light transmittance platelet aggregometry has changed little since its inception in the 1960s. The requirements of meticulous technical skills and the need to test live platelets within 4 hours of venepuncture make it largely the remit of specialist laboratories.

Aims: Sysmex CS-2x00 series analysers permit, for the first time, a more standardised and automated approach. To assess the potential advantages of the Sysmex platform, three of the UK's specialist haemostasis laboratories at Bristol Royal Infirmary, Guy's & St. Thomas' Hospitals and The Royal London Hospital, are undertaking a multi-specialist site evaluation comparing analyser outputs and diagnostic outcomes from their traditional manual platelet aggregometry with automated aggregometry using Hyphen BioMed platelet agonist reagents.

Methods: Platelet rich plasma from volunteer donors with no known history of platelet disorders have been assessed using a wide range of concentrations for the routine agonists of ADP, epinephrine, collagen, arachidonic acid and ristocetin, and second-line agonists of TRAP, U46619 (TXA_2 mimetic) and calcium ionophore.

Results: Results have been compared to those obtained using the existing manual PAP-8E technique to ensure consistency of response across the separate platforms. The results obtained to date using a range of ADP agonist concentrations are plotted in Fig 1 and show broad agreement between the two testing platforms.

Conclusions: The study proposes to collate results from each laboratory as they perform their routine diagnostic analyses to establish the optimal agonist profile for diagnostic purposes and to establish robust reference ranges for platelet aggregometry. If diagnostic equivalence with manual platelet aggregometry is confirmed, it is envisaged that these expert centres could adopt automated platelet aggregometry as their standard technique. This is expected to lead to improved throughput and efficiency on platelet diagnostic testing.

PB 415 | The Impact of Repeated Freeze-thaw Cycles on Antiphospholipid Antibody Titer

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Background: Solid phase assays (SPA) for antiphospholipid antibodies (aPL) are mostly analyzed in batch. Guidelines advice to avoid repeated freeze-thaw cycles (FTC). However, in daily practice, FTC might be necessary. In contrast to the effect of freeze-thawing in phospholipid-dependent clotting assays used for lupus anticoagulants, there is only limited literature for SPA. Consequently, a validation on the effect of FTC could provide useful information.

Aims: Evaluating the effect of repeated FTC on anticardiolipin (aCL) IgM/IgG and anti-beta-2 glycoprotein 1 (aβ2GPI) IgM/IgG.

Methods: Sample selection (n=42) was based on routine results to cover a wide range for all four aPL. Patient samples were frozen after double centrifugation. All analysis were performed on aliquots never thawed before the experiment. Further, samples were analyzed five consecutive days with an additional, standardized FTC every day. Samples were collected between 2012 and 2017. All analysis were performed by an automated chemiluminescent assay (HemosIL® AcuStar, Instrumentation Laboratory, Bedford, MA, USA). Statistical analysis was done in Medcalc. A Mann-Withney U test for statistical differences ($p < 0.05$) between the first and following FTC was performed. If no statistical difference was found after five FTCs, a concordance correlation coefficient (CCC) was calculated.

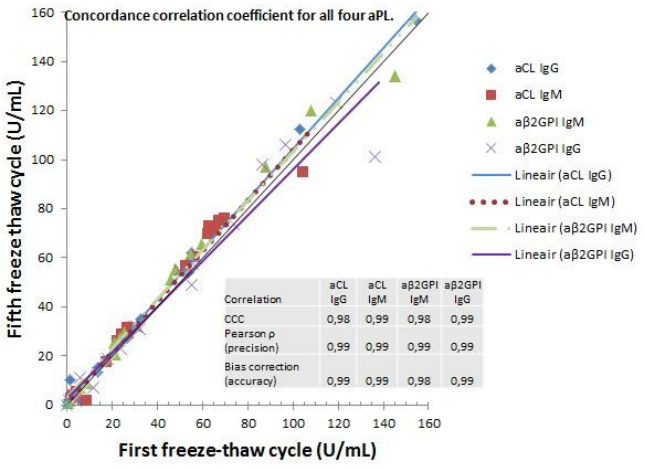

FIGURE 1 CCC between first and fifth FTC for all four aPL. Values above 200 U/mL are disregarded for visual purposes.

Results: APL titers showed no significant difference between earlier routine analysis and re-analysis at time of this study. After five FTC none of the samples, including those with values around the cut off, degraded from positive to negative. Mann-Withney U test for all analysis showed no statistical difference between the first and following repeated FTCs (table 1). The CCC between the first and fifth FTC were between 0.98 and 1 for all four aPL (figure 1).

Conclusions: ACL and aβ2GPI, over a broad titer range, are stable over time and after repeated FTC. With this study we excluded this pre-analytical factor as source for variability in aPL titer measured by SPA.

PB 416 | Suitability of a Liquid High-sensitivity Recombinant Human Prothrombin Time Reagent with Two Year Helf Life on an Automated Coagulation Laboratory Platform

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Background: In the management of vitamin K antagonist (VKA) therapy, few liquid prothrombin time (liqPT) options are available with comparable performance and shelf life to lyophilized (lyoPT) reagents. A 2 year shelf life liqPT provides benefits of reduced reconstitution variability versus lyoPT, user convenience and easier laboratory inventory control.

Aims: The study assesses the suitability of a ready-to-use high-sensitivity recombinant human liqPT reagent with two year shelf life versus a lyoPT predicate.

Methods: On automated coagulation laboratory platforms (ACL TOP series) at 9 large coagulation centers from five EU countries, PT results were obtained for a large cohort (N=3372) of normal donors, VKA patients, or donors with lupus anticoagulants (LA) to compare performance for the liqPT (ReadiPlasTin, ISI 0.97) vs. lyoPT (RecombiPlasTin 2G, multiple lots, ISI range 0.98-1.01).

TABLE 1 Results of 5 consecutive FTC. P-values were calculated with Mann-Withney U test. med: median, IQC: interquartile range

	FTC1 med(IQR)	FTC2 med(IQR)	p-value FTC 1-2	FTC3 med(IQR)	p-value FTC 1-3	FTC4 med(IQR)	p-value FTC 1-4	FTC5 med(IQR)	p-value FTC 1-5
aCL IgG	30(8-93)	32(13-263)	0.8692	33(13-274)	0.7418	35(147-237)	0.7349	34(13-276)	0.7143
aCL IgM	55(13-87)	57(11-88)	0.8528	58(11-90)	0.7650	59(11-86)	0.7259	57(8-76)	0.8481
aβ2GPI IgM	31(8-93)	28(9-98)	0.8234	31(8-99)	0.6908	29(9-102)	0.7784	31(8-81)	0.7949
aβ2GPI IgG	74(22-676)	73(18-724)	0.9354	79(15-694)	0.8642	74(11-748)	0.9058	74(13-723)	0.9551

Results: A method comparison of PT(sec) for liqPT vs. lyoPT (liqPT range 8.2-197.6 sec) yielded slopes within 2% of 1.00 (Table 1), indicating PT (sec) is independent of reagent form (liq or lyo).

TABLE 1 PT (sec) method comparison, liqPT vs. lyoPT

EU Location:	#samples(N)	Slope (liqPT vs. lyoPT)	Intercept	R^2
ALL	3372	1.01	0.129	0.995
Germany	1302	1.02	0.158	0.995
UK	557	1.02	-0.039	0.995
Benelux	1513	0.99	0.307	0.995

A liqPT vs. lyoPT PT (sec) comparison of the LA positive samples ranging from 11.1-13.4 sec gave results similar to Table 1 (slope = 1.02, int = -0.047, R^2 = 0.9923), indicating similarly low sensitivity performance to LA with both reagents. In Table 2, the mean vitamin K-dependent extrinsic factor recoveries from the VKA patient samples were comparable for both reagents with slopes within 6% of 1.00.

TABLE 2 Comparison, liqPT vs. lyoPT, % Factor Recoveries from VKA samples (N=7)

VKA Extrinsic Factor	Slope (liqPT vs. lyoPT)	Intercept	R^2	Range, liqPT Factor %
II	1.04	-0.842	0.996	14.4-108.2
VII	0.94	1.919	0.990	9.8-148.1
X	0.94	1.162	0.998	6.6-127.6

Conclusions: The results show excellent agreement between the liqPT reagent and reconstituted lyoPT predicate reagent, and demonstrate that a convenient ready-to-use liquid PT reagent with two year shelf life is a suitable replacement for the traditional lyophilized PT reagent in assessing VKA therapy on automated coagulation platforms.

PB 417 | Discrepancy in Optical and Impedance Platelet Count in Platelet Rich Plasma Prepared for Light Transmission Aggregation as a Possible Sample Quality Indicator

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Background: Mechanical forces during platelet rich plasma (PRP) preparation can activate platelets that subsequently form platelet clumps. We have observed differences in platelet counts measured with optical (PLT-O) and impedance (PLT-I) method, performed prior to light transmission aggregation testing. Currently, there is no recommendation on the preferred method for platelet count determination.
Aims: Aim was to explain observed differences by investigating platelet indices (PI) [mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (PLCR)] in PRPs with discrepant PLT-O and PLT-I, compared to PRPs with consistent platelet counts.

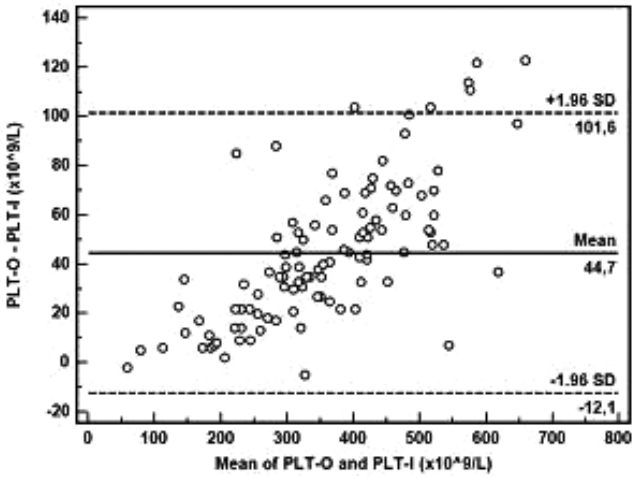

FIGURE 1 Comparison of optical (PLT-O) and impedance (PLT-I) platelet count according to Bland and Altman.

Methods: PRPs were prepared from 105 samples by centrifugation (200g, 10min). Platelet counts and PI were measured on Sysmex XE5000 analyzer (Sysmex, Japan). Total allowable error (13.4%) based on biological variation was used to discriminate PRPs with significantly different PLT-O and PLT-I. Kolmogorov-Smirnov test, Bland-Altman analysis, paired samples- and independent t-test, and Pearson correlation were used for statistical analysis. P< 0.05 was considered significant.
Results: PLT-O method gave consistently higher counts compared to PLT-I method (P< 0.001) with a mean difference of 44.7x10^9/L (Figure 1). Neither PLT-O nor PLT-I count correlated with PI. 50 of 105 (48%) PRPs showed significant difference between PLT-O and PLT-I with lower MPV, PDW and PLCR (P< 0.001 for all PI). All PI correlated weakly with the difference in PLT-O and PLT-I count (r=-0.37, r=-0.38, r=-0.35, respectively; P< 0.001).
Conclusions: The reason for discrepancies between PLT-O and PLT-I is not completely clear. Higher PLT-O counts could be due to platelet clumps that are only counted by optical method. Consequently, smaller platelets are counted using impedance method which explains lower PI in PRPs with discrepant platelet counts and greater difference between counts. This finding can be used as quality indicator of PRP prepared for aggregation testing.

PB 418 | Thrombophilia: Women-specific Reference Ranges May Prevent Overdiagnosis

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Background: Thrombophilia is a state where abnormalities of the haemostatic system are present that predispose to thrombosis. Some coagulation factors are generally lower in women than in men. Therefore, the use of standard reference ranges, based on male or

mixed-sex groups, may be misleading in the diagnosis of thrombophilia in women. This may be relevant in the analysis of thrombophilia after pregnancy complications. Therefore, the aim of this study was to evaluate the use of women-specific reference ranges in thrombophilia.

Aims: Investigate the effect of women-specific reference ranges on the interpretation of thrombophilia in women after an uncomplicated pregnancy or women who experienced preeclampsia.

Methods: Coagulant and anticoagulant parameters (Prothrombin Time; Antithrombin; Protein C-Activity; Protein S-Activity; Activated Protein C-resistance; Lupus anticoagulans with diluted Russell´s viper venom time and lupus-sensitive Activated Partial Thromboplastin Time; and anticardiolipin and β2-glycoprotein-I antibodies) were measured three months postpartum in 61 healthy women with an uncomplicated pregnancy and in 197 women who experienced preeclampsia (PE). Of the healthy women, 55 were also measured at least 6 months after an uncomplicated pregnancy. These measurements were used to calculate women-specific reference ranges. This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam and informed consent was obtained at inclusion.

Results: In total, 52% of healthy women had abnormal results when using routine reference ranges compared to 8% when using women-specific reference ranges ($p < 0.05$). In the women with PE, there were abnormal results in 74% of PE women when using routine reference ranges compared to 26% when using women-specific reference ranges ($p < 0.05$) (See table 1).

TABLE 1 Number of prothrombotic abnormalities in women 3 months after pregnancy based on routine reference ranges (RRR) / women reference ranges (WRR)

	Uncomplicated pregnancy (n=61)			Preeclampsia (n=197)		
	RRR	WRR	p	RRR	WRR	p
Antithrombin (U/ml)	1	0		3	1	
Protein C activity (E/ml)	1	0		5	4	
Protein S activity (E/ml)	7	0		32	5	
APC-resistance ratio	26	1		88	15	
APTT-Lupus (sec)	1	0		2	1	
DRVVT Ratio	5	0		54	9	
Antiphospholipid antibodies	3	4		59	31	
Total of patients ≥ 1 abnormality	32 (52%)	5 (8%)	< 0.05	144 (74%)	51 (26%)	< 0.05

Conclusions: When using women-specific reference ranges less abnormalities are seen in women, which may prevent overdiagnosis of thrombophilia.

PB 419 | A New Chromogenic DTI Assay for the Quantitative Determination of Dabigatran in Human Plasma

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Background: Dabigatran etexilate is an oral direct thrombin inhibitor used for the prevention of venous thromboembolism after elective hip and knee replacement, the prevention of stroke during arterial fibrillation, and the treatment of acute deep vein thrombosis. The active metabolite, dabigatran, has a predictable pharmacokinetic profile with no need for routine monitoring in general. Nevertheless, in certain clinical situations it is beneficial to quantify the dabigatran concentration in patient specimens.

Aims: The development of a reliable automated chromogenic assay for the quantitative detection of dabigatran in human plasma.

Methods: The new DTI Assay* is a thrombin-dependent assay using a chromogenic measuring principle and respective Standards* and Controls*. Applications were developed for Siemens BCS® XP System (BCS XP) and Sysmex® CS-2000i/2100i/CS-2500/CS-5100 Systems (CS-Systems). For verification of the assay performance the following studies were performed: Linearity, Limit of Quantitation, Precision, On-board Stability and Method Comparison to liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The assay is calibrated in a range of 0-500 ng/mL dabigatran. A clinical reportable range (CRR) of 20-1000 ng/mL is achieved by sample predilution. On all systems, linearity was assessed over the whole CRR. A total precision CV of 5.73% (CS-Systems) and 6.90% (BCS XP) was shown for a sample with 50-60 ng/mL and 2.27% (CS-Systems) and 2.19% (BCS XP) for a sample with 340-370 ng/mL dabigatran. An on-board stability of assay reagents of 54 hours (CS-Systems) and 24 hours (BCS XP) was achieved. Method comparison to LC-MS/MS revealed a correlation coefficient of r=0.997 (BCS XP).

Conclusions: The new DTI Assay offers together with respective Dabigatran Standards and Controls a reliable method for the determination of dabigatran in human plasma.

(*) Due to regulatory reasons product availability varies by country. Not available for sale in the U.S.

PB 420 | Comparison of Thrombin Generation Profiles among Patients Treated with Three Different Direct Oral Anticoagulants and Warfarin

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TABLE 1 Patient, clinical indications, drug levels and TG analysis. * p<0.0001 between DOAC vs Warfarin

Drugs	NVAF pt/n° VTE pt/n°	DOAC ng/ml warfarin PT/ INR mean (min-max)	Lag time (min) mean (min-max)	Peak (nM) mean (min-max)	ETP (nM-*min) mean (min-max)
dabigatran	NVAF/15 VTE/15	70.7 (15.2-207.7) 86.1 (31.9-94.2)	5.9 (2.3-8.8) 5.7 (3.3-7.8)	278 (150-383)* 290 (205-383)*	1484 (1070-1979)* 1547 (1439-1655)*
rivaroxaban	NVAF/15 VTE/15	37.0 (19.5-105.8) 25.7 (15.4-42.5)	4.5 (3.3-6.3) 4.9 (2.9-7.3)	124 (53-206) 160 (67-266)	1193 (609-1702)* 1674 (1135-2775)*
apixaban	NVAF/15 VTE/15	133(47.7-282.9) 117.2 (48-392.5)	5.7 (3.2-11.1) 5.03 (3.0-9.0)	82 (32-197) 118 (77.6-167)	1083 (647-1728)* 1312 (1097-1592)*
warfarin	NVAF/15 VTE/15	2.35 (2.1-2.9) 2.5 (2.0-2.9)	4.3 (2.7-7.3) 4.8 (3.1-7.0)	86 (46-156) 77 (37-113)	467 (259-836)* 421 (225-641)*

Background: Apixaban, dabigatran and rivaroxaban are three direct oral anticoagulants (DOAC) administered at fixed dose with different posology in relation to clinical indications, individual characteristics and renal function while warfarin daily dose is adapted to maintain the therapeutic range. Previous studies showed large inter/intra individual variability both at trough (Cthrough) and at peak (Cmax) in plasma DOAC levels measured with specific coagulation test.

Aims: To evaluate Thrombin Generation (TG) in patients affected by non valvular atrial fibrillation (NVAF) and venous thromboembolsim (VTE) treated with DOAC or warfarin.

Methods: 120 patients (60 NVAF and 60 VTE) were enrolled, 30 for each drug. Plasma samples were collected after three months from starting anticoagulant treatment at Cthrough for DOAC patients while, for warfarin patients, nearly 16 hours after the last dose intake. PT INR for warfarin, diluted thrombin time (dTT) calibrated for dabigatran, FXa calibrated for rivaroxaban or apixaban were performed. TG was assayed using the calibrated Automated Thrombogram (CAT-Stago). measuring lag time (min), endogenous thrombin potential (ETP- nM-*min) and peak (nM). The activation of coagulation was obteined by adding tissue factor 5pM.

Results: Results are shown in Table 1. High DOAC inter-individual variability, both in NVAF and in VTE patients, was found as measured with specific coagulation test (mean CV%= 52) and in TG analysis. Mean ETP was significantly higher in DOAC treatments both in NVAF and VTE, without differencies among them, compared with warfarin (p< 0.0001).

Conclusions: Our study confirms the high DOAC inter-individual variability as measured both with specific coagulation test than with TG analysis. In comparison with warfarin, ETP is significantly higher in patients treated with DOAC, suggesting a possible more stable coagulation inhibition associated to half life of warfarin. These data open the question if individualized patient dosing could improve the daily anticoagulant action.

PB 421 | Multicenter Evaluation of D-Dimer on Two New Coagulation Systems

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Background: D-dimer testing is widely utilized for exclusion of venous thromboembolism.

Aims: To evaluate the INNNOVANCE® D-dimer assay on two new coagulation systems in a multicenter study with 24 participating sites and to validate the exclusion of pulmonary embolism (PE) and of first event of deep vein thrombosis (DVT).

Methods: Frozen specimens were collected prospectively from consecutive outpatients presenting to the emergency or ambulatory department with a suspected PE or DVT. All potentially eligible patients were evaluated using the Wells´ rules to estimate their pre-test probability (PTP) with regard to PE or DVT. Patients with a high or likely PTP score were excluded from enrollment. Patients with no or a positive D-dimer result with the D-dimer assay used at the respective study center were evaluated by imaging methods, e.g. ultrasound, spiral CT or V/Q scan. Patients with a negative internal D-dimer result underwent imaging at the physician´s discretion. All patients with a negative diagnosis of PE or DVT were followed up after three months to evaluate potential DVT or PE. Patients with unobtainable follow-up data were excluded from analysis resulting in 1317 DVT and 1467 PE patients, respectively, available for analysis. A multiple imputation analysis was performed in order to investigate the possible impact of missing diagnosis of DVT and PE at follow up on the results.

Results: The overall prevalence was 6.1% for DVT and 6.9% for PE. The instrument specific sensitivity, specificity and negative predictive value (NPV) with their respective lower bound of the two-sided 95% confidence interval (LCL) were calculated. The results are shown in the tables below.

TABLE 1 First event of DVT

System	sensitivity	95% LCL	specificity	95% LCL	NPV	95% LCL
Sysmex CS-2100i	97.5	91.3	46.1	43.3	99.7	98.7
Sysmex CS-5100	97.5	91.3	45.1	42.3	99.6	98.7

TABLE 2 Pulmonary embolism

System	sensitivity	95% LCL	specificity	95% LCL	NPV	95% LCL
Sysmex CS-2100i	97.0	91.6	54.5	51.9	99.6	98.8
Sysmex CS-5100	98.0	93.0	54.5	51.9	99.7	99.0

Conclusions: In this study the results of the D-dimer assay fulfill the criteria of the CLSI Guideline H59-A for the exclusion of PE and first event DVT on both systems. The results of the multiple imputation analysis confirm the conclusions based on the complete case approach.

PB 422 | Innovative Method for the Description of Human Platelets Population

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Background: Disorders of platelets may lead to serious pathologies and it could be difficult to diagnose, especially at the earliest stages of the disease. Currently, many methods have been developed for the evaluation of platelet function. These methods are usually based on platelets aggregation and blood coagulation. However, none of them could provide information about the shape of individual platelets. Although the shape change is the first reaction to different influences including special agonists.

Aims: To develop a new method for diagnostics of:

· morphological abnormalities of platelets' populations.

· risks of bleeding and thrombotic events related to platelets.

Methods: We used the light-scattering flow cytometry. This method is based on measurement of angle-resolved light-scattering patterns (LSPs) of individual cells and solution to the inverse light-scattering (ILS) problem. We measured LSPs with the Scanning Flow Cytometer. After we found the solution to ILS problem for each cell, we obtained the distribution of platelets over the shape aspect ratio. This distribution was used to characterize the shape change during activation and other external influences.

Results: The study of platelets with the scanning flow cytometry was performed for different donors. Informed consent was obtained. Also, we created a new mathematical model for the identification of platelets distribution based exclusively on cell shape. We described three different subpopulations of platelets in native blood (resting, partially activated and fully activated). We also studied find out a correlation between shape and volume during platelets activation. And, this method needs no use monoclonal antibodies for identified subpopulation.

Conclusions: The study of platelets is very important for diagnostics related to hemostasis. We tested new method for this study and got consistent results. This method allows us to study shape change in live platelets during their platelets activation.

PB 423 | Thromboelastometry to Detect Anticoagulant Effect of Apixaban in Patients with Non Valvular Atrial Fibrillation

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Background: Rapidly available tests are needed to measure the anticoagulant effect of non-vitamin K oral anticoagulants in emergency

TABLE 1

Parameter (mean±SD, CV)	Control (15)	Peak sample (10)	Trough sample (10)	P *	P §	P +	AUC (95% CI)
CT (sec)	138.00±37.37, 0.27	303.30±86.54, 0.28	262.70±119.04, 0.45	<0.001	0.005	0.508	0.917 (0.822-1.012)
Alpha (°)	74.20±3.00, 0.04	66.80±11.92, 0.18	70.20±7.84, 0.11	0.045	0.105	0.082	0.283 (0.110-0.457)
CFT (sec)	78.00±16.11, 0.21	133.40±93.44, 0.70	104.20±47.08, 0.45	0.023	0.048	0.074	0.755 (0.593-0.917)
MCF (mm)	61.40±4.20, 0.07	64.40±6.96, 0.11	64.50±6.67, 0.10	0.277	0.451	0.833	0.610 (0.421-0.799)
CT/CTc	1.09±0.23, 0.21	1.75±0.28, 0.16	1.78±0.82, 0.46	<0.001	0.011	0.799	0.880 (0.766-0.994)
Alpha/Alphac	1.00±0.03, 0.03	0.90±0.13, 0.15	0.96±0.08, 0.09	<0.001	0.052	0.037	0.163 (0.029-0.297)
CFT/CFTc	0.98±0.13, 0.13	1.52±0.53, 0.35	1.24±0.29, 0.24	<0.001	0.005	0.047	0.880 (0.760-1.00)
MCF/MCFc	0.99±0.04, 0.04	0.99±0.06, 0.06	1.00±0.05, 0.05	0.643	0.578	0.953	0.437 (0.243-0.630)

SD= Standard Deviation; CV= Coefficient of Variation; CT= Clotting Time; CFT= Clot Formation Time; MCF= Maximum Clot Firmness; CTc= Clotting Time after anti-FXa catcher was added; Alphac= alpha angle after anti-FXa catcher was added; CFTc= Clot Formation Time after anti-FXa catcher was added; MCFc= Maximum Clot Firmness after anti-FXa catcher was added. AUC= area under the curve; CI= Confidence Interval. * comparison between controls and peak sample in patients; § comparison between controls and trough sample in patients; + comparison between trough sample and peak sample in patients.

situations (such as bleeding, emergency surgery and need for thrombolytic treatment). No global test is currently available to measure the anticoagulant effect of apixaban.

Aims: To assess the anticoagulant effect of apixaban by whole rotational thromboelastometry (ROTEM).

Methods: In patients with non-valvular AF treated with apixaban, peak and trough samples were obtained at steady-state. Healthy volunteers served as controls. Citrated blood samples were tested by ROTEM using diluted EXTEM assay with and without the addition of anti-FXa catcher. Clotting Time (CT), Alpha angle (Alpha), Clot Formation Time (CFT), Maximum Clot Firmness (MCF) were measured. Data are presented as means ± SD and comparisons were analyzed by Wilcoxon rank and Mann-Whitney tests. ROC curves for the evaluation of accuracy were performed.

Results: Ten patients and 15 controls were included. The mean CT of patients at trough was significantly longer than that of controls (Table). In patients, the mean CT was significantly shortened after addition of the anti-FXa catcher (peak CT 303.30±86.54 seconds without and 176.70±55.58 with anti-FXa catcher, p=0.005; trough CT 262.70±119.04 seconds without and 147.90±32.02 with anti-FXa catcher, p=0.009). CT, CT/CT+catcher (CTc) and CFT/CFT+catcher (CFTc) revealed a good accuracy in measuring apixaban anticoagulant activity AUC 0.917, 0.880 and 0.880, respectively. The CT/CTc and CFT/CFTc of patients were significantly higher compared to those of controls (p< 0.001 at peak in both and 0.011 and 0.005 at trough, respectively). The combination of a CT value >150 sec and a CT/CTc > 1.3 or a CFT/CFTc > 1.2 had a positive predictive value of 95% for antiXa activity in patients treated with apixaban (accuracy 92%).

Conclusions: Thromboelastometry showed a good accuracy in detecting apixaban activity in patients with non-valvular AF.

PB 424 | Assessment of Single-factor Deficiency Sensitivty of Three Thromboplastin Reagents

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Background: Prothrombin time (PT) sensitivity for detecting isolated factor deficiency can vary according to different reagents, due to thromboplastin origin, composition and concentration in phospholipids.

Aims: Performances of single-factor deficiency sensitivity of three thromboplastin reagents from Stago were assessed, including STA®-NeoPTimal, the new Stago thromboplastin from brain rabbit origin with an ISI close to 1.0. Agreement with H47A2 CLSI recommendations were evaluated for each reagent.

Methods: For each exogenous factor (II, V, VII, X), normal pooled plasma containing normal level of factors underwent 8 serial dilutions in respective deficient-factor plasma to achieve ranges between 10 and 100%. Each sample was tested with STA®-NeoPTimal, rabbit brain origin (1 lot for each packaging: 5, 10 and 20mL), STA®-Neoplastin R, recombinant (2 lots of 15mL) and STA®-Neoplastin CI+, rabbit brain origin (3 lots of 10mL) on STA®-R analyser.

PT ratio was expressed by dividing the result in seconds by the Mean of Normal Prothrombin Time (MNPT) defined with 20 fresh plasma samples from healthy donor. The factor level of each sample was tested using one-stage clotting assay from Stago.

The sensitivity is defined as the maximum factor level producing a PT ratio result out of the reference range, i.e. > 1.2.

Results: For each factor/thromboplastin combination, the sensitivity obtained with different lots of reagents are consistent from one to each other.

All the 3 reagents reached H47A2 CLSI recommendations (factor sensitivity between 30% and 45%) except for factor X with STA®-Neoplastin R and STA®-NeoPTimal where factor sensitivity is slightly higher than 45%.

Conclusions: Results show a very good lot-to-lot consistency for single factor deficiency sensitivity with the 3 reagents from Stago, STA®-Neoplastin CI+, STA®-Neoplastin R, and STA®-NeoPTimal. Besides, this study strongly confirm the reliability of these reagents to CLSI guideline and attest their robustness for daily practice in the coag lab.

PB 425 | Genetic Analysis for Investigation of Heritable Bleeding Disorders - Update on the UK NEQAS (Blood Coagulation) Programme

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Background: Genetic analysis for investigation of heritable bleeding disorders is an important part of the diagnosis of haemophilia and accurate testing and interpretation of results is important. External Quality Assessment of such analysis can help improve laboratory performance, and highlight potential errors of interpretation.

Aims: The UK National External Quality Assessment Scheme (UK NEQAS) for heritable bleeding disorders distributes samples from patients with haemophilia A, B or vWD to both UK and non-UK genetics laboratories for investigation and reports are assessed by an expert panel of clinicians/clinical scientists.

Methods: We describe here the findings from the past 2 years of this EQA programme.

Results: Survey 26, May 2015, comprised samples from a Haemophilia A carrier with the *F8* IVS22 mutation and a wild type male. 1 centre failed this exercise by failing to identify the mutation in the carrier,

and 1 failed through incorrect interpretation of data for the wild type male. Survey 27, November 2015, comprised a sample from a haemophilia A donor with a *F8* c.575T>C mutation in exon 4, and discrepant 1-stage and chromogenic FVIII assay results. 1 centre failed this exercise through clerical errors in the report.

In survey 28, May 2016, comprised of a sample from a donor hemizygous for a *F8* c.541G>A substitution, 1 centre failed the exercise through an incorrect amino acid substitution in their report. A questionnaire distributed in 2014 revealed that 17/18 centres always repeat genetic investigations and therefore the impact of such errors may be minimised.

Conclusions: This programme assesses both the analytical phase of genetic investigations and also the post-analytical phase through interpretation of information based on a specific clinical scenario. Identification of errors by participants and evidence of improved quality of reporting since the introduction of this programme highlights the need for EQA programmes in this area of haemostasis investigation.

PB 426 | Platelet Reactivity in Patients on Aspirin and Clopidogrel Therapy Measured by a New Bed-side Whole Blood Assay

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Background: There are various tests available for measuring on-treatment platelet reactivity. The pharmacologically most specific assays are time-consuming and require extensive laboratory skills. In this study, we implemented the novel ROTEM® platelet impedance aggregometry assay, a bed-side test which measures platelet aggregation in a whole blood sample within six minutes.

Aims: We aimed to investigate its ability to evaluate P2Y12- and cyclooxygenase-1 platelet inhibition and compare it to already established assays.

Methods: Platelet function was investigated in 93 patients after informed consent. 47 patients were on permanent aspirin therapy and 46 on dual antiplatelet therapy (DAPT) with aspirin and clopidogrel. We used ROTEM® platelet impedance aggregometry (ROTEM-PTL), light transmission aggregometry (LTA), Multiplate® electrode aggregometry (MEA) and VASP flow cytometry. Aspirin antiplatelet effects were evaluated by arachidonic acid, clopidogrel effects by adenosine diphosphate stimulation. The study was approved by the ethics committee of the Heinrich-Heine University Düsseldorf.

Results: ROC-analyses showed ROTEM-PTL differentiates well between patients on medication and healthy individuals: Aspirin: ROC_{AUC} 0.99; (95% CI, 0.97-1.01); p< 0.0001; DAPT treatment: ROC_{AUC} 0.80; (95% CI, 0.69-0.91); p< 0.001. Pearson regression analyses showed moderate correlations, for aspirin: MEA vs. ROTEM-PTL r^2=0.435, p=< 0.001; LTA vs. ROTEM-PTL r^2=0.048, p=0.180. For DAPT: MEA vs. ROTEM-PTL r^2=0.398, p=0.001; LTA vs. ROTEM-PTL r^2=0.409, p=0.001; VASP vs. ROTEM-PTL r^2=0.164, p=0.055).

Conclusions: The novel ROTEM® platelet assay excellently distinguished between treated patients and healthy individuals but correlated only moderately with other assays. By now, different studies failed to demonstrate superiority in tailored antiplatelet therapy guided by already established assays. Clinical trials are needed to investigate the ability of this new assay to identify patients at risk of adverse events.

PB 427 | Usefulness of Waveform Analysis of APTT on the ACL-TOP® When Troubleshooting Unexpected Abnormal APTT

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Background: Although atypical 2nd derivative curve on the ACL-TOP® family of hemostasis analyzers in the assay of activated partial thromboplastin time (APTT) is associated with coagulation abnormalities such as single factor deficiency, factor inhibitor, and lupus anticoagulant (LA), the reliability is not established until now (Solano C., et al. *Int J Lab Hematol*, 2011;33:67-78. Tokunaga N., et al. *Blood Coagul Fibrinolysis* 2016;27:474-6).

Aims: We assessed the usefulness of waveform analysis of the 2nd derivative curve of the ACL TOP® representative analyzer on the identification of causes of APTT prolongation by comparing the prevalence of abnormal curves among each group.

Methods: We measured APTT by the combination of APTT-SP® which includes silica and ACL TOP® (Instrumentation laboratory, Bedford, MA, USA), and then counted the presence of an atypical curve of the 2nd derivative curve in 28 patients with factor deficiency including FVIII (n=21), FIX (n=2), FXI (n=1), FV (n=1), von Willebrand factor (n=3), 6 patients with FVIII inhibitor, 70 patients with LA, 75 patients treated with unfractionated heparin, 151 patients treated with dabigatran (n=71) or ribaroxaban (n=80), and in 36 healthy volunteers.

Results: The prevalence of abnormal curve in the group of factor deficiency, factor inhibitor, or LA was 100.0% (28/28), 100.0% (6/6), 95.1% (67/70), respectively (Table). On the contrary, in the remaining groups including healthy volunteers, we never found an abnormal curve on the 2nd derivative curve of APTT assay.

TABLE. Frequency of an abnormal curve on the second derivative curve in APTT assay

Group	Number of sample	APTT-sec (Mean±SD)	Frequency of an abnormal curve
Factor deficiency	28	59.0±16.0	100.0% (28/28)
Factor VIII inhibitor	6	98.3±19.5	100.0% (6/6)
Lupus anticoagulant	70	46.8±17.3	95.7% (67/70)
Unfractionated heparin	75	62.7±37.6	0% (0/75)
Dabigatran	71	56.7±18.4	0% (0/71)
Rivaroxaban	80	44.5±6.3	0% (0/80)
Controls (Healthy volunteers)	36	31.8±2.8	0% (0/36)

Conclusions: We assessed the usefulness of waveform analysis on the 2nd derivative curve of ACL TOP® when troubleshooting an unexpected abnormal APTT by comparing the prevalence of abnormal curve in each patient group. The presence of an abnormal curve on the 2nd derivative curve suggests that APTT-prolongation might be caused by factor deficiency, factor inhibitor or LA.

PB 428 | Evaluating the Analytical Performance of Six New Coagulation Assays

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Background: Coagulation tests are widely used in healthcare, including for screening, diagnosis and assessment of coagulopathies, and monitoring of anticoagulant therapy. The ability to accurately, reliably and quickly measure indicators of haemostatic function is important for patient health.

Aims: We evaluated the analytical performance of 6 new coagulation assays, developed to measure prothrombin time (PT), fibrinogen, thrombin time (TT), antithrombin, D-Dimer, and PT-derived fibrinogen on the Roche Diagnostics cobas t 711 (high-throughput; 400 tests/hour) and cobas t 511 (mid-throughput; 200 tests/hour) analysers.

TABLE 1 Within-run precision and reproducibility of PT, TT, Fibrinogen and PT derived Fibrinogen using the cobas t 711 and cobas t 511 analysers

	within run precision acceptance criteria	within run precision Range of % CV or SD	within run precision Range of % CV or SD	Total reproducibility %CV (acceptance criteria ≤25%	Total reproducibility %CV (acceptance criteria ≤25%
		cobas t711	cobas t511	cobas t711	cobas t511
PT sec	CV ≤3%	0.2-0.7	0.1-0.4	1.9-3.2	2.2-3.7
Fib 60-400 mg/dl	CV ≤4%	0.8-2.3	0.8-1.5	2.1-3.0	1.6-2.6
Fib 400-600 mg/dl	CV≤6%	0.7-2.6	0.7-0.9	3.3	2.9
Fib >600 mg/dl	CV≤10%	1.8-2.6	0.6-1.4	4.3	4.3
PT derived Fib mg/dl	CV≤5%	0.4-1.4	0.4-1.3	1.4-2.2	1.1-3.1
TT sec	CV≤4%	0.6-2.9	0.6-4.1	1.1-4.5	0.9-4.0

TABLE 2 Within-run precision and reproducibility of DDimer and Antithrombin assays using the cobas t711 and cobas t511 analysers

	Within-run precison acceptance criteria	Within-run precision range of %CV or SD	Within-run precison range of % CV or SD	Total Reproducibility %CV (acceptance criteria: CV≤25%)	Total Reproducibility %CV (Acceptance criteria: CV≤25%)
		cobas t711	cobas t511	cobas t711	cobas t511
D-Dimer, µg FEU/ml	SD≤0.02 (<0.56µg FEU/ml)	0.0119-0.0172	0.0096-0.0160	3.5-8.6	3.3-6.7
D-Dimer, µg FEU/ml	CV≤3.5%(0.56-1.7µg FEU/ml)	1.5-2.4	1.4-1.5	5.4	5.5
D-Dimer, µg FEU/ml	CV≤3% (>1.7µg FEU/ml)	0.3-0.7	0.2-0.3	1.0-1.8	0.9-2.2
Antithrombin IU/dL	SD≤2.4IU/dL (Absolute ≤80 IU/dL)	0.727-1.359	1.06-1.35	2.3-5.8	2.5-6.3
Antithrombin IU/dL	SD≤3.0%(>80 IU/dL)	0.9-1.3	0.8-1.4	1.8-2.0	1.4-2.1

Methods: The analytical performance of the 6 assays was independently tested on the cobas t 711 (4 sites: UK/Germany/Hungary/Austria) and cobas t 511 analysers (2 sites: UK/Germany). Following a familiarisation phase, analytical performance was assessed by measuring within-run precision and reproducibility. Within-run precision: 2 controls and 5 human plasma pool samples of different analyte concentrations or clotting times were tested; 1 run was performed with 21 replicates from each sample. Reproducibility: 5 aliquots of each control/human plasma pool sample were prepared and tested over 5 days across the sites and evaluated in combination. Anonymised residual 3.2% (0.109M) sodium citrate plasma samples were used for all experiments. The coefficient of variance (CV) and standard deviation (SD) were calculated for within-run precision and reproducibility and compared against specified acceptance ranges.

Results: For all assays tested, within-run precision and reproducibility results were within the specified acceptance ranges (Table). Across all assays, the CVs for within-run precision were ≤4.1% and none of the CVs for reproducibility were over 8.6%.

Conclusions: All six coagulation assays demonstrated a good to very good analytical performance using the Roche Diagnostics cobas t 711 and cobas t 511 analysers.

PB 429 | Performance Evaluation of the microINR® Point-of-Care INR-testing System

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Background: Point-of-care (POC) International Normalised Ratio (INR) testing is becoming more varied and widely available. Multicentre validation of these instruments' performance is desirable, as it helps to confirm their suitability for clinical use in varied settings.

Aims: To evaluate the microINR® POC system for accuracy, precision, measurement repeatability, instrument & test chip variability, and error rates.

Methods: Venous blood INRs obtained with Thromborel® S on the Sysmex CS-2100i® analyser, were compared with capillary blood microINR® values, from 210 patients on warfarin. Precision was assessed using control materials and measurement repeatability was calculated on 41 duplicate finger-prick INRs. Triplicate finger-prick INRs using three different instruments (30 patients) and three different test chip lots (29 patients) were used to evaluate instrument & test chip variability.

Results: Linear regression analysis of microINR® and Sysmex CS2100i® values, showed a correlation coefficient of 0.96 ($p < 0.0001$) and a positive proportional bias of 4.4%. Dosage concordance (93.8%) and clinical agreement (95.7%) was high. All acceptance criteria based on ISO standard 17593:2007 system accuracy requirements were met. Control material Coefficients of Variation (CV) varied from 6.2% to 24.7%, depending on control type, and the capillary blood measurement repeatability CV was 7.5%. There was no significant instrument ($p=0.93$) or test chip ($p=0.81$) variability, and the error rate was low (2.8%).

Conclusions: The microINR® instrument is sufficiently accurate and precise, with adequate dosage concordance and clinical agreement, to monitor warfarin therapy. It has a low instrument & test chip error rate and is not subject to significant instrument or test chip variability.

PB 430 | Statistical Evaluation of APTT Clot Waveform Patterns (CWP) in Sequential Samples of Severe Haemophilia A Patients Receiving Long Acting Clotting Factor Concentrates (CFC) - An Observational Study

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Background: We have evaluated APTT CWP on sequential samples of previously treated patients of Severe Haemophilia A, who were on prophylactic therapy of low dose long acting Fc fusion protein recombinant FVIII CFC on weekly basis.

Aims:

1) Statistical comparison between APTT CWP of control and patients

2) To evaluate changes in APTT CWP in sequential samples of the same patient at the trough levels of FVIII activity before the administration of CFC.

3) Anticipating the development of inhibitors from APTT CWP.

Methods: Total 290 APTT CWP of 34 patients along with 30 from normal healthy subjects (control) performed on ACL TOP 300 using Synthesil APTT reagent was evaluated on following parameters:

1) Primary(P) and Secondary(S+ & S-) Derivative evaluation on:

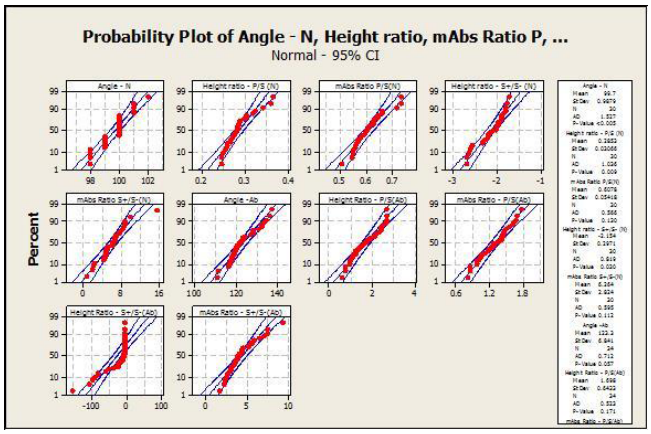

FIGURE 1 Plot 1: Probability plots for Angle, Area ratio, Height ratio, mAbs ratio within control and Haemophilia A

TABLE 1 showing Comparison Between Control and Haemophilia A APTT CWP data

Sample		Angle	Area ratio P/S+	Height Ratio P/S+	mAbs Ratio P/S+	Area Ratio S+/S-	Height Ratio S+/S-	mAbs Ratio S+/S-
Control	Mean	99.7	0.991666667	0.285314259	0.607759387	0.746666667	-2.153852157	6.264040461
Control	Standard Deviation	0.987857	0.131943184	0.030661102	0.054177589	0.043417249	0.297092108	2.82374574
Haemophilia A	Mean	123.2595	10.1903594	1.697696619	1.29191806	1.0604598	-27.432027	4.3471577
Haemophilia A	Standard Deviation	4.802224	4.19013906	0.605967474	0.243944142	0.5056843	60.13778183	1.83182041

Height, mAbs, Area under Curve (AUC), Ratios of AUC, Height, mAbs between P & S,

2) Angle of the Clot curve made with the baseline.

3) Changes in sequential APTT CWP within a patient so as to anticipate inhibitors.

Results: APTT CWP of control showed good correlation between AUC, Height and mAbs ratio between P & S and with angle of the clot curve. There was significant difference between mean of AUC ratio, Height ratio, mAbs ratio, between control and Patient samples. Height ratio and mAbs ratio was well correlated within sequential samples of a patient. Appearance of biphasic (P) with prolongation of plateau and variation in AUC ratio indicate possible presence of inhibitors. Since APTT value at the trough level was beyond 180 sec in many samples, correlation with APTT value was not possible.

Conclusions: Statistical evaluation of the APTT CWP is a reliable tool in anticipating the disease process in Haemophilia A patients. Long acting CFC do not change APTT CWP at the trough levels of factor VIII activity, although there is incidence of development of inhibitors in those patients which can be anticipated from APTT CWP.

Support: This study was supported by educational grant from Instrumentation Laboratory.

PB 431 | Divergence of Normal Ranges Using Thromboelastography as Measured by Clot Viscosity versus Clot Harmonic Resonance

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Background: Thromboelastography measuring clot viscosity and resultant resistance to rotation has been used for many years (TEG 5000). Recently, an instrument utilizing harmonic resonance-based clot detection (TEG 6S) has been approved by the FDA for use in adult cardiac and cardiac surgery patients.

Aims: To compare manufacturer's normal range to a locally defined healthy donor range for both the TEG 5000 and TEG 6S systems.

Methods: 30 healthy donors (without bleeding/clotting history or use of antiplatelet/anticoagulant medications) were identified. Venous blood was collected and run on the TEG 5000 and TEG 6S (Haemonetics, Braintree, MA) instruments using the following activators: kaolin, kaolin with heparinase, kaolin with tissue factor (rapid TEG), and tissue factor with abciximab (functional fibrinogen). Two 5000 instruments and four 6S instruments were employed, rotating through the instruments. FDA approval of the TEG 6S was limited to certain curve parameters; software blocks reporting of non-approved parameters.

Relevant TEG 5000 and 6S curve parameters were analyzed by nonparametric statistics using Microsoft Excel and GraphPad Prism software.

Results: Our local normal ranges were generally narrower than the manufacturer recommended range. This is of particular relevance for the Lysis 30 parameter on the TEG 5000, which may allow detection of more cases of hyperfibrinolysis.

In most instances the 5000 and 6S normal ranges were not identical (Table).

TABLE Local TEG Parameter Normal Ranges Obtained using Kaolin Activator

	R (min)	K (min)	Angle (degrees)	MA (mm)	LY30 (%)
TEG 5000	4.0-8.0	1.0-2.1	60-73	57-74	0.0-5.0
TEG 6S	5.0-8.6	0.8-2.6	61-78	52-69	N/A

Specifically comparing the TEG 5000 and TEG 6S kaolin data, the tightest agreement is for maximal amplitude, with the R square reaching 0.613 (Figure). For the R, K, and Angle values, the scatter is much greater, with R square values of 0.189, 0.234, and 0.287 respectively.

Conclusions: Local donor ranges differ from manufacturer recommended ranges. While TEG 5000 and TEG 6S normal ranges are quite similar, results are not interchangeable as a single distribution does not apply to both TEG 5000 and TEG 6S data.

PB 432 | Identification of Triple Positivity Patients with Major Risk for Thromboses Is Dependent of Methodology Applied at the Lab Investigation

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Background: The diagnosis of antiphospholipid syndrome (APS) is based on the persistent detection of antiphospholipid antibodies (aPL), which two kinds of assays are applied: Functional assay Lupus anticoagulant (LA), and immunological assays anticardiolipin (aCL) and anti-β2 glicoprotein I (aβ2GPI). Due to the heterogeneous antibodies profiles, the aPL assays has high coefficient of variation, evidenced for external quality control programs, close to 50% for aCL/aβ2GPI.

Aims: In order to evaluated when the functional and immunological assays present better results concordance, the objective of this study was to compare the aCL and aβ2GPI performance with different methodologies, in two group of patients, LA(+) and LA(-).

Methods: The IgM/IgG aCL or aβ2GPI were tested by *in house* ELISA and by chemiluminescent (CU). The profiles of aPL antibodies were evaluated as: single positive (aCL-/aβ2GP1-/LA+), double positive (aCL-/aβ2GP1+/ LA+) and triple positive (aβ2GP1+/aCL+/LAC+).

Results: In a total, 280 patients, 140 patients were LA (+) evaluated in two times (12 weeks) and 140 LA (-). For LA (+) group, when the IgM/IgG aCL and IgM/IgG aβ2GPI were compared with both methods, close 35% of patients had results unmated, which rendered a discordant interpretation of the antibodies profile. The concordance of CU with LA (+) were higher when compared in house ELISA with LA (+). In general, most of samples showed false negative results for in house ELISA assay, table 1. The triple positivity was 3.5 folds higher for CU when compared with ELISA. When the persistence of antibodies was evaluated, some patient that has consecutive negative results for ELISA, also showed consecutive positive results for CU. For group LA(-), in house ELISA assay may role out patients who need of treatment.

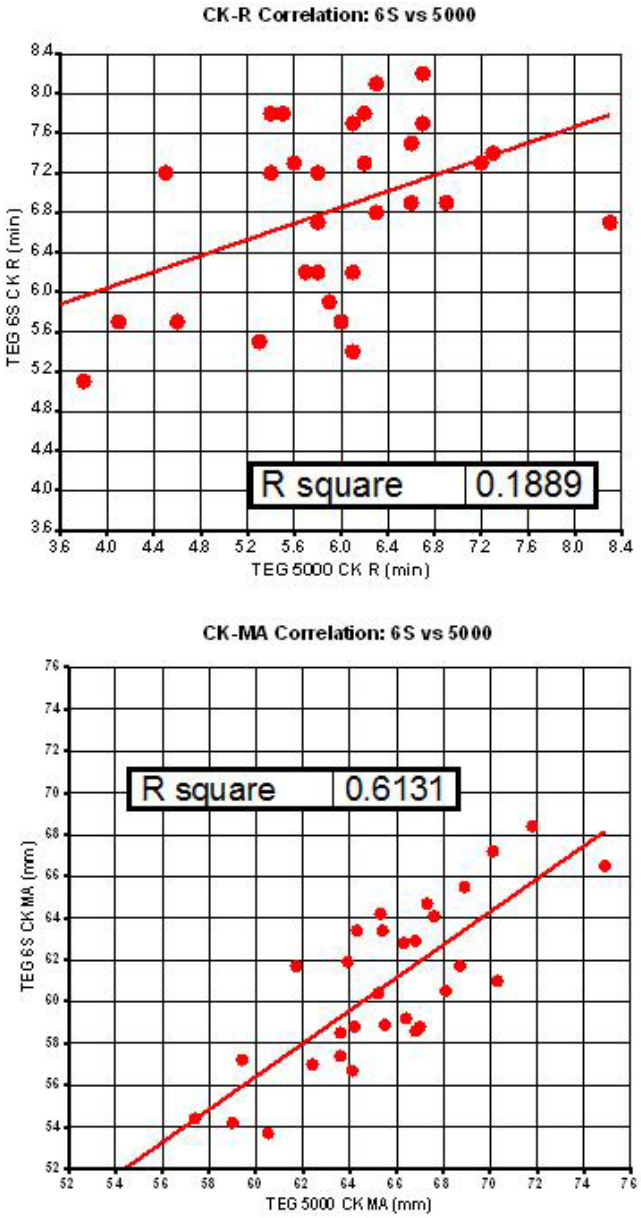

FIGURE 1 Correlation of TEG 5000 and 6S parameters R and MA using kaolin activator

TABLE 1 The aPL assays performed following the international guidelines ISTH for functional and immunological evaluation.

Group 1 LA(+)	Positive results N=140	Median (Minimum to Maximum) LA(+) and LA(-)	Antibodies profile (%)
In house ELISA Methodology			
Anticardiolipin IgG	7	15 GPL (5-50 GPL)	Triple 5%
Anticardiolipin IgM	1	15 GPL (5-40 GPL)	Duple 20%
Anti-β2 glicoprotein IgG/IgM	40	N.D	Single 65%
Chemiluminescent Methodology			
Anticardiolipin IgG	26	2.9 CU (2.6 to 2026.0 CU)	Triple 17%
Anticardiolipin IgM	13	2.5 CU (1.0 to 280 CU)	Duple 8%
Anti-β2 glicoprotein IgG	36	3.6 CU (3.6 to 4833.0 CU)	Single 65%
Anti-β2 glicoprotein IgM	14	1.1 CU (1.1 to 391.0 CU)	

Conclusions: Our results highlight the importance of assays election which presents adequate sensitivity and specificity for diagnosis, once these results affect the clinical decision to indicate continuous anticoagulation for these patients.

PB 433 | Performance Evaluation of the ZL 6000i Cone Plate Viscometer

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Background: Elevated whole blood viscosity (WBV) is associated with increasing blood flow resistance in the microcirculation and is used to predict the occurrence and outcome of cardiovascular diseases, such as myocardial infarction and stroke. The ZL 6000i (ZONCI Technology, Beijing, China) is a fully automated blood viscosity analyzer based on

the cone-plate method. We evaluated the analytical performance of the ZL 6000i to measure WBV.

Aims: We established a reference range and validated precision using quality control (QC) and human blood samples. Storage time and temperature effects were also evaluated.

Methods: Samples were collected from 287 normal adults (162 males and 125 females) to establish a reference range. We evaluated total precision for 20 days using QC viscosity materials at shear rates of 1, 50, and 200 s^{-1}, and within-run precision using phlebotomy subjects at shear rates of 1, 5, 30, and 200 s^{-1}. Aliquots of the phlebotomy samples were stored at room temperature and 5°C, respectively, for the stability analysis. All samples were tested at baseline (control) and 6, 24, 48, 72, and 96 hours later to assess changes over time.

Results: Reference intervals differed between males and females (Table 1).

TABLE 1 Reference intervals for specific shear rates in health men and women

	Male (n = 162)		Female (n = 125)	
Age (years)	43 ± 10.9		46 ± 11.0	
Hct (%)	46.2 ± 2.63		40.5 ± 2.29	
Shear rates	Median WBV	Central 95 percentile RI	Median WBV	Central 95 percentile RI
1	21.88	16.554-36.248	17.44	12.168-37.303
5	9.48	7.561-12.847	7.69	6.058-13.172
30	5.35	4.311-6.369	4.51	3.582-5.915
200	3.93	3.261-4.643	3.38	2.816-3.960

The coefficients of variation (CVs) for within-run and total precision with QC material and human whole blood were < 7.5% at all shear rates. The CVs for between-day precision with QC material were < 2.5% at all shear rates. WBV of the samples stored at room temperature for up to 6 hours and at 5°C for up to 2 days did not change compared to the control.

Conclusions: We recommend that separate reference intervals be used for men and women. The results indicate that the ZL-6000i provides rapid, accurate and reproducible WBV data. Viscosity measurements should be finished within 6 hours after sample collection, and samples that cannot be measured within the 6-hour window should be refrigerated until ready to test.

PB 434 | Standardization and Automation of Thrombin Generation Assay: on the Way to the Clinical Lab

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Background: Thrombin generation (TG) is known for more than 60 years. Several developments have been done through the years to improve its usability but it remains a research use tool because of a lack of standardization of methods. Typical inter-day precision of TG assays is around 10 to 30% depending on the parameter analyzed. It depends on the concentration and the source of tissue factor (TF) in the reagent, the use of external or local normal plasma to normalize the results, the operator as well as the method (1-2).

Aims: ST Genesia, a new analyzer intended to measure thrombin generation in a fully automated way, was evaluated in our lab for validation purposes.

Aside from biological outcomes of our protocol in the anticoagulant treatment setting, the purpose of this evaluation was also to determine how precise could become TG measurement.

Methods: 41 independent runs of measurement were performed with the same batch of STG - DrugScreen on ST Genesia. On each run, 3 freeze-dried samples were tested prior to testing fresh or frozen patient samples. 2 of these samples were internal quality control samples (hypocoagulable and normocoagulable) and 1 is intended to be used as reference plasma for normalizing results (2).

Results: Mean, standard deviation and coefficient of variation achieved are reported in table 1.

Conclusions: Automation, enhanced control of temperature throughout the assay and standardization of thrombin generation measurement help to achieve highly reproducible results, first step to introduce this assay in the clinical lab.

References:

(1) Dargaud Y et al *Evaluation of a standardized protocol for thrombin generation measurement using the calibrated automated thrombogram: An international multicentre study* Thromb Res 2012; 130(6): 929-934.

(2) Perrin J et al *Large external quality assessment survey on thrombin generation with CAT: further evidence for the usefulness of normalisation with an external reference plasma* Thromb Res 2015; 136: 125-130.

TABLE 1 Performances of ST-GenesIla on reference, hypo- and normo-coagulable plasma

	Sample 1 = STG - QualiTest Norm DS			Sample 2 = STG - QualiTest Low DS			Sample 3 = STG - RefPlasma DS		
n=41	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Lag time (min)	0,92	0,04	4,6%	1,18	0,05	4,1%	0,98	0,05	5,2%
Peak (nM)	501,92	16,17	3,2%	206,80	10,12	4,9%	486,25	16,50	3,4%
Time to Peak (min)	1,97	0,07	3,6%	2,32	0,10	4,3%	2,17	0,08	3,5%
ETP (nM.min)	1602,66	65,24	4,1%	522,55	20,55	3,9%	1740,53	67,63	3,9%
Velocity Index (nM/min)	718,69	55,87	7,8%	244,02	23,37	9,6%	598,74	49,27	8,2%

PB 435 | Accurate Recovery of N9-GP in a One-stage Ellagic Acid Based Clot Assay on the ACL Top Analyzer

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Background: FIX activity measurements for N9-GP exhibit large variations in a one-stage clotting assay (OSCA) owing to reagent specific variability. Previous studies have shown the most accurate N9-GP recovery with SynthAFax [1], an ellagic acid based APTT reagent available for use on IL coagulation analyzers.

Aims: To demonstrate a new test application using SynthAFax on the ACL TOP Family hemostasis test systems that accurately measures FIX activity for N9-GP samples.

Methods: The ACL TOP analyzer was calibrated with Calibration plasma using 8 dilution levels. The FIX-SynthAFax (FIX-SFX) application showed linearity with N9-GP from 1 to 150%. N9-GP spiked samples were prepared at three levels (100, 40 & 5%, n=40 each level) and tested with SynthAFax as the APTT reagent over five days on two representative ACL TOP instruments.

A multi-site internal study was also conducted with three spiked N9-GP samples (100, 40 & 5%, n=50 each level) and the SSC/ISTH secondary standard over 5 days on the ACL TOP analyzer. Data was compared across sites and mean results from each site were subsequently used to calculate the percentage of target recovery. For the purpose of this study, a target recovery range of ± 30% was considered to be acceptable.

Results: The FIX-SFX calibration was validated and used for data analysis. Results from all N9-GP samples recovered with precision comparable to IL controls. N9-GP samples at 100% and 40% recovered values within the acceptance criteria of %CV within device ≤ 15%. Samples at all levels consistently recovered at 100± 30% of the target. Results from internal testing compared well across days and all sample levels. Percent target recovery calculated for each site, for each N9-GP level was comparable and consistent, recovering within the target range.

Conclusions: N9-GP showed consistent results and accurate recovery using the new FIX-SFX application on the ACL TOP test systems with the SynthAFax in an OSCA.

PB 436 | A Performance Evaluation of a New Lupus Sensitive APTT Reagent

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Background: We report on the performance of a new APTT reagent (NewAPTT, Sysmex Corp. Japan), containing ellagic acid and synthetic phospholipids, which is sensitive to lupus anticoagulant (LA).

Aims: To compare the performance of New APTT with two widely used APTT reagents; reagent A (LA sensitive) and reagent B (LA insensitive).

Methods: The performance of NewAPTT was compared to that of two commercial APTT reagents, both containing ellagic acid and purified phospholipids (Reagents A and B), on a Sysmex CS-5100 coagulometer.

Results: Normal reference ranges were established in 123 normal healthy donors (see table). Acceptable between-day imprecision was obtained for all 3 reagents and excellent on-board stability was observed. Good correlation was observed between NewAPTT and reagent A with NewAPTT showing greater sensitivity to LA. The sensitivity to deficiencies in FVIII, FIX, FXI and FXII was assessed by testing deficient plasmas supplemented with normal plasma to achieve a range of concentrations of 10 - 100%. The sensitivity of NewAPTT was similar to Reagent B and superior to Reagent A. All three reagents had similar sensitivity to coagulation defects in liver disease. NewAPTT and Reagent B had similar sensitivity to unfractionated heparin; both were more sensitive than reagent A.

TABLE 1

	NewAPTT	APTT Reagent A	APTT Reagent B
Normal range (s) n = 50	24.73 - 35.29	23.12 - 31.49	21.04 - 28.92
Between Day imprecision %CV normal QC; abnormal QC	0.34; 0.47	0.29; 0.45	0.36; 0.32
4 day on-board stability (% change from day 0 in 3 levels of QC material	0.33 to 1.46	0.55 to 0.76	1.26 to 3.09
Mean APTT ratio in LA plasmas (n=30)	1.75	1.30	1.10
Correlation vs reagent A (r)	0.92	-	0.36
Correlation vs reagent B (r)	0.19	0.36	-
% above normal range	70	60	20
Factor VIII, IX, XI & XII sensitivity %	62; 35; 55; 47	48; 13; 22; 20	60;28;60; 18

Conclusions: NewAPTT showed comparable or improved performance relative to two widely used APTT reagents and is suitable for use in the detection of LA, inherited factor II, V, VII and X deficiency and assessment of coagulopathy in liver disease.

PB 437 | Standardization and Automation of Thrombin Generation (TG) Assay Measured in Presence and Absence of Thrombomodulin: TG on its Way to the Clinical Lab

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TABLE 1 Thrombin generation in quality controls

n=18	Sample 1 = STG - QualiTest Norm TS			Sample 2 = STG - QualiTest Low TS			Sample 3 = STG - QualiTest High TS		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Lag time (min)	1.52	0.05	3.07%	1.97	0.07	3.73%	1.36	0.03	2.49%
Peak (nM)	231.75	19.89	8.58%	90.48	11.95	13.20%	419.37	14.23	3.39%
ETP (nM.min)	1198.33	93.27	7.78%	519.55	28.54	5.49%	1380.11	80.59	5.84%
ETP Inhibition induced by TM (%)	23.17	3.42	14.76%	39.42	5.92	15.02%	ND (many results < 5%)		
Lag time (ratio)	0.87	0.03	3.43%	1.12	0.05	4.32%	0.78	0.03	3.83%
Peak (%)	82.51	3.63	4.40%	32.18	2.74	8.51%	150.07	9.34	6.22%
ETP (%)	86.46	4.20	4.86%	37.64	3.01	8.00%	99.80	5.12	5.14%

Background: Thrombin generation (TG) is known for more than 60 years and remains a research use tool despite usability improvements done through the years. This is due to a lack of standardization of methods leading to impaired precision. Typical inter-day precision of TG assays is around 10 to 30% depending on the parameter analyzed. It depends on the concentration and the source of tissue factor (TF) in the reagent, the use of external or local normal plasma to normalize the results, the operator as well as the method (Perrin J *et al*, Thromb Res 2015).

Aims: ST Genesia, a new analyzer intended to measure TG in a fully automated way, was evaluated in our lab for validation purposes in thrombophilia patients.

Aside from biological and clinical outcomes of our protocol on how useful could be to evaluate the thrombotic risk in thrombophilia patients, with and without thrombomodulin (TM), the purpose of this evaluation was also to determine how precise could become TG measurement.

Methods: 18 independent runs of measurement were performed with the same batch of STG - ThromboScreen (medium TF activity in presence or absence of TM) on ST Genesia analyzer. On each run, 4 freeze-dried samples were tested prior to testing fresh or frozen patient samples. 3 of these samples were internal quality control samples (hypo-, normo- and hypercoagulable) and 1 is intended to be used as reference plasma for normalizing results (1) in absence of TM only.

Results: Mean, standard deviation and coefficient of variation achieved are reported in Table 1 and Table 2.

TABLE 2 Thrombin generation in reference plasma

n=18	Sample 4 = STG - RefPlasma TS		
	Mean	SD	CV
Lag time (min)	1.75	0.06	3.69%
Peak (nM)	280.96	21.84	7.77%
ETP (nM.min)	1387.78	109.47	7.89%
ETP Inhibition induced by TM (%)	NA	NA	NA
Lag time (ratio)	NA	NA	NA
Peak (%)	NA	NA	NA
ETP (%)	NA	NA	NA

Conclusions: Automation, enhanced control of temperature throughout the assay, normalization of results and standardization of TG measurement help to achieve more reproducible results all over the TG profile range. This is an important step to introduce this assay in the clinical lab.

PB 438 | Establishment of Oral Anticoagulant Monitoring Facility at Tertiary Care Teaching Hospital in Western India: Four Years Experience

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Background:

- In India, majority of the patients requiring oral anticoagulation are on Vitamin K antagonists requiring monitoring of PT-INR. Establishment of regular facilities for **precise** monitoring of PT-INR is still a major concern.
- Our institutional coagulation laboratory (Routine) handles > 10,000 patients per annum with ~1500 requests for oral anticoagulation monitoring.
- Here we give an account of our experience of establishing quality systems in the coagulation laboratory, liasoning and coordination between different departments in the hospital, education of the Post-Graduate Students, Faculty and patients; resulting in better patient care.
- We believe and hope that our experience would be of practical benefit for establishing such facilities in other parts of the world.

Aims: To establish the systems for effective monitoring of patients on oral anticoagulants.

Methods: Our laboratory has a fully automated random access coagulation analyser and back up semi-automated instruments from IL. Following measures were implemented:

- Developing protocols for sample collection and processing.
- Establishing the „Quality Systems" in the coagulation laboratory.
- Training of the technical staff, resident doctors and faculty.

- Education of the patients.
- Coordination and liasoning protocols with clinical faculty.

Results:

- Steady increase in the number of patients regularly availing laboratory facilities reflecting the trust of clinicians and patients.
- Number of cases with untoward effects / complications decreased over the period of time.
- Patients´ education resulted in better compliance.
- Training of the doctors and coordination resulted in better patient care.

Conclusions:

- The monitoring of patients on oral anticoagulants improved with systematic efforts.
- Patients were benefited in terms of decreased overall cost of management due to accuracy of results, decreased morbidity and complications, decreased visits for check ups, reduced mental stress.
- Multi-pronged approach results in precise monitoring of patients on oral anticoagulants.

PB 439 | Do Chinese Patients with Heart Valve Replacement Benefit from Warfarin Pharmacogenetic Test: A Pilot Retrospective Study

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Background: How much the patients could benefit from warfarin pharmacogenetic test has been discussed for years since genetic backgrounds are different among races, and polymorphisms in CYP2C9/VKORC1 account for less than 70% of interindividual variability in warfarin dosing requirements.

Aims: We carried out a pilot retrospective study to investigate the in-hospital warfarin adjusting time according to pharmacogenetic test in Chinese patients.

Methods: Patients receiving heart valve replacement in Wuhan Asia Heart Hospital were included in analysis. Warfarin pharmacogenetic tests for CYP2C9*3 (rs1057910) and VKORC1 (-1639G>A) were applied before warfarin therapy. Recommended dose was calculated based on parameters according to IWPC pharmacogenetic algorithm. Therapeutic INR was determined by surgeons. Days for in-hospital adjusting warfarin dose were analyzed. Appropriate statistic tests were performed according to the data.

Results: 210 patients having genotype-guided warfarin adjustment were included in analysis. 100 patients following INR-guided warfarin adjustment were included as control.There were 187 patients with *1*1 (89.05%), 22 with *1*3 (10.48%), and 1 with *3*3 (0.48%) in CYP2C9*3 genotyping. 172 patients had AA (81.9%), and 38 had AG (18.10%) in VKORC1. Patients having genotype-guided warfarin adjusting reached their therapeutic INR in shorter time comparing with control (3.9±1.6 vs 4.9±2.8 days, P< 0.01). Pharmacogenetic test not only reduced adjusting time for those whose therapeutic dosing was lower than 5.0/day between investigation and control groups (3.9±1.6 vs 4.6±2.2 days, P< 0.01), it also cut off nearly two fold in adjusting time comparing with control when patients needed more than 5.0 mg/d for their warfarin therapy (4.7± 1.5 vs 11.6±5.3 days, P< 0.01).

Conclusions: Pharmacogenetic test could significantly reduce the in-hospital adjusting time for optimal warfarin dosing in Chinese patients with heart valve replacement, especially for those who may require high dose for warfarin therapy.

PB 440 | Analytical Performance of an Anti Xa Assay with Rivaroxaban Specific Calibrators

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Background: Rivaroxaban (RIVA) have been developed without the need of routine monitoring, although laboratory testing may be required in select situations. The gold standard method to measure this drug is de HPLC-Tadem mass spectrometry but some chromogenic methods have been developed to measure the RIVA concentration.

Aims: Evaluate the performance of anti Xa dependent chromogenic assay with no external antithrombin using RIVA calibrators.

Methods: RIVA level was measured by HemosiL Liq Anti Xa .Two levels of specific calibrators are used to create a 5-point calibration curve (HemosiL Rivaroxaban calibrators) on ACL TOP 500. Controls high (CH,313 ng/mL) and low (CL,83 ng/mL) (HemosiL Rivaroxaban controls) were used to assessment the precision and accuracy by EP15 A2. The linearity range were investigated by successive dilution of a patient sample with 845 ng/ml . Detection limit was also determined doing 60 runs during 5 days. Bilirubin and hemoglobin interference were investigated spiking them into three samples with different level of RIVA.

Population: 20 patients who were anticoagulated with RIVA 15 or 20 mg once daily.(4- 12 hs since last dose) and 10 healthy controls.

Results: RIVA test showed a CV inter assay 1,8 % for CH and 1,4 for CL ; the bias was 5,7 % for CL and 15,8 % for CH according EP 15 A2 CLSI protocol . The linearity range was 0 - 500 ng/mL without reflex test. The interference from hemoglobin was negligible up to 500 mg/dl instead no interference was observed for bilirrubin. In all normal samples RIVA levels was lower than 0,001 ng/ml and the detection limit was 8 ng /ml. The RIVA level detected in the patients was the expected according with the doses and the times from last intake.

Conclusions: In our experience this method with rivaroxaban specific calibrators has been a robust assay for detection rivaroxaban in plasma. The test has a satisfactory calibrator/control stability, good sample precision.with minimum interference from pre analytical variable.

PB 441 | Multicenter Study on the Interference of Spontaneous Hemolysis at Blood Sampling on Five Main Hemostasis Tests

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Background: Hemolysis is the first cause of rejection in hemostasis samples. Many studies demonstrate the effect of hemolysis on coagulation tests using artificially hemolyzed samples. New coagulometers able to identify optical interferences in order to replace visual inspection have been released.

Aims: To evaluate the impact of spontaneous in vitro hemolysis on the main five hemostasis tests, hemolyzed samples (Es) were compared with another non-hemolyzed (NEs) specimen obtained from the same patient within 4h.

Methods: A multicenter study involved 15 hospitals for the collection of paired samples (Es + NEs). 200 pairs of frozen plasma were analyzed with ACL TOP 750 CTS (IL) for 5 tests: PT, aPTT, D-Dimer (DD), Fibrinogen (Fib) and Antithrombin (AT). Critical difference (CD) between Es and NEs for each analyte was calculated as $2.77x(CVa^2+CVi^2)^{1/2}$ (CVa=analytical coefficient of variation,CVi =within-subject biological variation). Bias between Es and NEs was evaluated by Bland-Altman analysis.

Results: Median values for all tests and median free hemoglobin (IQR, min-max) for Es and NEs are shown in Tab1. Mean bias (95% of the differences Es-NEs) was -0.1s(-7.9 to 7.6) for PT, -1.1s(-12.3 to 10.0) for aPTT, 1366ng/mL(-12650 to 15383) for DD, -4mg/dL(-130 to 122) for Fib and 1.2%(-17 to 19) for AT. Differences beyond critical limit based on CVa and CVi were 9.5% for PT, 37% for aPTT, 13% for DD, 5.5% for Fib and 5% for AT. Correlations (r) between free hemoglobin concentration and E-NE differences were 0.17(PT), 0.17(aPTT), -0.04(DD), 0.22(Fib) and -0.08(AT). All DD differences >10000ng/mL(n=10) had aPTT ratio < 0.86. Mean bias for DD, without these samples, falls to 61.7ng/mL(-1977 to 2100).

TABLE 1 Median values (IQR, min-max) for PT, aPTT, DD, Fib and AT and median free hemoglobin (IQR, min-max) for Es and NEs

	Hemolyzed samples Median (IQR; min-max)	Non Hemolyzed samples Median (IQR; min-max)	P (Paired-samples t-test)
PT sec	13.0 (11.5-21.0; 9.6-246.9)	13.0 (11.4-21.0; 9.9-286.8)	p=0.687
PT Ratio	1.14 (1.00-1.84; 0.84-21.7)	1.14 (1.00-1.84; 0.87-25.2)	p=0.691
aPTT sec	31.9 (28.2-37.5; 19.2-77.2)	33.5 (29.4-37.4; 22.0-80.6)	p=0.005*
aPTT Ratio	1.04 (0.92-1.22; 0.62-2.51)	1.09 (0.95-1.22; 0.71-2.62)	p=0.005*
D-Dimer ng/mL	412 (208-1016; 25-67784)	329 (188-762; 36-28695)	p=0.007*
Fibrinogen mg/dL	378 (304-506; 74-1098)	389 (304-506; 64-1050)	p=0.376
Antithrombin %	103 (88-116; 25-153)	102 (89-115; 26-186)	p=0.066
Free hemoglobin g/L	1.97 (1.34-3.14; 0.52-15.3)	0.29 (0.29-0.29; 0.13-1.47)	p<0.001*

Conclusions: Our data suggest that in vitro hemolysis does not affect PT, Fib and AT. A moderate bias and a significant difference was instead observed for DD, and to some extent to aPTT, but at least some largest discrepancies are likely due to interferences different than hemolysis, such as activation of the coagulative cascade.

PB 442 | Performance Evaluation of the Coag-Sense® Point-of-Care INR-testing System

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Background: Novel point-of-care (POC) instruments for International Normalised Ratio (INR) testing are regularly entering the market. New instruments should ideally be subjected to multicentre validation studies to confirm that they are fit for purpose.

Aims: To evaluate the Coag-Sense® INR monitoring system for accuracy, measurement repeatability, instrument & test strip variability, and error rates.

Methods: Capillary blood Coag-Sense® values from 202 patients on warfarin were compared with venous blood INRs obtained with Thromborel® S on a Sysmex CS-2100i® analyser. Control materials were used to assess precision, and measurement repeatability was calculated on 46 duplicate finger-prick values. Instrument & test strip variability was evaluated on triplicate finger-prick values obtained on three different instruments (30 patients) and three different test strip lots (28 patients).

Results: A correlation coefficient of 0.93 (p< 0.0001) and a positive proportional bias of 15.3% was found on linear regression analysis. Although overall dosage concordance was low (70.3%), broader clinical agreement was high (91.0%). Control material coefficients of variation (CV) varied from 3.6% to 19.0%, depending on the control type, and the capillary blood measurement repeatability CV was 17.6%. There was no significant instrument (p=0.59) or test strip (p=0.36) variability, but the error rate was high (16.4%).

Conclusions: The Coag-Sense® instrument is generally adequate in terms of accuracy, broader clinical agreement, precision and instrument & test strip variability. The measurement repeatability, dosage concordance, and instrument & test strip error rate, can be improved.

PB 443 | Thrombin Generation in Patients with Coronary Complications during the First Year after Percutaneous Intervention

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Background: Thrombin generation test (TGT) may be useful for dynamic assessment of the patients undergoing percutaneous coronary intervention (PCI). However, data about differences between patients with and without stent thrombosis or restenosis are few.

Aims: To determine thrombin generation, measured by TGT, in patients after PCI according the presentation of stent thrombosis or restenosis during the first year of observation.

Methods: 100 patients with stable coronary artery disease and PCI were observed during first year: 13 with stent thrombosis or restenosis (group I), 87 - without any thrombotic events or restenosis (group II) and 28 healthy donors (group III). Lag time, min; ETP, nmol/min; Peak Thrombin, nmol; ttPeak, min and its' modifications with thrombomodulin (TM) were determined before PCI, on the 3, 7, 90, 180 and 365 days after by Thrombinoscope BV, Holland and ThermoFisher SCIENTIFIC, Finland.

Results: Before PCI: Group I and Group III were different in ETP (1947±74058 and 1644±28443, p=0.01), ETP with TM (1054±69772 and 724±52317, p=0.0008), Peak Thrombin with TM (191±3653 and 148±2864, p=0.04) and ttPeak with TM (5.8±0.5 and 5.3±0.4, p=0.03). Between Group II and Group III only a difference in ETP with TM were found (975±144994 and 724±52317, p=0.001). Both patients' groups showed the same dynamics after surgery: decrease ETP and ETP with TM from 3 to 90 days. However, Group I ETP seemed to be higher then in Group III (1794±53033 and 1644±28443, respectively, ns) in 90 days of observation. In Group II, the ETP reached those of healthy donors (1666±90761 и 1644±28443, respectively). The medium value of ETP with TM in groups I and II tended to decrease up to day 90, but was higher than in Group III (1037±104329, 1044±162742 and 724±52316, respectively).

Conclusions: ETP is the most informative component TGT suitable for dynamic observation of the coagulation activity in patients with different outcomes after PCI.

PB 444 | Prediction of Partial Thromboplastin Time (PTT) and Prothrombin Time (PT) on the Basis of Factor Assay Activities Using Data from a Clinical Data Warehouse

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Background: Clinically, when a patient has multiple factor deficiencies it is unclear to what degree each factor influences the overall screening results. This is important when assessing complex patients with PT/PTT abnormalities who may have multiple factor deficiencies. The data repository at our hospital contains data from patients with tandem screening and factor assays. This data may be useful in determining PT/PTT results given multiple factor abnormalities.

Aims: To determine if factor assay activities can be used in combination to predict PT/PTT results using retrospective data from tested patients.

Methods: We collected 15 years' worth of data with >10,000 instances of tandem PT/PTT and factor assays. We created univariate models on log transformed values and then trained multivariate linear regression (LM) and random forest (RF) models with five-fold cross-validation to predict the PT/PTT value given factor assay results.

Results:

TABLE 1 Models for PT results

Factor	Beta	P-Value	R-Squared
Factor X	-7.549	6.01E -121	0.37372
Factor VII	-5.523	3.93E -288	0.43188
Factor V	-4.655	4.63E -32	0.09346
Fibrinogen	-2.474	1.08E -19	0.03082
Factor IX	-0.907	3E -12	0.03131
Factor XI	-0.169	0.11	0.00183
Factor XII	-0.023	0.879	-0.00178
Factor VIII	0.896	2.55E -45	0.02512

TABLE 2 Models for PTT results

Factor	Beta	P-Value	R-Squared
Factor XI	-18.55	2.59E -138	0.4921
Factor V	-17.9	1.91E -35	0.1125
Factor II	-15.35	1.27E -45	0.1722
Factor XII	-12.44	5.19E -23	0.1392
Fibrinogen	-10.63	1.19E -27	0.0468
Factor VII	-9.17	8.79E-66	0.1363
Factor X	-8.56	2.34E -17	0.0649
Factor VIII	-7.23	2.37E -211	0.1136

Tables 1 and 2 represent the results for univariate regression for PT and PTT, respectively. Factors X and VII associated strongly with PT results, as expected. While factor VIII was significantly associated with PT, the R^2 value indicated an overall poor correlation. Factor XI was found to be most strongly associated with PTT results. Interestingly factor VII was found to be associated with PTT as well, likely due to covariation with other liver dependent factors. Evaluation of multivariate models demonstrated fair performance of the LM for PT and PTT with R^2 values of 0.47 and 0.38, respectively. In contrast, the RF model performed significantly better with R^2 values of 0.65 and 0.47 for PT and PTT, respectively.

Conclusions: We found the expected relationship between factors and PT/PTT using retrospective laboratory data. Furthermore we created regression models that can be used to predict PT and PTT results. We hope to demonstrate the utility of these models in lab order decision making and in guiding coagulation reflex algorithms.

PB 445 | Use of a Synthetic Collagen to Monitor Platelet Function in Patients Receiving Dual Antiplatelet Therapy

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Background: Patients with coronary artery disease (CAD) are treated with aspirin and a P2Y12 antagonist to prevent myocardial infarction or stent thrombosis. Variable clinical response to both types of agents has been reported. Existing assays to identify sub-optimal response to anti-platelet therapy do not meet reported medical needs.

Aims: To characterize the utility of a synthetic collagen (SynC) to monitor platelet function in patients on dual-antiplatelet therapy.

Methods: Blood samples from healthy individuals (n=5) and CAD patients treated with clopidogrel (75-600 mg) with or without aspirin (75-325 mg) (n=51) were centrifuged to produce plasmas for aggregometry studies (PAP 8E, Bio/Data Corp. Horsham, PA). The aggregation response to 20 µM ADP, 500 µg/ml arachidonic acid and 190 µg/ml Type 1 biologic collagen was compared to that induced by SynC (2-200 ng/ml; JNC Corp., Yokohama, Japan). The aggregation response was characterized in terms of % aggregation, slope and AUC.

Results: Using plasmas from healthy individuals and CAD patients, two lots of SynC produced a concentration-dependent aggregation response over a concentration range of 16 to 128 ng/ml. Patients treated with 81 mg aspirin and 75 mg clopidogrel (33 of 51 patients) were stratified into two groups based on their % aggregation response to SynC, using the median % aggregation as the cut point. While the two groups had a distinct response to SynC, the median responses to ADP, arachidonic acid and biologic collagen were nearly the same and there was a strong overlap between the responses to these agonists in the two sub-groups. This is most evident at the lower concentrations of SynC.

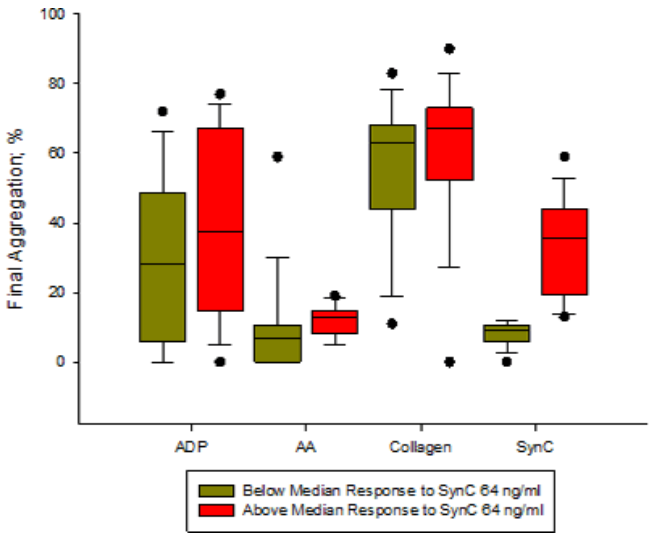

FIGURE 1

Conclusions: SynC-induced platelet aggregation may identify subgroups of patients with sub-optimal response to dual antiplatelet therapy that go undetected with traditional platelet agonists. Studies to clarify why biologic and synthetic collagens have different sensitivities to aspirin and clopidogrel are warranted.

PB 446 | Stability of TGA Parameters in Patients Treated with Anticoagulants Using the Fully Automated ST-Genesia System

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Background: Current thrombin generation assay (TGA) systems are semi-automated with samples tested in batch. Thus, the method lacks automation and standardization for homogeneity in results from study to study. TGA may be more informative than plasma concentration to assess the intensity of anticoagulation in a particular individual. A new automated and standardized TGA assay (i.e: ST-Genesia) has been recently developed by Stago.

Aims: To assess if TGA results in healthy subjects and patients treated by DOACs (dabigatran, rivaroxaban, apixaban), vitamin K antagonist (acenocoumarol) or LMWH (enoxaparin) are stable along time on samples stored at around -80°C during 10 months.

Methods: 6 healthy individuals and 30 samples of patients treated with anticoagulants were planned to be collected according to standard pre-analytical conditions. Samples were taken at peak and anticoagulant activity was measured according to current recommendations. Each sample were planned to be tested fresh (D0) and after storage at -80°C during 1 day (D1), 1 month (M1), 2 months, 3 months, 6 months, 9 months and 10 months. Impact of freezing (D1 vs D0) was to be assessed through paired-sample analysis (Student or Wilcoxon test) and stability through evaluation of trends overtime vs D1.

Results: Currently 6 healthy subjects, 7 apixaban, 3 dabigatran, 6 rivaroxaban, 5 VKA and 6 LMWH patients were included. Only results for D0, D1 and M1 are currently available. Table 1 summarizes, the mean difference relative to D0 or D1 and the range of relative deviations.

Conclusions: Freezing slightly affects all TG parameters. Once plasmas are frozen, TG parameters are also slightly influenced during the first month of storage. As it is frequently impossible to ensure that storage duration of samples is strictly equivalent throughout a study on a plasma bank, working with fresh samples would help in avoiding the outliers observed. This demonstrates the utility of a fully automatized TGA analyzer that could be integrated in the routine lab.

TABLE 1 Stability of TGA parameters after 1 day and 1 month storage at -80°C.

		Lag time (min)		Peak Height (nM)		Time to Peak (min)		ETP (nM.min)	
		D1 vs D0	M1 vs D1	D1 vs D0	M1 vs D1	D1 vs D0	M1 vs D1	D1 vs D0	M1 vs D1
Healthy subjects	Mean diff. (range)	-1% (-15 to 11%)	-8% (-12 to 4%)	-8% (-12 to 4%)	5% (0 to 7%)	-1% (-19 to 20%)	-7% (-10% to -3%)	-6% (-27% to 24%)	-2% (-8% to 3%)
Apixaban	Mean diff. (range)	0% (-10% to 12%)	1% (-20% to 33%)	15% (-4% to 28%)	-2% (-12% to 9%)	-4% (-12% to 6%)	1% (-8% to 15%)	2% (-9% to 7%)	0% (-23% to 18%)
Dabigatran	Mean diff. (range)	-10% (-14% to -4%)	-10% (-19% to 2%)	2% (-3% to 6%)	3% (-2% to 8%)	-7% (-12% to 1%)	-9% (-16% to -2%)	2% (1% to 3%)	-6% (-8% to -3%)
Rivaroxaban	Mean diff. (range)	-7% (-11% to -4%)	-6% (-12% to 7%)	13% (-5% to 25%)	-1% (-14% to 16%)	-7% (-12% to -3%)	-4% (-12% to 2%)	6% (-9% to 20%)	-2% (-6% to 5%)
VKA	Mean diff. (range)	-1% (-6% to 1%)	-3% (-13% to 2%)	1% (-4% to 5%)	-1% (-11% to 10%)	-1% (-4% to 2%)	-1% (-11% to 5%)	3% (-3% to 11)	-2% (-16% to 8%)
LMWH	Mean diff. (range)	-1% (-9% to 6%)	-9% (-20% to 3%)	15% (-5 to 69%)	0% (-26% to 18%)	1% (-8% to 10%)	-6% (-15% to 3%)	14% (-1% to 54%)	-3% (-18% to 17%)

PB 447 | First European Evaluation of ClarityCor Plasma Set for Instrument to Instrument Reproducibility Assessment

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Background: In Accreditation context, labs have to manage reproducibility between instruments.

To perform such testing, the lab can archive patient samples spanning the reportable range or can purchase pre-assayed validation plasma sets from commercial suppliers.

In this context, Stago (Asnières, France) has developed the ClarityCor (CC) Plasma Set, a set of frozen plasmas enabling to verify the correlation between different coagulation analysers.

Aims: Evaluation of CC Plasma Set to assess between-instrument reproducibility in comparison to locally selected frozen plasma samples.

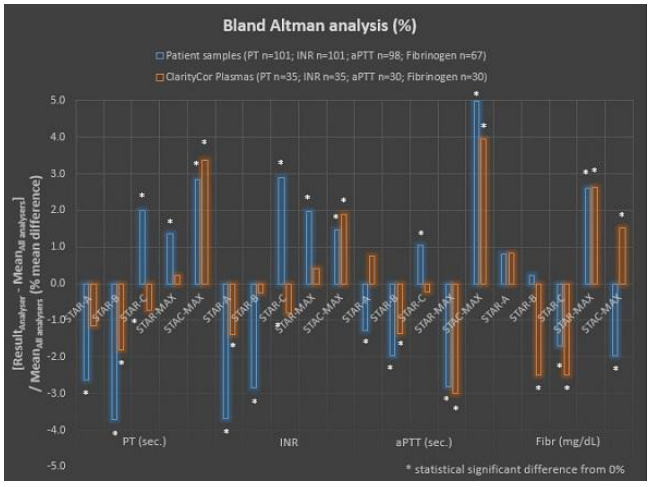

FIGURE 1 Overview of %mean bias comparing a single analyser (3 STAR Evolutions [A,B,C],STAR-MAX,STAC-MAX) with the mean of all analysers for routine parameters

Methods: One CC Plasma Set (n=30 for PT, INR, aPTT and Fibrinogen (Fibr) + n=5 for INR across the reportable range) was used for each instrument in the lab (Figure 1). In addition, citrated patient plasmas were collected. Method comparison to the mean of all analysers was done by Passing-Bablok (PB) regression and Bland-Altman (BA) analysis. Comparison was made between CC Plasma and patient sample datasets.

Results: Rank correlations (Rs) were between 0.967 and 0.999. PB slopes revealed no proportional bias for PT(sec) and INR of patient samples and CC Plasma Set except for STAR-MAX. 2/5 and 3/5 analysers showed concordant slope results between both data sets for aPTT and fibrinogen.. The %mean difference was ≤5% and ≤3.7% for all analyser/parameter combinations as compared to the mean and between datasets respectively (figure 1). All manufacturer's criteria were met (Rs>0.95, proportional(slope:0.9< x< 1.1) and systematic bias(BA %bias: 5-10%)).

Conclusions: Between instrument evaluation with CC Plasma Set results in sufficient data across the measuring range of PT, INR, aPTT and Fibrinogen. No major deviations were noted between frozen samples and CC Plasma Set. Given the advantages of sufficient plasma volume for all 3 parameters on a multitude of analysers and no need for time-consuming selection of suitable patient samples, the CC Plasma Set is a good alternative for local patient samples to evaluate between-instrument reproducibility.

PB 448 | Comparison of Activated Clotting Time Measured by I-STAT, Sonoclot and ACTPlus and Correlation with Anti-Xa during Cardiopulmonary Bypass Procedures

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Background: Activated clotting time (ACT) is used to monitor anticoagulation therapy with high heparin concentrations.

Aims: This study evaluated ACT measurements by 3 point-of-care instruments [I-STAT (Abott), ACTPlus (Medtronic) and Sonoclot (Sienco, Inc)] and their correlation with anti-Xa values during cardiopulmonary surgery (CPS).

Methods: One ACTPlus instrument (measuring in duplicate) was compared with simultaneous, parallel measurements on two I-Stat and two Sonoclot. For 30 adults receiving heparin during CPS, ACT was measured at four time points: before heparin administration, 3min following administration, 15min and 45min after starting CPS. Acceptance criteria for duplicate (intra-assay, ACTPlus) and parallel (inter-assay, I-Stat and Sonoclot) measurements were set as ≤12% spread error. Anti-Xa was determined on citrated plasma by a chromogenic assay (BioPhen Heparin LRT) at each time point. Method comparison was done using Passing-Bablok regression analysis.

Results: Results per instrument type are displayed in the table. Overall, only 51,2% of ACTPlus measurements fulfilled the 12% acceptance criteria; large differences were observed in cases with >12% spread error (mean difference 143.1%). In comparison, 8.4% of parallel measurements on I-STAT and 32.8% on Sonoclot differed >12%. Correlation between anti-Xa and ACT was moderate for all instruments. Method comparison showed no proportional or systematic error between I-STAT and ACTPlus. However, we observed both proportional and systematic error when comparing Sonoclot with I-STAT and ACTPlus. For each time point, ACT values were significantly lower on Sonoclot in comparison with ACTPlus and I-STAT.

TABLE 1 Comparison of ACT measurements performed during cardiopulmonary surgery, per instrument type.

	ACTPlus	I-STAT	Sonoclot
Number of instruments	1	2	2
Type of measurement	Duplicate (D)	Parallel (P)	Parallel (P)
Number of measurements	119	119	120
D/P with valid result for both measurements	100 (84.0%)	112 (94.1%)	113 (94.2%)
Measurement ≤12% spread error	61 (51.2%)	102 (85.7%)	74 (61.7%)
Measurement >12% spread error	39 (32.8%)	10 (8.4%)	39 (32.5%)
Mean spread error between all D/P	143.1%	5.2%	30.6%
Minimum-maximum spread error between D/P	0.0 - 1781.5%	0.0 - 34.8%	0.0 - 800.0%
Correlation between anti-Xa and ACT (correlation coefficient; 95% CI)	R^2 = 0.57 (0.38 - 0.71)	R^2 = 0.62 (0.50 - 0.72)	R^2 = 0.45 (0.31 - 0.58)

Conclusions: Overall, I-STAT performed better than ACTPlus and Sonoclot for ACT measurement. Failed duplicate and parallel measurements were 48.8% on ACTPlus and 38.3% on Sonoclot. Correlation between anti-Xa and ACT was moderate for all instruments. These results are unacceptable in clinical practice and require further investigation.

PB 449 | Presence of Residual Venous Thrombus at Warfarin Withdrawal: A Predictor for Recurrence After a First Episode of Symptomatic Provoked Proximal Deep Venous Thrombosis in an Asian Population?

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Background: The presence of residual venous thrombus (RVT) has been associated with an increased risk of thrombotic recurrence in patients with venous thrombosis. Recurrent DVT occurs not only in previously thrombosed veins but at other sites as well. There are no reported clinical studies on the relation between RVT and the risk of recurrent DVT after a first provoked proximal DVT in an Asian population.

Aims: To assess the risk for deep venous thrombosis (DVT) recurrence by presence of residual venous thrombus (RVT) at warfarin withdrawal following symptomatic first provoked proximal DVT.

Methods: Medical records of 45 consecutive patients with symptomatic first provoked proximal DVTs who had undergone warfarin surveillance for ≥3 months were reviewed retrospectively. Altogether, 22 patients discontinued anticoagulation after ≥3 months regardless of duplex ultrasonography results or RVT diagnosed by compression US. Another 23 patients discontinued anticoagulation after the RVT disappeared. Primary outcome was recurrent DVT. The ethics committee of the Siriraj Institutional Review Board approved this study. This study was supported by the Faculty of Medicine Siriraj Hospital, Mahidol University. This study was supported by Chalermphrakiat grants, Faculty of Medicine Siriraj Hospital, Mahidol University.

Results: Four of the 45 patients experienced recurrent DVT (8.89%), including 2 (9.00%) of 22 patients who had discontinued anticoagulant regardless of duplex ultrasonography results and 2 (8.70%) of 23 patients who discontinued anticoagulation after RVT disappearance (p = 0.963). The survival analysis showed no differences in the recurrent DVTs between patients who had a surgically provoked risk factor and those who had non-surgically provoked risk factors (p = 0.997).

Conclusions: RVT at warfarin withdrawal was not a predictor for recurrence following a first symptomatic provoked proximal DVT.

PB 450 | Hemolyzed Samples: Should Be Accepted in Hemostasis Laboratories?

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Background: Spurious hemolysis reflects a generalized process of endothelial and blood cell damage during blood sample collection. This is a common phenomenon in many laboratories.Different authors

recommend reject them, but in the other hand some authors affirm that results from patients with in vivo hemolysis should be reported to avoid repeat.

Aims: We compared hemolytic samples from random patients, with samples without hemolysis from the same patients; over 24Hs of the rejection. Samples were collected all over 2015 year. a) We compared the flag levels in Prothrombin time (PT); APTT, Fibrinogen (FBG) and Factor VIII (VIII) performed employing optical automated analyzer. b.) We compared optical automated analyzer vs an electromechanical one.

Methods: In all samples (with and without hemolysis) PT, APTT, FBG and VIII were performed. All tests were carried out in an ACL TOP 300 coagulometer with IL reagents and Sta Compact with Stago reagents. Hemoglobin level (Hg) was measured in a Sysmex XR-2100 analyzer. A Deming regression was performed by using EP evaluator 9 software(Data innovations). As a quality requirement, allowed total error of 15 % (CLIA) for PT and APTT, 10% for FBG and VIII. Another established requirement was that confidence interval (CI) includes 1 value and intercept 0, so to get an R > 0.85, and to check as a quality requirement (QR) that each point was inside it.

Results:

TABLE 1 Samples results from ACL TOP 300/ Sta Compact]

Coagulometer	Test	n	R	Slope (95%CI)	Samples out of QR
ACL TOP 300	PT	51	0.63	0.96 (0.88-1.04)	2
	APTT	51	0.86	1.01 (0.86-1.17)	2
	FBG	50	0.84	1.07 80.84-1.24)	5
	VIII	51	0.86	1 (0.85-1.15)	13
Sta Compact	PT	32	0.97	0.88 (0.81-0.96)	1
	APTT	32	0.97	1.07 (0.98-1.18)	1
	FBG	30	0.72	0.65 (0.45-0.86)	3

Hg levels of hemolyzed samples were0.05-0.12 mg/dl. Samples results from ACL TOP 300 and STa Compact coagulometers are shown in table. QR for the comparison of samples with and without hemolysis were achieved for PT and APTT in both coagulometers, but not for VIII and FBG.

Conclusions: We did not find any differences between methods (optical and electromechanical). Our recommendation is to reject hemolytic samples for FBG and VIII and accept them for TP and APTT.

PB 451 | Coag S: A Professional INR Monitoring Device. Comparison with the Laboratory INR Testing

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Background: The deep vein thrombosis (DVT) still remains a challenge for the communities struggling for an aging population. The frequency and quality of OAC monitoring by prothrombin time is decisive.

Aims: The number of reports comparing the diagnostic performance of the POC devices is still not to be found in high number.

Diagon Ltd., (Budapest, Hungary) developed a small POC coagulometer ("Coag S"). The detection system is based on photooptical monitoring of fibrinogen-fibrin transition. A comparison was made between this POC and the traditional PT testing.

Methods: 100 patients were enrolled in this study. From the threated patient 50% were anticoagulated by Warfarin (Marfarin) and the other 50 % with acenocoumarol.

p { margin-bottom: 0.1in; direction: ltr; color: rgb(0, 0, 0); line-height: 120%; }p.western { font-family: „Liberation Serif","Times New Roman",serif; font-size: 12pt; }p.cjk { font-family: „Droid Sans Fallback"; font-size: 12pt; }p.ctl { font-family: „FreeSans"; font-size: 12pt; } The separated blood plasma was measured at the same time as the fingertip blood drop for prothrombin time. (TOA Sysmex 1500, Siemens recombinant thromboplastin, "Innovin".

Results: Regarding the INR results as continuous value, we performed Passing Bablok regression test and Blandt Altman difference plot. The Passing Bablok slope was 1.00, the intercept -0.075. The correlation coefficient square (R^2) was 0.955.

The Blandt Altman difference plot showed in average -0.0641 difference, without any graphical sign of a systemic bias, as the samples' INR-s were increasing.

The Cohen kappa coeff: was 0.7489 (C.I. 95%:0.621-0.8762).

Conclusions: This POC PT device has proven it´s equal value in comparison with laboratory PT method in the monitoring of coumarin therapy.

PB 452 | Evaluation of the New Tcoag Pre-calibrated TriniLIATM D-Dimer II Assay

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Background: Plasmin degrades fibrin into various breakdown products including D-Dimer following the formation of a fibrin clot. D-Dimer is measured in suspected cases of disseminated intravascular coagulation and has been applied for a number of years as a negative predictive indicator (NPI) of venous thrombo-emboli (VTE) as a biological marker to exclude recent thrombus formation.

Aims:

1. To evaluate the new Tcoag TriniLIATM D-Dimer II for application in the routine diagnostic service as a NPI of VTE and the robustness of a pre-calibrated assay on the Tcoag DT100 and Destiny Max analysers.
2. To compare results of Tcoag TriniLIATM D-Dimer II with the Biomerieux Vidas D-Dimer Exclusion II (gold standard) and Tcoag Destiny Max TriniLIA D-Dimer.

Methods: D-Dimer assays were performed using citrated samples received routinely for patients with suspected VTE using pre-calibrated TriniLIATM D-Dimer II on the Tcoag DTI00 and Destiny Max analysers, TriniLIA D-Dimer on the TCoag Destiny Max and the Vidas D-Dimer Exclusion II using Biomerieux Vidas analyser. Where required assays were calibrated using reagent specific calibrators and quality control material.

Results: Results showed good correlation of the TriniLIATM D-Dimer II with current reagents and analytical platforms. Correlation of TriniLIATM D-Dimer II between DT100 and Destiny Max analyses r^2 = 0.98, between DT100 and Vidas r^2 = 0.94. Correlation of TriniLIATM D-Dimer II with TriniLIA D-Dimer performed on the DT100 and Destiny Max analyses r^2 = 0.90. There was no significant difference in results between TriniLIATM D-Dimer II and Vidas D-Dimer Exclusion II p = 0.72. All low and high quality controls run over a 6 month period were within acceptance range.

Conclusions: Study has demonstrated good performance of the TriniLIATM D-Dimer II against the gold standard. A robust pre-calibrated assay has advantages in a busy diagnostic laboratory with the need for rapid turnaround times of results. The proposed cut-off value for use as a NPI for VTE is 0.5ug/ml (FEU).

PB 600 | A Systems Biology Approach to Elucidate the Post-translational Regulome of Coronary Artery Disease

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Background: Coronary artery disease (CAD) is the major cause of mortality and morbidity in the world and is at endemic levels in Indian population. There is an urgent need to comprehensive analysis of post translational regulation of genes and pathways associated with CAD which in turn may give better understanding of CAD.

Aims: Our aim was to evaluate the important post-translational modifications (PTMs) regulating all the pathways and genes associated with CAD.

Methods: Using PolySearch 995 genes were found to be associated with CAD (Integrative Cardiome database). The protein information was taken from SWISSPROT database using which distribution and co-evolution of PTMs were identified. The co-evolutionary PTMs were further used to construct the network and based on topological parameters regulatory PTMs and enzyme were validated by immunoprecipitation assays and ELISA in age and gender matched 300 CAD affected and 300 control subjects samples.

Results: Of all the PTMs regulating 995 genes, we found phosphorylation, ubiquitylation and acetylation and N glycosylation to be highly represented. The network construction and analysis of co-evolving and conserved PTMs, we found that serine phosphorylation, acetylation and n- glycosylation as important regulators. Immunoprecipitation assays performed CAD affected and control subjects demonstrated clear increase in serine phosphorylation in disease condition. The top 5 kinases involved in serine phosphorylation were GSK3beta, PRKCA, PRKCD, SRK and PRKACA. We identified that SK3B could be potential master regulator modulating several protein functions. Furthermore, a 1 U/l increase of phsophoGSK3B (on a log scale) increased the risk of CAD by 6.4 fold (OR, 6.349; 95% CI, 2.29-17.64; P=0.0003) upon adjustment with conventional risk factors and diet.

Conclusions: We have identified serine phosphorylation by GSK3B as highly enriched PTM regulating several pathways associated with CAD.

PB 601 | A Translational Approach for Identifying Shared Molecular Function and Pathways between CAD and its Risk Factors

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Background: Coronary artery disease (CAD) is multi factorial disease influenced by several classical risks factors. Herein we implemented a novel approach using gene ontology for understanding the link between CAD and conventional risk factors (CRFs) such as Hypertension, Obesity and Diabetes.

Aims: To understand pathway physiology between CAD and classical risk factors.

Methods: Gene ontology (GO) based approach has been used to identify interplay of pathway between CAD and CRFs. We extracted CAD associated genes from In-Cardiome database and CRFs associated genes from Text-mined Hypertension, Obesity, and Diabetes candidate genes database (T-HOD). GOIDs for each gene were extracted from Uniprot database which were further grouped into Functional communicating ontologies (FCO) based on same functions and processes. In order to identify the risk association with individual and combination of risk factors, patient samples were taken from IARS database and statistical analysis was performed using SPSS software.

Results: We have selected 10,217 patient samples for this study. For risk assessment model, a new variable with 8 different categories was created using all 3 CRFs followed by logistic regression to assess the risk predictive ability. The best risk association was found with the combination of all 3 CRFs

(OR: 29.848; 95% CI 24.64-36.15), with enriched functions as acute inflammatory response and calcium ion homeostasis. However, second highest odds ratio was found with ‚Hypertension+ Diabetes' category (OR: 15.16; 95% CI 12.25-18.77) with enriched functions as blood coagulation and disease mutation.

All models built were tested for best-fit using the Hosmer-Lemeshow test.

Conclusions: This novel approach enabled the identification of important pathways for the disease and its risk factors communication. The information derived from such bioinformatic analysis can give better understanding in predicting the CAD risk.

PB 602 | Electronic Alerts, Comparative Practitioner Metrics, and Education Effect on Thromboprophylaxis and Thrombosis in Community Hospitals

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Background: We previously reported an intervention to improve appropriate venous thromboembolism (VTE) chemoprophylaxis and reduce VTE in tertiary care teaching hospitals [Woller SC *Am J Med* 2016]. Yet, VTE chemoprophylaxis remains underutilized and even lower rates have been reported in community hospitals [Kahn SR *Thromb Res* 2007]. We now report the performance of this intervention in community hospitals.

Aims: Our three aims are to report the rate of appropriate thromboprophylaxis, thrombosis, and bleeding among hospitalized medical patients before and after implementation of a multifaceted intervention (**Table**).

TABLE 1

Components of the venous thromboembolism reduction intervention applied in community hospitals
An EMR chart interrogation tool generated a daily VTE risk score, and classified each patient as being at high risk for VTE or not, based on a validated VTE risk assessment tool^
A second EMR electronic tool interrogated the MAR daily for whether guideline-recommended# chemoprophylaxis or therapeutic anticoagulation was being administered
If the patient was at high risk for VTE and appropriate chemoprophylaxis was not ordered, then a text alert was sent to the hospitalist of record
A monthly audit and feedback performance report was provided to each hospitalist
A proprietary targeted online CME activity tailored to each hospitalist' performance was sent monthly
LEGEND: EMR: electronic medical record; VTE: venous thromboembolism; CME: continuing medical education ^Kucher N, etal. N Engl J Med 2005; 352:969-977 #Kahn SR etal. Chest 2012; 141:e195S-e226S

Methods: We prospectively enrolled sequential medical patients admitted to one of 3 hospitals from 2011-2013. Patients were members of the "control" (2011), "intervention" (2012), or "subsequent year" (2013) group. Beginning in the intervention period, if a patient was high risk and not receiving chemoprophylaxis, then a text alert was sent to their hospitalist. Hospitalists were credited with applying appropriate chemoprophylaxis if within 36 hours of an alert they prescribed appropriate chemoprophylaxis or identified a contraindication in the EMR. Anonymous comparative physician performance was communicated monthly along with educational content tailored to each physician's performance.

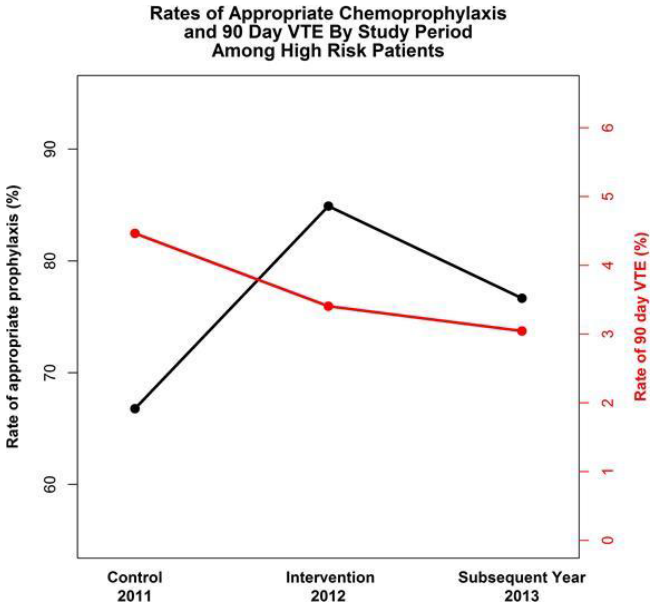

FIGURE 1 Rates of appropriate chemoprophylaxis and 90 day VTE among high risk patients

Results: 27,778 patients (35% high risk) constituted a total of 95,236 patient days. The rate of appropriate chemoprophylaxis among high risk patients increased then declined (67% control vs. 85% intervention vs. 77% subsequent year; p< 0.001). A reduction of 90 day symptomatic VTE was seen (4.5% control period, 3.4% intervention period, 3.0% subsequent year; p=0.008; **Figure**). No difference in major bleeding was seen (0.72% control, 0.84% intervention, 0.59% subsequent year; p=0.61).

Conclusions: Appropriate chemoprophylaxis among high risk patients improved during the intervention, but subsequently declined. Patient outcomes improved, but the proportion of improvement that resulted from the intervention is uncertain.

PB 603 | Efficiency of Bayesian Logic in Reagent Batch Change Management: Application to aPTT

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Background: A preliminary study („Use of prior manufacturer specifications with Bayesian logic eludes preliminary phase issues in quality control: An example in a hemostasis lab", Blood Coagul Fibrinolysis 2015, 26: 590-596) showed how the conventional preliminary phase can be avoided using Bayesian logic. Two new Bayesian tools are introduced, aiming to identify such trends in the process. The dynamic nature of Bayesian IQC results management enables these new tools which are capable to identify persistent shifts and in case of no reaction to absorb them in longitudinal monitoring of IQC results during reagent batch change.

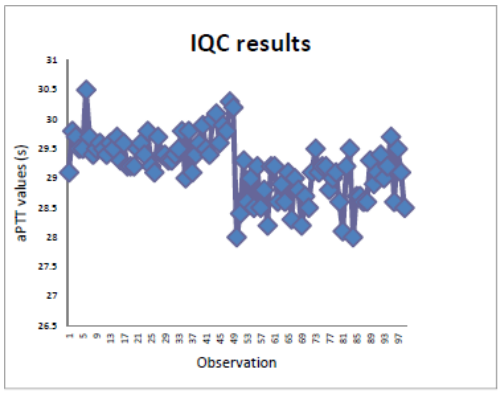

Figure 1 (a): Raw IQC results with reagent batch change at observation 50

FIGURE 1(A) Raw IQC results with reagent batch change at observation 50

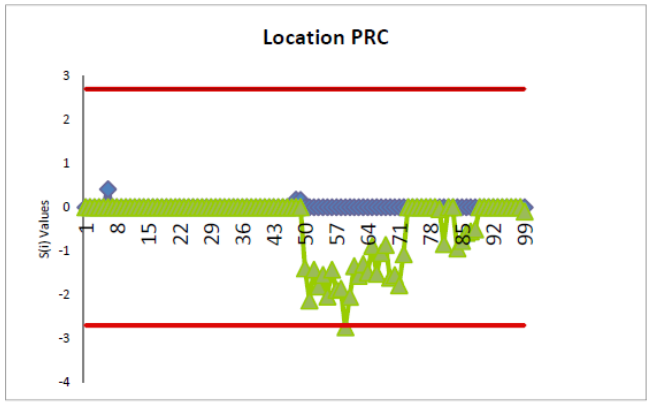

Figure 1 (b): Location PRC control chart with reagent batch change at observation 50

FIGURE 1(B) Location PRC chart with reagent batch change at observation 50

Aims: A case study is used to illustrate detection of a shift liable to impact patient results, and automatic updating of the control chart.
Methods: From Instrumentation Laboratory (IL), Bedford, MA, USA: ACL TOP 750 analyzer, HemosIL Synthasil and HemosIL Normal Control Assayed (aPTT (activated partial thromboplastin time) prior target = 29 seconds (s)). The 2016 ECAT screen exercises enable calculation of the acceptable bias size for z-score of 2 corresponding to a 30 s consensus value (ISO 17043). The last IQC result with the initial Synthasil batch is reported at control point 49, and the first result with the new reagent batch at control point 50.
Results: At the 10th IQC point, an alarm signaled that the reagent batch change was inducing a significant step change liable to impact patient results. From the 1st control point with the new reagent batch, a clear trend emerged, confirmed at the 2nd point. The laboratorian would thus have advance warning. The location PRC chart further identified a capacity for automatic updating of the control chart parameters in response to the reagent batch change (Figures 1(a) and 1(b)).
Conclusions: These new Bayesian tools enable efficient management of reagent batch change issues.

PB 604 | aPTT as an Independent Risk Factor for 30-day Survival for Patients on Extracorporeal Membrane Oxygenation (ECMO): 8-year Experience at Albert Einstein College of Medicine, Montefiore Medical Center

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Background: Survival of patients on ECMO has remained stable in every population.
Aims: Laboratory values predictors of survival are required to improve patient care.
Methods: Clinical Looking Glass software was used to assess Electronic Medical Records (EMRs) of patients at Albert Einstein College of Medicine, Montefiore Medical Center (2007-2014).
Results: Our population comprises of 166 adults and was divided in survivors and non-survivors, within 30 days. Indications for ECMO were cardiac (65%), respiratory (25%) and infectious diseases (< 10%). Eighty six patients (51.8%) survived the procedure. Gender, body weight, ejection fraction, diastolic blood pressure, and socio-economic status did not differ among survivors and non-survivors. In contrast, younger patients (45 yo *vs* 55 yo, p=0.0001) and higher systolic blood pressure (115 mmHg *vs* 103 mmHg, p=0.025) have favorable outcome. Univariate analysis shows that pre-cannulation values for creatinine (p=0.019), chloride (p=0.0026), and bicarbonate (p=0.026) have prognostic value. Post-cannulation aPTT, pH, platelet and lymphocyte counts also have discriminative power. Notably, multiple logistic regressions for Multivariate Analysis identified chloride (OR 1.07; 95% CI 1.02-1.13; p=0.004), pH (OR 3.35; 95% CI 1.89-5.9; p< 0.0001) and aPTT (OR 0.98; 95% CI 0.976-0.998; p=0.024) as independent risk factors for 30-day mortality. These results imply that pre-existing renal conditions and hemostatic dysregulation contribute to poor outcome. Finally, patients on VV-ECMO have increase odds of survival (OR 1.88; 95% CI 1.06-3.34; p=0.029).
Conclusions: The aPTT and other laboratory markers identified herein may guide the management of patients on ECMO.

PB 605 | Sensitivity of Spatial Thrombin Generation and Fibrin Clot Growth to Clotting Factors Deficiency

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Background: Blood coagulation is a space- and time-dependent process of plasma coagulation that leads to formation of a clot which prevents blood loss in the case of vascular injury.

Aims: To investigate the effect of tissue factor (TF) distribution in space on blood coagulation in normal and coagulation factors deficient plasmas and to identify the underlying molecular mechanisms.

Methods: We employed an in vitro reaction-diffusion experimental model of coagulation with a Thrombodynamics Analyzer device. Clotting in platelet free plasma supplemented with phospholipids (2 μm) was activated with TF homogeneously distributed in plasma volume (5 pM) or localized on surface (100 pmoles/m^2). Fibrin clot growth was registered via light scattering; clotting time or clot growth velocity was calculated. Spatial and homogeneous thrombin generation was determined using the registration of the fluorescence of thrombin-activated fluorogenic substrate; the amplitude of thrombin peak or thrombin wave was calculated.

Results: The comparison of clot formation and thrombin generation parameters in FII-, V-, VII-, VIII-, IX-, X- and XI-deficient plasmas of patients (37 samples total) demonstrated that in the case of spatially localized TF plasma clotted in all samples, while in the case of uniformly distributed standard TF concentration (5 pM) clotting was not observed in FV-, FVII- and FX-deficient plasma. When we increased uniformly distributed TF concentration up to 50-200 pM, clotting occurred in all deficient plasmas. In the case of spatially localized TF clotting parameters were normal (difference between measured parameters and baseline was less than 30%) when factor concentration was higher than 5%, while in the case of uniformly distributed standard TF concentration clotting parameters normalization occurred when factor concentration was higher than 30-70%.

Conclusions: We found that TF localization on surface could decrease coagulation system sensitivity to clotting factors deficiency.

PB 606 | In-cardiome: A Knowledgebase Integrating Molecular and Clinical Worlds for Translational Research and Drug Development in Coronary Artery Disease

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Background: Coronary artery disease (CAD) is a leading cause of death and major hurdle in the improvement of diagnosis and treatment is the lack of integration of knowledge from different areas research.

Aims: Our aim was to create "Integrated Cardiome - In-Cardiome" a knowledgebase integrating molecular and clinical worlds.

Methods: We used PolySerarch datamining tool along with manual curation for genes associated with CAD. Other data such as genetic, gene, expressions, pathway modulations, functional, phenotype, knockout gene etc, and clinical information such as clinical trials, drugs and their target networks, patient data from Indian population (Indian Atherosclerosis Research Study-IARS) were integrated. We have implemented network graphics for visualization of results and the backend integration was performed using MYSQL and PHP, JavaScript, D3 script and cytoscape.js in the web platform.

Results: In-Cardiome (http://tri-incardiome.org/) contains 995 highly curated genes, 1204 completed clinical trials, 12 drug classes with 138 drug targets. In-Cardiome provides 4 different kinds of search criteria i.e. Therapy based, Function based, Risk factors based and direct link to "Search genes".Therapy based criteria gives information on 12 different drug classes according to American Heart Association classification where each class has individual page of drugs, drug targets and their interactome are included. The 13 functional categories showing the disease progression is represented in a hierarchical system integrated with all the other clinical and molecular information. In the Risk factor categorization analysis we have selected 10,217 Patient data from IARS for 3 risk factors; hypertension, diabetes and obesity. Demographics, distribution and association data for each and combinations of risk factors can be accessed.

Conclusions: In-Cardiome is one of its kind novel knowledgebase which can enable better translational research and drug development for CAD.

PB 607 | Addressing Reproducibility Concerns for Mechanistic Models of Clot Formation

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Background: Coagulation and Fibrinolysis are complex phenomena including hundreds of biochemical interactions, and a few biophysical processes. Mechanistic models have become established as tools to simulate the quantitative behaviour of these phenomena, and to elucidate their design principles. However, the reliability of results from such models is complicated due to concerns about reproducibility.

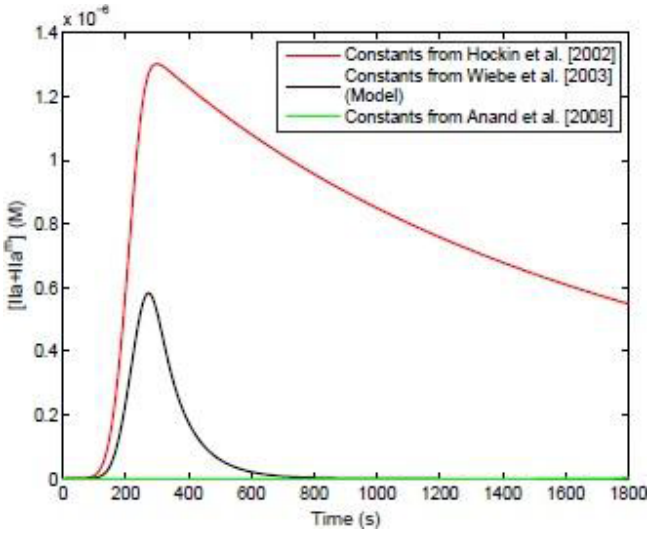

FIGURE 1 Variation of total thrombin for ATIII inhibition constants from different sources

Aims: The large number of parameters that are used in mechanistic models of hemostasis pose a challenge in terms of reproducibility as well as possible utility in patient-specific simulations. We address the challenge of reproducibility by shaping a consensus on the parameters that can be used in mechanistic models.

Methods: We show, using simulations of a sample model, that model predictions vary significantly with the use of different values available in the literature for the same kinetic constant. We thus highlight the importance of having consensus of kinetic constants used in mechanistic models for coagulation. The literature is then reviewed to document values of each kinetic constant corresponding to different reaction conditions (namely synthetic, in vitro, or in vivo).

Results: The variation in model predictions due to different literature values of a given kinetic constant can be as high as ±100% (see Fig 1). A table is proposed that documents values of kinetic constants that can be used in mechanistic models of coagulation. A portion of the table, which is available in Susree and Anand, (MMNP 2016), is given for illustration purposes in Table 1.

TABLE 1 Illustrative table depicting consensus kinetic constants for reactions of coagulation

Binding of TF and VII	**k1 = 3.2x10^-3 nM-1s-1**	**OBrien et al 1994 Krishnaswamy 1992**	**synthetic, in vitro**
Dissociation of TF-VII	k2 = 3.1x10^-3 s-1	OBrien et al 1994 Krishnaswamy 1992	synthetic, in vitro
Binding of TF and VIIa	k3 = 0.023 nM-1s-1	OBrien et al 1994 Shobe et al 1999	synthetic, in vitro
Dissociation of TF-VIIa	k4 = 3.1x10^-3 s-1	OBrien et al 1994 Shobe et al 1999	synthetic, in vitro

Conclusions: The proposed table of kinetic constants are the consensus values for the reactions in a particular reaction network. Other reaction networks could throw up different reaction mechanisms for which a separate literature review needs to be performed.

PB 608 | Epigenetic Regulation of PAR-4-mediated Platelet Activation Underlies Smoking-related Cardiovascular Disease

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Background: Smoking is a known risk factor for cardiovascular disease, with nearly 10% of cases worldwide attributed to tobacco use. The underlying mechanisms for this association remain largely unknown; however, increasing evidence has arisen suggesting epigenetic modifications may play a role. Several methylation loci have emerged as robust indicators of smoking, including within *F2RL3*, where methylation is reduced. *F2RL3* codes for Protease-Activated Receptor 4 (PAR-4), a GPCR expressed on the surface of platelets that stimulates platelet activation and thrombus formation in response to thrombin.

Aims: To determine whether the methylation status of *F2RL3* influences platelet function.

Methods: Pyrosequencing was used to assess methylation at four CpG sites in *F2RL3*. For *F2RL3* epidemiology, participants from the Copenhagen City Heart Study had methylation status determined and were classified based on smoking status. For platelet function analysis, participants from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort were recruited based on methylation of *F2RL3*. Activation of platelet $a_{IIb}b_3$ integrin and surface exposure of P-selectin in response to PAR-1 and PAR-4-specific peptides were determined by flow cytometry. Experiments were conducted in a double-blind manner. For *F2RL3* methylation cell model, human coronary endothelial cells (HCAEC) were exposed to cigarette smoke extract (CSE) in culture and assayed for methylation status and cell response.

Results: Low *F2RL3* methylation corresponds to higher platelet responsiveness to PAR4 activator peptide.

Conclusions: Epigenetic regulation of PAR-4-mediated platelet activation underlies smoking-related cardiovascular disease.

PB 609 | Polymorphic Variation in Glutathione-S-transferase Genes among Chronic Myeloid Leukemia Patients in Pakistan

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Background: Glutathione S Transferases are Phase II drug metabolizing enzymes which help in detoxification carcinogens of catalyses of conjugation of glutathione with reactive electrophiles. Human GSTs: GSTM1 and GSTT1 are two genes which detoxify or protect against reactive oxygen species and xenobiotics. Reduced individual ability in drug detoxification activity may leads to cancers upon prolonged exposure to xenobiotics. It is known that polymorphism of GSTs genes affects the detoxification of xenobiotics which leads to genetic susceptibility of chronic myeloid leukemia (CML).

Aims: To determine the polymorphism of GSTs enzymes in CML patients.

Methods: *Multiplex PCR of GSTM1* and *GSTT1* genotypes of 60 DNA samples of CML were performed.

Results: A total of confirmed 60 cases (7 females and 53 males) of CML with mean age of 35 (median) range (9-64) and 63 normal healthy individuals of same sex and age as control were analyzed. GSTM1 null deletion was significantly more frequent in controls (41%) as compared to CML patients (31%). CML patients (14%) have low frequency of GSTT1 null deletion as compared controls (21%). Double deletion was present in CML (7%) and has double frequency in controls (14%) and these differences were statistically significant (p= 0.025).

Conclusions: This study has allowed determining the frequency of GSTM1 and GSTT1 polymorphism in a sample of our patients. A significant increase has also been observed in control group which suggest they might be susceptible to disease occurrence. Further studies with large sample size are required to draw a valid conclusion.

PB 610 | Gene Expression Profile of Schistosoma Mansoni Adult Worms after Treatment with an Anti-schistosomicidal Drug that Inhibits Lysine Demethylase LSD1

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Background: Schistosomiasis is ranked as the second most common human parasitic disease in the developing world after malaria. *Schistosoma mansoni*, the most widespread blood parasite moves through the intestinal veins by means of the host circulatory system. To date no vaccine is available for schistosomiasis, and the treatment is done by long-term application of a single drug, praziquante (PZQ).

Aims: The aim of this project is to check how the parasites respond to different drugs developed by our partners in the project "Anti-Parasitic Drug Discovery in Epigenetics" (A-ParaDDisE) that target histone-modifying enzymes. The drugs are tested at sub lethal doses and we identify the genes that are differentially expressed (DEGs) using high-throughput RNA sequencing technology. In the present work, we treated the adult parasites with compound A, a potential lysine demethylase (LSD1) inhibitor and compared transcriptomic profiles of drug treated and untreated parasites.

Methods: An in-house bioinformatics pipeline involving up-to-date software is used to carry out the analysis. The various components of pipeline include quality control filtering, read mapping, transcript quantification, differential expression analysis and gene ontology (GO) enrichment.

Results: We found 938 significantly differentially expressed genes (629 down and 309 up regulated genes) in female and 2709 genes in males (1312 down and 1397 up) respectively. Based on GO classification, the genes were divided into three major functional categories (biological, molecular and cellular), of these the dominant terms were ion transport, regulation of cellular and metabolic process, chromatin modification, drug transport and glycoprotein metabolic process.

Conclusions: We hope this comprehensive differential gene expression profile of drug-treated adult parasites will provide useful information to the community in search of anti-schistosomicidal drug targets and new therapeutics for the control of schistosomiasis.

PB 611 | Nanoscale Measurement of Force Generation during Platelet-driven Clot Formation in Platelet Rich Plasma

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Background: We developed a nano-thrombelastographic (nTEG) method based on atomic force microscopy (AFM) that gives information about the viscoelasticity and contractility of fibrin network during clot formation and degradation in human plasma.

Aims: Our aim was to follow platelet-driven clot formation in platelet-rich plasma (PRP) by using nTEG.

Methods: Blood was collected into Vacuette tube (sodium citrate 3.2%) from five untreated and eight coumarine-treated individuals. PRP was prepared by centrifuging citrated blood with 150g at room temperature for 10 minutes. An AFM cantilever was submerged in a 300-µL sample and cyclically moved up and down with an amplitude of 1 µm and a speed of 1 µm/s. The sample contained PRP and Ca^{2+} 10 mM, and clotting was initiated with thrombin or collagen (at 1 IU or 5 µg/ml). A silicon shield surrounding the droplet prevented evaporation. As the sample clotted the cantilever increasingly deflected during its vertical travel.

Results: The peak-to-peak force amplitude, measured as the difference between the maximal upwards and downwards cantilever deflections, gradually increased during the formation of the elastic, platelet-rich fibrin network. The time delay until the initial rise of the force amplitude was 100-200 s and became prolonged three-fold in lieu of thrombin activation. Force hysteresis, measured as the difference between the areas under the upwards and downwards force-displacement curves, started to increase prior to that of the force amplitude, suggesting that the viscous response preceded the elastic one. Upon activation the platelets contracted the clot, leading to a permanent cantilever deflection the magnitude of which scaled with platelet number.

Conclusions: The presence of coumarine apparently did not influence the above nTEG parameters. In sum, by a quantitative monitoring of the time-dependent nanoscale changes of an AFM cantilever the nTEG method provides a unique insight into the microscopic mechanisms of clot formation and contraction.

PB 612 | Analysis of Platelet Activation by Stable Isotope-resolved Metabolomics (SIRM)

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Background: In response to vessel damage, platelets activate, aggregate, and pull against each other (*i.e.*, clot retraction), to form a stable clot. These processes require energy. Patients with metabolic diseases are particularly at risk of thrombosis, in part, due to hyperglycemia-linked platelet hyper-reactivity. Thus platelet metabolism is critical for modulating normal hemostasis and controlling pathogenic thrombosis. Early studies showed that platelet activation increases glycolysis and lactate production; however, these studies relied on crude analytics and inhibitors, so details are still ill-defined.

Aims: We sought to delineate platelet metabolism in both resting and activated states, utilizing state-of-the-art, stable isotope-resolved metabolomics (SIRM).

Methods: Human platelets were labeled with [U-$^{13}C_6$]-glucose and then treated with or without thrombin. Platelet pellets and releasates were separated by centrifugation and pellets were further extracted for polar and non-polar fractions. Metabolites in pellet fractions and releasates were analyzed by high-resolution nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

Results: Both NMR and MS analyses showed release of AXP (ATP, ADP and AMP) upon activation. AXP production clearly occurred in platelets, as the ribose-rings were synthesized from [U-$^{13}C_6$]-glucose *via* the pentose phosphate pathway. We noted increased production and excretion of [$^{13}C_3$]-lactate, indicating enhanced glycolysis and lactic fermentation, during activation. We directly monitored activation-dependent changes in a large panel of metabolites in glycolysis, the tricarboxylic acid (TCA) cycle, and glycogen, lipid, and nucleic acid metabolism.

Conclusions: Using SIRM, a more complete picture of platelet metabolism is emerging, leading to a better understanding of platelet energy utilization. Our work will yield critical insights into the causes of platelet dysfunction in metabolic disorders and uncover novel therapeutic strategies *via* modulating platelet metabolism.

PB 613 | Metabolites Associated with Massive versus Submassive Pulmonary Embolism - A Pilot Study

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Background: Outcomes after acute pulmonary embolism (PE) vary greatly. To improve our understanding and ability to risk-stratify PE patients, we performed the first-ever global metabolomic study comparing hemodynamicaly stable, "submassive" PE to unstable, "massive" PE.

Aims: To identify metabolites associated with massive vs. submassive PE.

Methods: We enrolled consecutive patients with acute PE in an academic emergency department from 2009-2012. We compared 28 submassive PE patients with 18 massive PE patients. All PE were lobar or more proximal with right heart strain or a positive troponin. Massive PE patients were hypotensive, required life support or thrombolysis within 5 days or died within 30 days. Blood samples were drawn within 24 hrs of diagnosis and processed within 60 min. We performed global metabolomic analysis using the Metabolon® DiscoveryHD4™ platform. We used Welch's two-sample *t*-test to identify biochemicals that differed across groups and Fisher's Exact Test for Metabolite Set Enrichment Analysis (MSEA) to determine pathways/metabolite categories driving differences. We calculated false discovery rates (FDR) to account for multiple comparisons.

Results: 42 metabolites differed between submassive and massive PE, at a nominal p value ≤0.05, with N-acetyl-beta-alanine, N-stearoyl-sphingosine and docosahexaenoylcholine the top hits. We performed a first MSEA at a global level, identifying the category Lipid to be significantly enriched with a nominal p value =0.03. A second MSEA, focused on sub-categories, identified ceramides to be significantly enriched with a nominal p value ≤0.05. Additionally, Xanthine Metabolism, Tyrosine Metabolism and Urea cycle; Arginine and Proline Metabolism had a nominal p value ≤0.2. However, the small sample size made it difficult to identify significant results after multiple testing correction.

Conclusions: While the small sample size did not allow firm conclusions, several metabolites, including lipids and ceramides, may differentiate massive from submassive PE.

PB 1133 | A Single Analysis for Simultaneous Measurement of All 4 DOACs

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Background: Commercial kits are available for functional measurements of the direct oral anticoagulants (DOAC) dabigatran, rivaroxaban, apixaban and edoxaban by coagulation assays. However, as each DOAC requires its own assay with separate kit, calibrators and quality controls, laboratories are confronted with high reagent costs and a cumbersome work load for a limited number of clinical samples.

Aims: To develop a single in-house method for measurement of all 4 DOACs.

Methods: A liquid chromatography-tandem mass spectrometry (*LC-MS/MS*) analysis was set up using a single extraction procedure for the simultaneous measurement of all 4 DOACs. A single in-house calibrator series was prepared for dabigatran, rivaroxaban and apixaban by spiking normal pooled plasma. For edoxaban, a commercial calibrator (Stago, France) was used. Different optimization steps led to a final LC-MS/MS protocol including sample extraction in acid phosphate buffer pH2, dabigatran dissolution in DMSO HCl5% and the use of an analytical precolumn backflush system. HemosIL Liquid Anti-Xa assay (Werfen, USA) and Hemoclot Thrombin Inhibitors assay (Hyphen Biomed, France) were used for functional measurements.

Results: Results for commercial quality control material where within the manufacturer's range for all 4 DOAC's. Within and between run imprecision CV's were < 9%, limit of quantitation was < 10 ng/mL and correlation coefficients of linearity studies were >0.999 for all 4 DOACs. Passing-Bablock analysis revealed excellent correlations with the respective functional measurements using patient samples, spiked samples and commercial quality controls. LC-MS/MS analysis could be performed on a high number of samples in less than one hour. Commercial kits are not required, although commercial control material can be used when desired.

Conclusions: A single LC-MS/MS assay measures all 4 DOACs in a sensitive, accurate, rapid and a cost-effective way, even when the type of DOAC is unknown to the laboratory or when a mixture of anticoagulants is present.

PB 1134 | Interest of PERC Rule (Pulmonary Embolism Rule-out Criteria) to Exclude Pulmonary Embolism in European Patients with Low Implicit Clinical Probability: PERCEPIC Study

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Background: Pulmonary Embolism Rule-out Criteria (PERC) is a clinical rule designed to exclude pulmonary embolism (PE) without further testing including D-dimer. Its accuracy has not been confirmed when it was retrospectively applied to European populations with relatively high overall PE prevalence. Nevertheless, one study suggests PERC reliability in patients with low clinical probability (CP) assessed by implicit judgment.

Aims: To prospectively assess the reliability of a negative PERC rule among European patients with low implicit CP to rule out PE.

Methods: Multicenter observational prospective study (9 French and 3 Belgian centers) of patients suspected of PE. The study received the approval of ethic committee. The main outcome was the rate of thromboembolic events (TEE) among patients with low implicit CP and a negative PERC rule: PE diagnosed during the initial admission, TEE and unexplained sudden deaths occurring during the 3 month follow-up. To consider PERC rule as reliable, the upper limit of the 95% confidence interval (CI) of TEE rate had to be less or equal to 3 %. The sample size of patients with low implicit CP and negative PERC has been calculated as 300 patients (NCT02360540).

Results: 1757 suspected PE patients were included, 201 had PE and/or TEE during follow-up leading to an overall prevalence of 11.4% (CI:10.0-13.0%): 4.7% (CI:3.5-6.1%) in patients with a low CP (n=1052), 16.1% (CI:13.3-19.4%) in patients with moderate CP(n=558) and 41.5% (CI:33.7-49.6%) in patients with high CP (n=147). Among patients with a low implicit CP, 336 had a negative PERC rule (32%). In this group, 4 PE were diagnosed: 1.2% (CI: 0.5-2.8%), including 1 isolated subsegmental PE. The tests performed in this group included 322 D-Dimer tests, 46 CTPA, 3 V/Q scan and 1 leg ultrasonography.

Conclusions: In European patients with low implicit CP of PE, PERC rule may exclude PE with a low rate of false negative and could avoid a substantial number of exams.

PB 1135 | Validation of STA-Liatest D-Di Assay for Exclusion of Deep Vein Thrombosis According to the Latest Clinical and Laboratory Standard Institute/Food and Drug Administration Guideline. Results of a Multicenter Management Study

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Background: Diagnosis of venous thromboembolism (VTE) is challenging. Objective confirmation is needed: failure to treat due to missed diagnoses can be life-threatening, while anticoagulation carries risk. Imaging studies are considered the most reliable diagnostic tool although time-consuming and expensive. Using assays with high negative predictive value (NPV), a negative D-dimer (DD) excludes clinically relevant VTE when used in conjunction with a pre-test

probability (PTP) score. In 2011, the Clinical and Laboratory Standards Institute (CLSI) issued a guideline on the use of DD in VTE exclusion and, with the US FDA, recommendations for testing patients with low/moderate VTE PTP.

Aims: Demonstrate the performance of STA®- Liatest® D-Di assay used in combination with a PTP score for deep vein thrombosis (DVT) exclusion according to CLSI guideline.

Methods: International, multicenter, prospective non-randomized, noninterventional clinical outcome management study conducted in standard of care setting. DD (STA®- Liatest® D-Di, Stago) measured in consecutive, ambulatory outpatients suspected of DVT, with low/moderate PTP, and without medical conditions known to alter DD values regardless of thrombosis presence using a 0.5 µg/mL (FEU) threshold for DVT exclusion.

Results: 1234 patients signed informed consent, of which 1031 underwent DD testing. 980 (mean age: 55y) had valid results (494 negative DD) and completed the study as planned. DVT prevalence was 8.4%. STA® - Liatest® DDi assay performance exceeded the CLSI/FDA guidance requirements: sensitivity: 100% (95% confidence interval [CI]: 95.8-100%), specificity: 55.2% (CI: 51.9-58.5%) NPV: 100% (CI: 99.3100%).

Conclusions: STA® - Liatest® DDi used in combination with PTP score has an excellent performance in relevant patients for DVT exclusion. Similar assay performances were recently demonstrated for PE. Altogether, the assay allows safe VTE exclusion, while avoiding unnecessary and expensive imaging tests.

(CI: 99.3100%).

PB 1136 | Variation in Practice for FVIII and FIX Inhibitor Investigations - Results from a UK NEQAS (Blood Coagulation) and UK HCDO Multicentre Exercise

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Background: Detection and accurate measurement of FVIII and IX inhibitors is important in the management of patients with haemophilia. Previous studies by UK NEQAS (BC) have shown variability in approaches to performing inhibitor assays, and variability in results obtained on samples with FVIII inhibitors.

Aims: In this study, we sought further information on inhibitor assay performance, including adoption of heat inactivation to improve inhibitor accuracy, and the precision of laboratory testing for FIX inhibitors.

Methods: Samples from a patient with an acquired FVIII inhibitor and a patient with haemophilia B with a FIX inhibitor were distributed to 95 centres. Participants were asked to perform FVIII and IX inhibitor assays, and to complete a questionnaire on assay methodology and the laboratory approach to inhibitor investigation. Results were obtained from 74 centres.

Results: Variation in practice for inhibitor assays was observed. For example, only 25 centres report use of a heat inactivation step prior to assay - 8 of which carry this out before all assays, whilst 17 do so only if FVIII or IX is above a certain level (from >0.01IU/ml to >0.2IU/ml). Temperatures for heat inactivation ranged from 56-60oC and incubation times from 56-90min. The effect such variation has on inhibitor assay results is unknown. For the sample from a patient with an acquired FVIII inhibitor, the median titre reported was 3BU, range 0.7-7BU, CV 43%, excluding one outlying result of 15.4BU. For the sample from a patient with a FIX inhibitor, the median titre reported was 18BU, range 0-43.8BU, CV 33%, excluding one outlying result of 117.54BU. The outlying centre was the same in each assay. For the FVIII assay, several labs reported a non-linear dilution effect.

Conclusions: Variation in practice may contribute to the lack of precision in FVIII and IX inhibitor assays. Further standardisation is required to improve detection and quantification of these inhibitors.

PB 1137 | Improving aPTT Test and Resource Utilisation in Manitoba, Canada - A Successful Provincial Experience

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Background: aPTT is one commonly performed test that is non-sensitive in predicting bleeding risk. An abnormal aPTT may lead to cancellation or unnecessary delays in surgical procedures, and haemostasis work-ups. The bleeding score is recommended in assessing patients' bleeding risk. Manitoba has been advocating evidence-based practice in all facets of medicine.

Aims: To improve test utilisation, we reviewed our practice, and the evidence of routine aPTT testing.

Methods: We audited aPTT ordering in all Manitoban hospitals. We found aPTT frequently being requested in situations where it was not evidence-based. After extensive consultations with various stakeholders, we decided to restrict the test availability and provided a consensus recommendation for proper utilization of aPTT.

Requests for indications other than unfractionated heparin monitoring and a selected number of specialty settings were processed after approval by hematopathologist or haematologist. In addition, the aPTT tick box was moved away from the INR/PT box on the requisitions and a reason for the test had to be specified. The laboratory staff enforced the initiative with 24/7 support from hematopathologists. The planning process lasted 6 months. The initiative commenced in June 2015. Data collected included test numbers, indications and clinical feedback. Some initial resistance required further education along with involvement of the province's senior medical leadership.

Results: Prior to June 2015, the use for aPTT was 16277 tests/month, with an 82% reduction to 2816 tests/month in 2016. The change did not impact patient care. Savings were realised in the following categories: 1) replacement of conventional coagulation analysers with point of care INR devices in small-medium sized sites: $462,000; 2) service contracts: $105,950 (33%) yearly and 3) annual reduction of reagents and consumables: $245,500 (21%).

Conclusions: Our local policy in restricting aPTT has successfully resulted in proper use, resource savings without impacting patient care.

PB 1138 | Prediction of Coagulation Testing in Underfilled Patient Samples

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Background: The common practice of rejecting blood samples for coagulation testing that are underfilled to < 90% of optimal volume, due to improper ratios of blood to anticoagulant, delays reporting of results and requires an additional blood draw.

Aims: This IRB-approved study was designed to determine the effect of addition of a standardized buffer solution to bring the underfilled specimen up to intended volume, thereby restoring proper blood to anticoagulant ratio, upon determinations of PT, aPTT, and fibrinogen.

Methods: Initially from eight healthy volunteers, citrated tubes were drawn to 50, 60, 70, 80, 90, and 100% of full volume. Imidazole-buffered Saline (IBS) was then added to each of the underfilled tubes to bring them to full volume, following which coagulation testing was performed. The results were well fit for PT (Fig. 1) and aPTT by exponential, and for fibrinogen by polynomial, models to create standard curves.

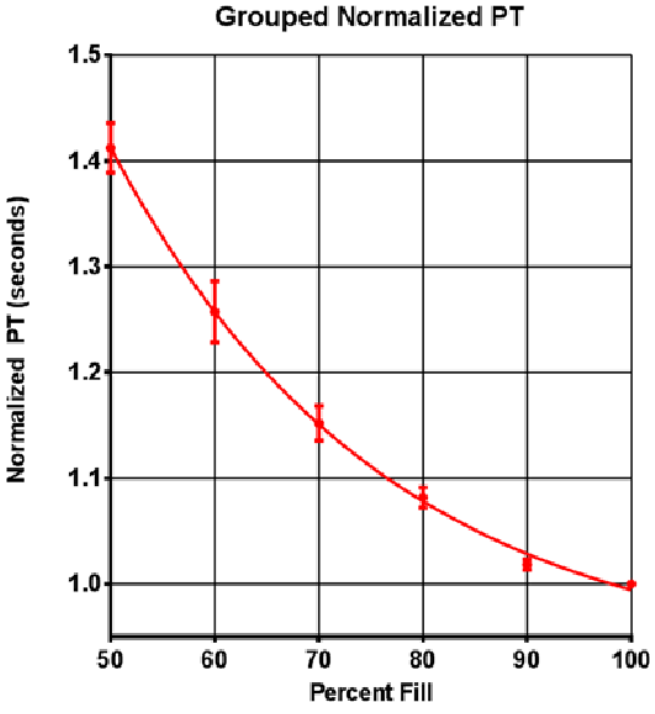

FIGURE 1 Prothrombin time curve from healthy volunteers

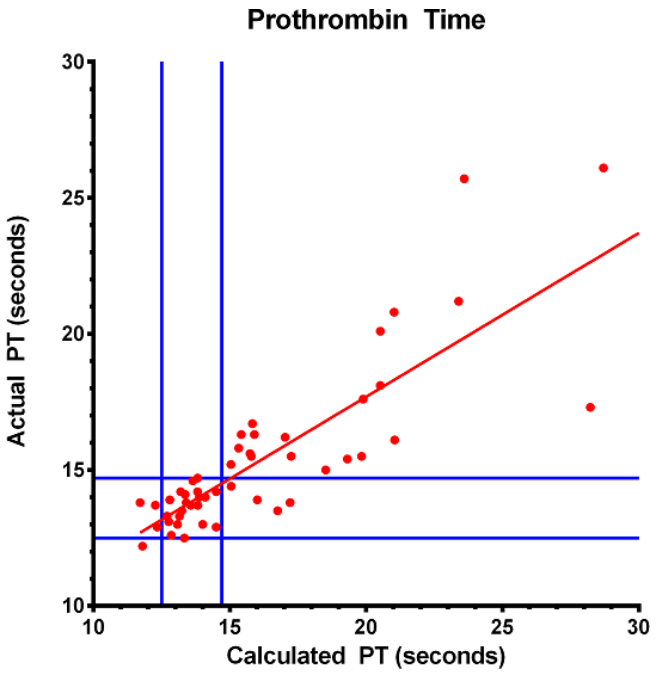

FIGURE 2 Comparison of calculated vs. actual prothrombin time

We next studied patients for whom underfilled samples were received by the coagulation laboratory. The underfilled tubes were brought to 100% volume with IBS, testing performed, and predictions of true values made based on the respective models. These predictions were then compared with actual values obtained on full volume patient tubes subsequently received.

Results: In the context of testing for exclusion of hemorrhagic tendency, where a false negative abnormality would pose the greatest clinical risk, no false negative misclassifications were made for PT (Fig 2) and aPTT, and in the case of fibrinogen, only one false negative misclassification in which the actual value was found to be only minimally below the lower normal limit.

Conclusions: While clearly obtaining a properly filled tube promptly would always be preferred, the approach employed in this study is encouraging, particularly in situations where additional delay of test results could have highly deleterious effects on patients, such as in the determination as to whether a stroke patient may be a candidate for tPA administration.

PB 1139 | Thrombin Generation Based Factor VIII Assay is Sensitive to Low FVIII Levels in Hemophilia

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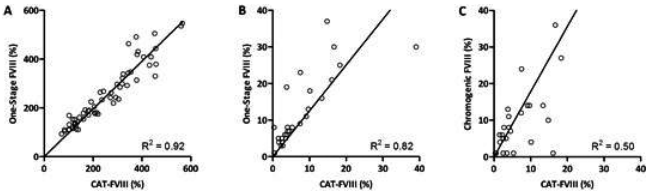

FIGURE 1 Correlation of FVIII-CAT and one-stage FVIII assay in healthy subjects (A) and hemophiliacs (B), and chromogenic FVIII assay in hemophiliacs (C).

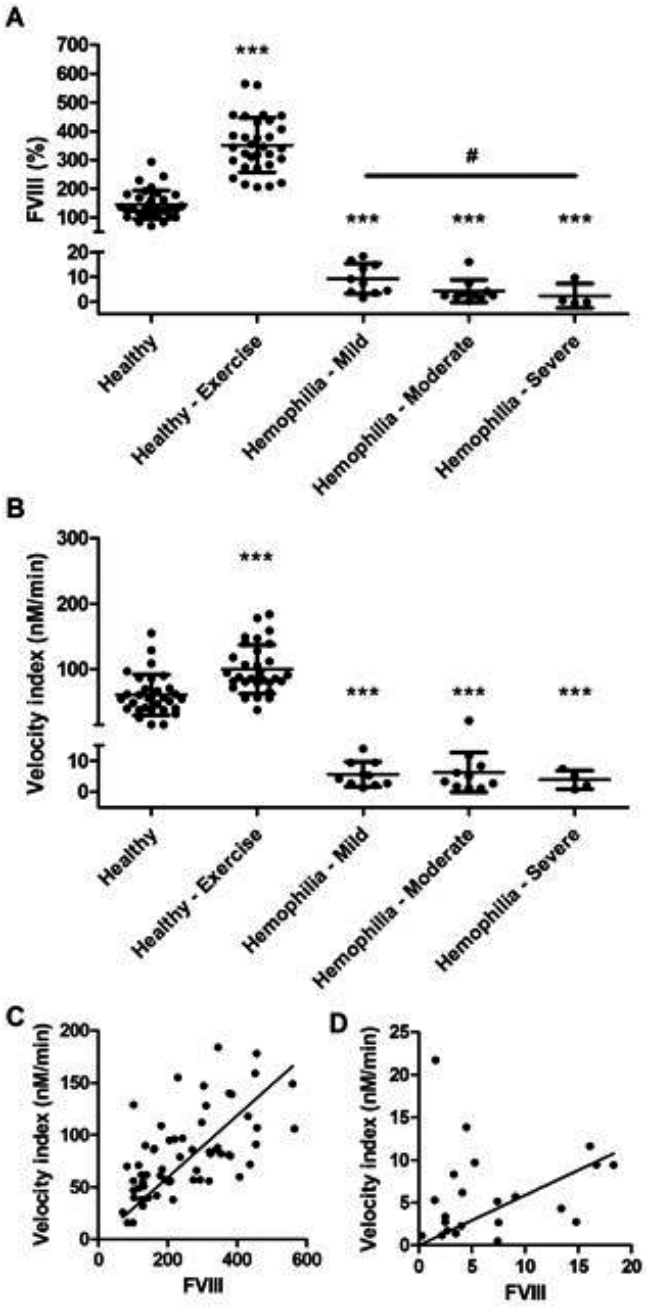

FIGURE 2 FVIII and velocity index in healthy subjects before and after exercise (A) and hemophiliacs (B) and their correlation (C-D).

Background: The adequate assessment of FVIII level is essential for treatment and monitoring of hemophilia A patients. Current tests include the one-stage assay and chromogenic assay. However, discrepancies have been reported between various assay results and both assays are not sensitive to detect FVIII level below 1%. Thrombin generation (TG) has previously been shown to be predictive of bleeding in hemophilia and a TG-based test for FVIII may be sensitive to FVIII levels below 1%.

Aims: To develop and validate a highly sensitive TG-based FVIII assay.

Methods: TG was triggered with 2 nM factor IXa in FVIII deficient plasma containing 12.5% standard plasma (containing 0-20% FVIII) or sample plasma and a linear calibration curve was constructed using the velocity index (FVIII-CAT). The test was validated in plasma samples with normal FVIII (healthy subjects, n=30), high FVIII (healthy subjects after strenuous exercise, n=30) and low FVIII (haemophiliacs, n=31) by comparing to the one-stage assay results. Samples were collected after informed consent.

Results: The intra- and inter-assay CVs of the FVIII-CAT were 3.1% and 7.4%. The FVIII-CAT correlated well with the one-stage FVIII determination in healthy subjects and hemophilia patients, but less with the chromogenic FVIII determination in hemophiliacs (figure 1).

FVIII levels were significantly elevated in healthy subjects after exercise and decreased in hemophiliacs (figure 2). Regular TG was measured at 1 pM tissue factor to assess the overall hemostatic potential of the subjects and the velocity index (VI) was elevated after exercise (+63%, p< 0.001) and reduced in hemophiliacs (-91%, p< 0.001). The FVIII level was significantly correlated to the VI in healthy subjects and hemophiliacs.

Conclusions: The FVIII-CAT can quantify FVIII in normal and hemophilia plasma, correlates well with the current golden standard (one-stage assay) and is sensitive to FVIII levels as low as 0.2 %. Global hemostasis in hemophiliacs is only partially determined by the FVIII level.

PB 1140 | Novel Biochip Detects Drug Effects in Patients with Cardiovascular Disease in a Near Patient Setting

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Background: Antiplatelet agents are used to prevent major events such as myocardial infarction in patients with cardiovascular disease (CVD), with over 50 million people in the US currently on anti-platelet therapy. Despite their proven benefit, major events still occur in CVD patients taking antiplatelet agents. Up to 30% of patients on anti-platelet drugs are non-responsive to the therapy and are at increased risk of adverse cardiovascular events. Consequently, a rapid, accurate assay of antiplatelet drug effect is needed.

Aims: To demonstrate the ability of a novel biochip to measure the effects of anti-platelet agents *in vitro* and *ex vivo*.

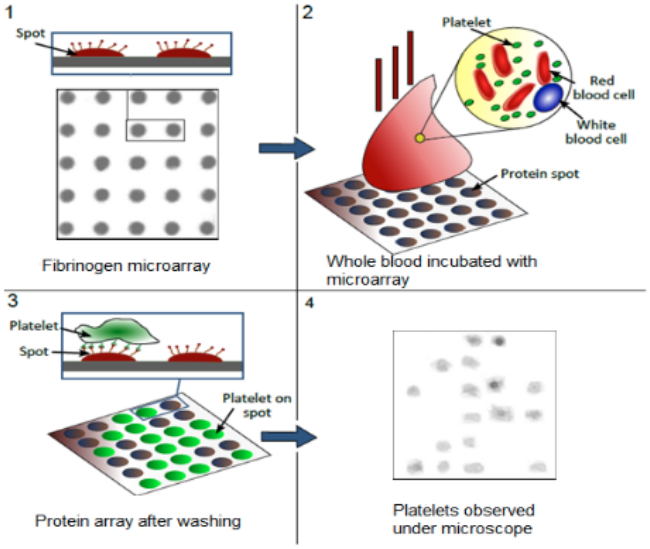

Figure 1 Assay principle. In the assay, unactivated platelets adhere to 6 µm fibrinogen spots. The addition of platelet agonists decreases platelet adhesion to fibrinogen spots by promoting platelet activation and aggregation. However, in the presence of an agonist-specific inhibitor, this decrease in platelet adhesion does not occur. Hence, the assay measures the inability of agonists to decrease adhesion in the presence of antiplatelet agents.

FIGURE 1 Assay principle

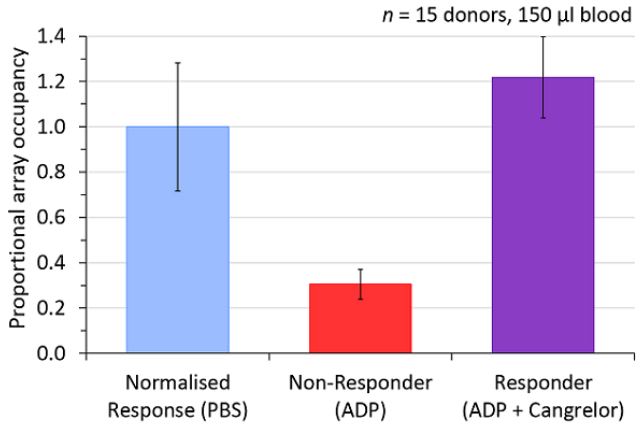

Figure 2. Cangrelor effect on platelet adhesion in vitro. In healthy volunteers, platelet adhesion is 72 ± 13 % (mean ± SD, Normalised response). The platelet agonist adenosine diphosphate (ADP, 20 mM) causes a significant decrease in platelet adhesion (non-responder). Cangrelor (10 mM) inhibits this ADP induced decrease in platelet adhesion (responder), demonstrating the ability of the assay to detect antiplatelet effects in vitro.

FIGURE 2 Cangrelor effect on platelet adhesion in vitro

Methods: We have developed a simple biochip-based approach to assess platelet function by measuring the specific adhesion of individual platelets from small volumes of whole blood to fibrinogen-patterned surfaces (Fig. 1). This biochip is capable of accurately measuring the effect of antiplatelet agents.

Results: The biochip can detect antiplatelet effects *in vitro* (Fig. 2). In CVD patients taking P2Y12 inhibitors such as Plavix, platelet adhesion in untreated and ADP treated blood samples is comparable, demonstrating the ability of the assay to detect antiplatelet effects *ex vivo*. The assay has also been optimised to detect the effect of other clinically relevant antiplatelet agents, including aspirin.

Conclusions: In conclusion, we have developed a simple, sensitive biochip that can accurately detect the effect of antiplatelet therapies *in vitro* and *ex vivo*. Due to its simplicity and sensitivity, the assay has potential for widespread use in the monitoring and tailoring of antiplatelet therapies in CVD patients.

Work was co-funded by Enterprise Ireland and the European Regional Development Fund under Ireland's European Structural and Investment Funds Programmes 2014-2020, grant no. CF/2015/0009. It was approved by the RCSI ethics committee and informed consent was obtained.

PB 1141 | Microfluidic Systems with Vascular Biomimetic Surfaces for Assessment of Platelets and Fibrin Components of Hemostasis: Comparison with a Classic Annular Device in the Evaluation of the Antithrombotic Effects of Apixaban

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Background: Blood flow devices have improved our understanding on the mechanisms of bleeding and thrombotic disorders. The main inconvenient is that use of the classic devices is not standardized between different laboratories. New microfluidic technologies have been widespread implemented by reducing blood volumes and simplifying the evaluation process. Studies comparing the performance of microfluidic and classic devices are needed.

Aims: Compare the results of a microfluidic model using a biomimetic vascular surface vs. a classic annular device exposing genuine subendothelial components. Validate their performance evaluating the *in vitro* antithrombotic effects of apixaban (APIX).

Methods: Microfluididic studies were carried out with a commercial slide-based chamber (µ-Slide^{VI} 0.4, IBIDI). Citrated-recalcified blood aliquots were perfused through channels coated with fibrillar collagen type I and tissue factor, for 5 min at 800/s. We tested the effect of different APIX concentrations (0, 10, 40 and 160 ng/mL) and evaluated platelet and fibrin deposition onto the biomimetic surface by dual immunofluorescence. Parallel studies were performed in annular perfusion chambers exposing damaged vascular segments in which platelets and fibrin were morphometrically evaluated in histological sections.

Results: APIX caused a dose-dependent reduction in platelet and fibrin interactions onto the different thrombogenic surfaces, with similar results and statistical trends in both flow devices. APIX at 40 ng/mL statistically reduced platelet coverage; this effect was further increased at 160 ng/mL, also affecting the fibrin deposition.

TABLE 1 Percentage of surface coverage by platelet aggregates and fibrin nets

[APIX]	Annular chamber		Microfluidic chamber	
ng/mL	Platelets	Fibrin	Platelets	Fibrin
0	46.6 ± 3.5	53.8 ± 7.4	23.0 ± 3.0	43.4 ± 4.8
10	42.1 ± 2.5	51.2 ± 3.5	17.9 ± 0.9	42.1 ± 1.9
40	35.5 ± 4.8 *	46.9 ± 6.3	14.0 ± 5.3 *	23.4 ± 7.7
160	21.0 ± 2.8 *#	19.3 ± 4.4 *#	5.4 ± 2.2 *#	14.1 ± 4.9 *#

Mean ± S.E.M. *p<0.05 vs. absence of APIX; #p<0.05 vs. APIX 10 ng/mL

Conclusions: Results of the evaluation of platelets and fibrin in the microfluidic model using biomimetic vascular components showed a high level of correspondence with those observed in the classic annular device. The microfluidic model explored offers an alternative to classical flow based systems and may be useful in the evaluation of drugs that interfere with hemostasis.

Grant: DTS16/00133

PB 1142 | Automated CH50-like Assays for Assessment of the Complement System and Complement Inhibitor Drugs

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Background: Complement dysfunction plays a role in many diseases including: PNH and thrombotic microangiopathies; a variety of pharmaceutical complement inhibitors are in development. Complement is assessed by lytic CH50 assays or commercial Elisa, which are cumbersome and time consuming.

Aims: We have automated a CH50-like test on a coagulation analyser, to provide a global complement test for hospital laboratories.

Methods: We initially established a CH50-like assay (auto CH50) on the CS-2100i (Sysmex) coagulometer using reagents from Siemens, but later developed a high sensitivity method using in-house sheep erythrocytes (SE) and hemolysin. The time taken until 50% SE lysis was measured at 660nm and standardized against dilutions of pooled normal serum (PNS). CH50 ELISA (Quidel Corp) and manual, in-house, lytic CH50 were reference methods.

Results: The auto CH50 was linear over a wide range of dilutions, detection limit 1.6%; intra- & inter-assay cv: 2.5 & 7.0%. Serum was stable for 4hr at RT. Good correlations were obtained with both manual lytic CH50 and CH50 ELISA (r=0.50 & 0.57, respectively, p< 0.001) in sera from normals (n=36), patients with liver disease (n=7), antiphospholipid syndrome (n=48) and their disease controls (n=25). In vitro spiking of PNS with C5 inhibitor coversin, showed dose responsiveness, but a difference in inhibition between auto CH50 and ELISA methods (IC50: 6 v 3ug/ml); weaker inhibition was observed with the C5 monoclonal antibody eculizumab (IC50: 32 v 10ug/ml). Phase I & II (PNH) coversin studies gave similar inhibition by all assays at peak dose, but faster normalisation by auto CH50. Using in-house reagent (more dilute SE), the auto CH50 gave good agreement with Elisa and manual CH50 for both complement inhibitor drugs in vitro and ex vivo.

Conclusions: The auto CH50 potentially allows routine assessment of complement activity in a standardized manner. The more sensitive in-house automated assay may allow pre-treatment screening and efficacy monitoring of C5 inhibitor drugs.

PB 1143 | Prenatal diagnosis for Inherited Bleeding Disorders in India

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Background: Inherited bleeding disorders are a group of heterogeneous disorders which includes quantitative and qualitative defects in the coagulation proteins and platelets. Although phenotypic assays still remain the first line in diagnostic testing, molecular diagnosis provides carrier detection and prenatal diagnostic opportunities. The latter is particularly significant where therapeutic options are limited. This is particularly relevant in southern India where such disorders are more common given the high level of consanguinity in the community. We have therefore established methods for genetic diagnosis for a wide range of bleeding disorders.

Aims: This report reflects our experience with prenatal diagnosis for inherited bleeding disorders carried out between 2002 and 2016 at our institution.

Methods: Prenatal diagnosis (PND) is carried out on DNA extracted from chorionic villous samples obtained at 10-12 weeks of pregnancy. Mutations were detected by a range of techniques including CSGE and Sanger sequencing. Maternal cell contamination was ruled out by analyzing STR markers. Diagnostic methods are established for Haemophilia (A&B), other factor deficiencies (II, V, VII, X, XI, XIII), platelet disorders (Glanzmann Thrombasthenia, Bernard Soulier Syndrome), von willebrand disease and Wiskott Aldrich syndrome.

Results: PND was offered to 303 couples with different bleeding disorders. Most of the PND done was for Haemophilia A (n= 240, 78%) followed by haemophilia B (n=37, 12%). Other disorders were Glanzmann Thrombasthenia (n=8), Wiskott Aldrich Syndrome (n=8), F13 deficiency (n=4), F7 deficiency (n=3), Von willebrand disease Type-3 (n=3) and F10 deficiency (n=1). Of these 143 fetuses were found to be normal, 78 were identified as carriers and 84 were affected.

Conclusions: Genetic counselling aided families with inherited bleeding disorders to make an informed decision. Prenatal diagnosis provides an important tool in the control and management of a wide range of inherited bleeding disorders in India.

PB 1144 | Availability of Age-specific Coagulation Laboratory Values in Pediatric Hospitals vs. Combined Pediatric/Adult Hospitals: An International Perspective

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Background: The diagnostic scoring system for disseminated intravascular coagulation (DIC) was developed by ISTH for adult medicine. Pediatric DIC care may be suboptimal if pediatric DIC diagnosis and management are guided by adult reference ranges.

Aims: To explore age-specific issues in DIC diagnosis including the availability of age-appropriate reference ranges worldwide.

Methods: A web-based questionnaire was developed using LimeSurvey software; 3 sections: Respondent Demographics; DIC Diagnosis; and DIC Management. International dissemination was done via pediatric and neonatal professional societies. Study coordinated by sites in Hamilton (Canada) and Melbourne (Australia) in collaboration with Manchester (England) and members of ISTH Pediatric and DIC SSC. Data were collected from January to September 2016.

Results: A total of 211 responses were obtained: 160 full and 51 partial. There were 133 (63%) respondents from pediatric hem/onc, 45 (21%) from NICU, and 23 (11%) from PICU. Geographic distribution of responses was: 96 (46%) from North America, 56 (27%) from Asia, 25 from Australia (12%) and 24 from Europe (11%). Overall, 25% reported that age-appropriate laboratory values for PT, INR, aPTT, fibrinogen, and platelet count were not available; 20% for protein C, S, anti-thrombin and thromboelastography. Lack of access to age-appropriate values varied by location: Asia 35%, North America 25%, Europe 14% and Australia < 10%. A lack of access to age-appropriate values was reported in 20% of respondents in pediatric-only hospitals and 35% in combined pediatric/adult hospitals.

Conclusions: Despite ISTH position statements emphasizing the importance of reporting age-appropriate data from diagnostic labs, a substantial proportion of respondents report that labs offering services to pediatric patients are still not compliant with this concept. Future studies should assess the impact of this finding on pediatric quality of care and research outcomes from centres that are not using age-appropriate reference values.

PB 1145 | The Use of the ADJUST (Age Adjusted) D-dimer Cut-off in Screening Patients Aged over 50 Years Presenting with Suspected DVTs Can Decrease the Need for Compression Ultrasonography

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Background: In patients with suspected DVT, D-dimer levels below the conventional cut-off of 500ug/l (CDD) can rule out DVT in patients with a non-high clinical probability score. As DD levels rise with age a higher proportion of older patients with suspected DVT may have a DD >conventional cut-off resulting in a greater need for unnecessary compression ultrasonography (CUS), as many will be normal. It has been suggested that the use of an age-adjusted DD cut-off, age x10ug/l, (AADD) in individuals of >50 years can improve specificity without significant loss of sensitivity, decreasing the need for unnecessary imaging.

Aims: To determine, in a primary care setting, whether the use of an AADD could safely decrease the number of CUS required in patients >50 years presenting with suspected DVT and a non-high Wells score (WS).

Methods: We analysed retrospectively data from two cohorts of patients presenting with suspected DVTs to a single community clinic. One, previously reported, from Sept 2012 to Sept 2014, and a second, new, cohort from Jan 2015 to Mar 2016. All patients were screened with a WS and a quantitative Point of Care DD. CUS was carried out on site as per standard protocols. All patients discharged with DVT excluded had a 90-day follow (90dFU) to determine the subsequent incidence of venous thromboembolism (VTE).

Results: 1411 patients were reviewed. 1173 (83%) were >50 years. Of these, 305 (26%) had non-high WS and a raised CDD but 127 (42%) had a normal AADD. 113 of these 127 either had a normal CUS and/or a 90dFU without recurrent VTE. 14 were lost to follow up but were not re-referred with suspected VTE within 90 days.

Conclusions: In patients >50 years with suspected DVT, a non-high WS and a raised CDD, the use of an AADD cut-off can safely exclude a DVT without the need for CUS. This could decrease the need for CUS in this group by 40%, improving the efficiency and cost effectiveness of a DVT service, especially in primary care, decreasing the need to refer to secondary care for CUS.

PB 1146 | Is the Application of a Single Conversion Factor Suitable for the Laboratory Monitoring of Single Chain rFVIII by One-stage FVIII:C Assay?

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Background: A novel single chain, recombinant factor VIII (SCrFVIII) for the treatment of haemophilia A has recently been licensed. The manufacturer recommends that either a chromogenic (FVIII:CR) or one-stage assay (FVIII:C1) is used to monitor levels but that a conversion factor of 2 be applied to all FVIII:C1 results regardless of APTT reagent used in the assay.

TABLE 1 Recovery of SCrFVIII using converted FVIII:C1 and six APTT reagents

Labelled Potency (IU/ml)	Converted Synthasil (IU/ml)	Converted APTT SP (IU/ml)	Converted Synthafax (IU/ml)	Converted Actin FSL (IU/ml)	Converted Actin FS (IU/ml)	Converted Pathromtin SL (IU/ml)
2.39	2.02	2.28	2.48	2.48	2.46	2.04
1.59	1.38	1.62	1.76	1.70	1.82	1.36
1.19	0.92	1.24	1.38	1.36	1.34	0.98
0.60	0.48	0.68	0.74	0.72	0.72	0.54
0.24	0.20	0.30	0.36	0.28	0.28	0.22
0.12	0.12	0.16	0.20	0.16	0.18	0.12
0.06	0.06	0.08	0.14	0.08	0.10	0.08
0.02	0.04	0.04	0.08	0.04	0.06	0.04

Aims: The aim was to assess whether a universal conversion factor for the FVIII:C1 is suitable for a range of APTT reagents used in the routine monitoring of SCrFVIII.

Methods: SCrFVIII was reconstituted in water then serially diluted using the labelled potency to 2.39-0.02 IU/ml in 0% FVIII. FVIII:C1 was assayed with Synthasil, APTT SP and Synthafax (all Werfen), Actin FLS, Actin FS and Pathromtin SL (all Siemens). All FVIII:C1 results were multiplied by a factor of 2. FVIII:CR was measured by Hyphen Biomed assay. All assays were performed on Sysmex CS5100i instrumentation at multiple dilutions.

Results: At higher FVIII:C1, >0.60 IU/ml, up to 23.3% difference was demonstrated between labelled potency and converted FVIII:C1. 43.3% difference was seen between highest and lowest results at the same potency with different APTT reagents. The greatest variation was at the 0.02 IU/ml dilution which was 100-300% higher than target with all APTT reagents (see table 1). The FVIII:CR overestimated the FVIII:C by 13-50% depending on the target dilution.

Conclusions: The application of a conversion factor improved the results but underestimation was still observed with some reagents and overestimation with others. At very low FVIII:C, similar to patient trough levels, a gross overestimation of converted FVIII:C1 was observed with all APTT reagents which may lead to under-dosing of patients. FVIII:CR demonstrated less dose dependant variation and was more accurate at very low FVIII:C. Clinicians should exercise caution when using the FDA and EMA approved single conversion factor in FVIII:C1 for all APTT reagents especially when measuring and interpreting trough levels.

PB 1147 | Impact of Pneumatic Tube System Transportation for the Monitoring of Heparin Therapy

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Background: Transport by pneumatic tube system (PTS) is a rapid and widely spread mode of transport for biological samples in laboratories. It allows reduced turnaround time but its impact on haemostasis assay results is unclear. PTS transport did not seem to have a major impact on several haemostasis parameters including aPTT in healthy subjects but there is no data regarding samples of patients anticoagulated with heparin.

Aims: To evaluate the impact of PTS transport on aPTT and anti-Xa assays of samples collected in patients treated with heparin.

Methods: Citrated blood samples of 156 patients treated with unfractionated heparin (UFH, n=126) or low molecular weight heparin (LMWH, n=30) were divided in two identical tubes. For each patient, one tube was hand-delivered to the laboratory and one tube was transported by PTS. Both tubes were then simultaneously centrifuged at 2500 *g* for 15 min and aPTT and anti-Xa assays were performed on a BCS XP automate (Siemens) during the same run.

Results: Compared to the hand-delivered samples, aPTT and anti-Xa assays were lower on samples transported by PTS (median difference of 2.8 sec, interquartile range [IQR]: 0.4-7.5, p< 0.001 and 0.03 UI/ml, IQR 0.0-0.09, p< 0.001 respectively). For patients with UFH, the difference was more obvious for aPTT (4.5 sec, IQR:1.3-9.0, p< 0.001) and for anti-Xa assay (0.04 UI/ml, IQR:0.03-0.07, p< 0.001), while there was no difference of anti-Xa results in patients with LMWH (0.0 UI/ml, IQR: 0.0-0.01, p=0.52). Bland&Altman plots showed that the difference of both parameters increased with the heparin concentration in patients treated with UFH. Among the 58 patients with UFH and with anti-Xa assay in therapeutic range as measured on hand-delivery samples, 12 (21%) would have been classified as infra-therapeutic if the samples had been transported by PTS.

Conclusions: PTS transport significantly impacts the results of aPTT and anti-Xa assays for UFH monitoring.

PB 1148 | Comparison of von Willebrand Factor Collagen Binding Activity (VWF:CB) Determined by a Chemiluminescent Assay on AcustarTM to an ELISA Kit

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Background: VWF collagen binding (VWF:CB) activity assay is a second-laboratory level test used to explore and phenotype Von Willebrand Disease (VWD). This assay is particularly sensitive to high molecular weight (HMW) of Von Willebrand Factor (VWF) and helps to differentiate VWF variants.

Aims: The aim of the study was to evaluate performance characteristics of a new chemiluminescent technology for the diagnosis of VWD. Results of VWF:CB were compared between the classical assay (ELISA method) and this new technology.

Methods: In this retrospective study, VWF:CB was measured by chemiluminescent assay (Acustar™, Werfen) and ELISA (Asserachrom® VWF:CB, STAGO) in samples from 53 patients. All patients were well-defined by phenotype evaluations and genetic reports: VWD type 2-2A (n=13), VWD type 2-2M (n=7), VWD type 2-2B (n=11) and VWD type 1 (n=22). We also analysed normal plasmas (n=15). By measuring antigen of VWF (VWF:Ag) on the same sample, we calculated VWF:CB/VWF:Ag ratio. The concordance of the ratio obtained with the two methods (> or < to 0.6) have been interpreted in relation to the genetic reports.

Results: We found adequate diagnostic performance (within-run, between-run, reference range), highly correlated results (r^2=0.9564) and a minimal bias (+4.7%) with the chemiluminescent assay. Evaluation of VWF:CB/VWF:Ag ratio on VWD plasmas (n=53) were concordant between the two assays for almost all subtypes and almost all mutations. We obtained 4 different results for the 5 patients with VWD 2M-2A like. For these samples, VWF:CB/VWF:Ag ratios with chemiluminescent assay were lower and more concordant to the genetic report: p.(R1374C) and p.(R1374H) mutations.

Conclusions: Automatized of VWF:CB assay is benefit for laboratories: better analytical performance and more sensibility to HMW of VWF. This phenotype assay based on chemiluminescent technology is also well correlated to genetic results of VWD patients.

PB 1149 | Validation of a Flow Cytometric Platelet Function Test in a Cohort of Patients with a Suspected Platelet Function Disorder

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Background: Light transmission aggregometry (LTA) is the most commonly used test for the diagnosis of platelet function disorders (PFDs). However, LTA is labor-intensive, requires a large blood volume, is poorly reproducible and has only moderate sensitivity for the detection of mild PFDs. Flow cytometry has been recommended for additional diagnostics of PFDs, but is hard to standardize and not readily applicable in diagnostic laboratories. We developed a standardized protocol for flow cytometric analysis of platelet function (FCAP) that measures fibrinogen binding to integrin αIIbβ3 and P-selectin expression as markers of platelet activation in response to stimulation with agonists.

Aims: To determine the additional value of FCAP with a panel of agonists in a cross sectional cohort of patients suspected of a PFD.

Methods: Platelet function was assessed with LTA and FCAP in 60 healthy controls and 108 patients suspected of a PFD in whom Von Willebrand disease and coagulation factor deficiencies were excluded. Bleeding scores (BS) of patients were determined with the ISTH bleeding assessment tool. Performance of both tests was analyzed with an Area Under a Receiver Operating Curve (AUC).

Results: Out of 108 patients, 81 patients had a BS>3. 42 out of 81 patients (51.9%) had abnormal platelet function measured with FCAP and 40 out of 81 patients (49.4%) were abnormal based on LTA. 25 patients (30.9%) had poor platelet function based on both tests and 24 patients (29.6%) had normal platelet function in both LTA and FCAP. Agreement between LTA and FCAP was poor to moderate (κ=0.29). The discriminative ability of FCAP was good (AUC 0.74), but moderate for LTA (AUC 0.68). Both tests combined had a better discriminative ability (AUC 0.82, p< 0.05) than LTA alone, but performed similar to FCAP (p=0.27).

Conclusions: Flow cytometric analysis of platelet function can be used to discriminate between healthy controls and patients with a PFD, especially combined with LTA.

PB 1150 | Comparison of light transmission aggregometry and multiple electrode aggregometry for the evaluation of platelet aggregation in patients with mucocutaneous bleeding

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Background: Light transmission aggregometry (LTA) is the "gold standard" diagnostic test for platelet aggregation, although required blood volumes can limit its use. Whole blood or multiple electrode aggregometry (MEA) using the Multiplate® analyzer requires much smaller blood volumes, but has not been directly compared to LTA for evaluation of patients with mucocutaneous bleeding.

Aims: To compare results of LTA and MEA for patients referred for platelet function analysis.

Methods: Blood samples were collected following informed consent; the study was approved by the Research Ethics Board. LTA and MEA assays were performed using threshold concentrations of ADP, arachidonate (AA), collagen and thrombin receptor activating peptide (TRAP). Concordance of LTA and MEA for each platelet agonist was calculated using the McNemar Chi-square test. Using LTA as the standard, the sensitivity and specificity for each MEA agonist were determined.

Results: 44 patients (30F/14M) were studied: median age 18 (range 2.2-84) years; mean platelet count 250±94 x 10^9/L. 6 patients had at least 1 abnormal agonist response with LTA; 8 had at least 1 abnormal agonist response with MEA. The two methods were concordant based on the McNemar Chi-square test: p > 0.6 for all agonists. Sensitivities of MEA relative to LTA were: ADP, 0.33; AA, 0.5; collagen, 0 [the small numbers of patients with abnormal results contributed to the low sensitivities]. Specificities of MEA relative to LTA were: ADP, 0.9; AA, 0.95; collagen, 0.93. Negative predictive values (NPVs) for MEA relative to LTA were: ADP, 0.95; AA, 0.98; collagen, 0.95. Values for TRAP could not be determined as no patient had an abnormal LTA result.

Conclusions: The two methods provided concordant results for responses to ADP, AA and collagen. MEA results showed good specificities and NPVs in relation to LTA; i.e., MEA performed well in identifying the patients who had no abnormal aggregation results by LTA. Comparison of additional patient samples is required for validation of results.

PB 1151 | Clot Waveform Analysis Has the Potential to Detect Fibrinogen Abnormalities

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Background: Fibrinogen abnormalities comprise two classes of plasma fibrinogen defects: quantitative and qualitative deficiencies. The Clauss fibrinogen assay, which has been used for an initial screening test, provides only fibrinogen clottable activity; however, we cannot distinguish between qualitative and quantitative defect of fibrinogen.

Aims: Automated coagulation analyzers can provide the additional information in conventional clotting tests. Clot waveform analysis (CWA) has been applied to evaluate coagulability in patients with bleeding disorders such as hemophilia. CWA is also available in Clauss fibrinogen assay determined by light transmittance, and in this study we investigated the CWA in fibrinogen analysis to detect fibrinogen abnormalities.

Methods: Citrated plasma samples were obtained from healthy volunteers and patients with dysfibrinogenemia. We performed both Clauss fibrinogen assay and latex agglutination assay to measure fibrinogen antigen. We compared fibrinogen activity, antigen, and CWA parameters; clotting time, changes in absorbance (dOD), |min1| value from 1st derivative curve, |min2| and |max2| values from 2nd derivative curve.

Results: The results showed that fibrinogen activity and antigen were strongly correlated in normal plasma, the discrepancies between activity and antigen were observed in dysfibrinogenemia plasma. The CWA parameters: dOD and |min1| were also highly correlated to fibrinogen antigen in normal plasma. The clotting time was prolonged due to low fibrinogen activities, and the dOD values were high as compared with normal low fibrinogen level in dysfibrinogenemia plasma. The |min1| values in dysfibrinogenemia were low, that indicated impaired fibrin polymerization of abnormal fibrinogen.

Conclusions: The CWA in Clauss fibrinogen assay had the potential and could be helpful to detect abnormal fibrinogen using |min1| values, with no additional costs in standard laboratory tests.

PB 1152 | Effect of Anticoagulant Adjustment on Prothrombin Time Test Using 2 Different Thromboplastins in Patients with Elevated Hematocrit

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Background: An elevated hematocrit(Ht) value changes the ratio of citrate anticoagulant concentration/volume of blood collected and the excess of the anticoagulant increases the prothrombin time (PT) results.The adjustment of sodium citrate volume is important on samples of patients with high hematocrit(HT>55%) since unadjusted citrate volume in these situations can lead to errors in the treatment.

Aims: The aim of this study was to compare the effect of adjusted and unadjusted citrate volume using two different thromboplastin on patients with Ht>55% and under anticoagulation therapy.

Methods: Paired citrate-adjusted and non-citrate-adjusted blood specimens were obtained from 179 patients with high Ht values on use of warfarin.Two different thromboplastins were used: one is a recombinant human tissue factor (PT-RTF) and the other is extracted from rabbit brain

(PT- Rabbit).Results are expressed as *international normalized ratio* (INR).

Results: The PT- RTF results from the citrate adjusted and citrate-unadjusted blood specimens did not differ significantly and presented a strong correlation (R^2=0.8226,p< 0.0001).The median of PT-RTF for citrate adjusted samples was 2.10(CI95% 2.06 to 2.38)and for citrate-unadjusted was 2.00 (CI95%2.10 to 2.41).From the 179 samples analyzed seventeen (9.5%) exceeded the Allowable Total Error for this test (>15%) and all of them presented hematocrit >62%.Already, the results using PT- Rabbit presented a moderate correlation between samples (R^2 =0.4267,p< 0.0001).The median of the results was 2.30 (CI95%:2.34 to 2.67) for citrate adjusted and 2.70(CI95%:3.11 to 3.81) for citrate-unadjusted. From the 179 samples analyzed seventy nine (44.1%) were above the Total Error and had Ht values between 56% and 72%.

Conclusions: Our data demonstrate that using PT-RTF reagent, samples with hematocrit up to 62% may be safely performed and interpreted with confidence without anticoagulant adjustment.Unlike for PT-Rabbit it will be necessary to adjust the citrate volume according to Ht.

PB 1153 | Methodology of Thrombogenicity Assessment Intended for Biomaterials Utilized in Blood Pumps

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Background: Construction of blood contacting devices (particularly blood pumps) requires materials which high biocompatibility and low thrombogenic potential. Platelet (PLT) activation followed by coagulation pathway and complementary system induction leads to thrombus formation and increases risk of thromboembolic complications.

Aims: The aim of study was to develop the set of examinations in order to in-vitro evaluation of thrombogenicity of various biomaterials.

Methods: Samples of biomaterials were prepared in form of discs. Surface roughness was measured optically. Samples were sterilized in a way appropriate for biomaterial (ETO, radiation). Citrated or anticoagulated by R-hirudin human blood was used.

First the PLT aggregation under ADP was measured. Static thrombogenicity test was as follow: samples incubation in human PRP (60min, 37°C), washing in PBS, fixing in formalin. Afterwards the qualitative surface assessment (number and morphology of PLT) was performed by means of AFM or SEM.

Thrombogenicity test under shear stress was evaluated utilizing Impact-R analyser. Our modification of method consisted in placing a disc of tested biomaterial into a polystyrene cell. The assessment of biomaterial thrombogenicity consisted of:

· platelet activation and aggregation (CD61, CD62P, CD45, CD14) in blood circulating above the examined biomaterial by means of flow cytometry,

· cells adhesion to the surface of biomaterial (CD62P, CD45) by means of fluorescent microscopy.

Depending on biomaterial the references were: polystyrene,Ti6Al7Nb or PU (Bionate2) -without any surface modification.

Results: Described methodology was applied to evaluate the thrombogenicity of bionanocellulose, Ti alloys, PU, PEEK, PET/DLA copolymers and their surface modifications (TiN, DLC). The comparison of obtained results to reference allowed to quantitative assessment of thrombogenicity of investigated biomaterials.

Conclusions: The proposed method allows to in-vitro, quantitative assessment of biomaterials in terms of thrombogenicity.

PB 1154 | LC-MS/MS Nonradioactive Serotonin Release Assay for Platelet Function Analysis in Whole Blood

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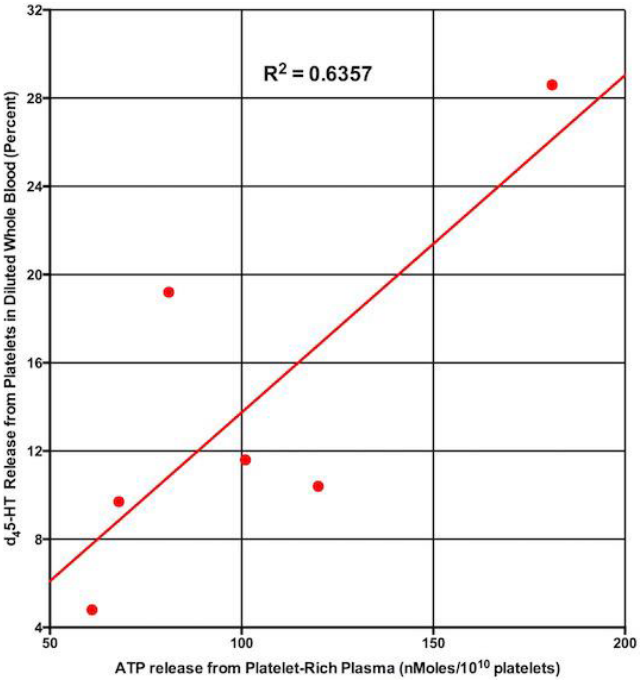

FIGURE 1 ADP-Induced Dense Granule Release: Comparison of d5-HT Release in Whole Blood and ATP Release in Platelet-Rich Plasma

Background: Assay of dense granule secretion as part of an evaluation of platelet function is presently most typically performed by study of ATP release in a lumi-aggregometer. Sensitivity of this approach limits its applicability both for very young patients and for adult patients with a significant degree of thrombocytopenia.

Aims: The aim of this IRB-approved study is the development of a highly sensitive, non-radioactive assay of dense granule secretion that could be performed using very small numbers of platelets in whole blood.

Methods: From a single pediatric size citrate tube, 200 µL of whole blood was incubated with exogenous deuterated serotonin ($d_4$5-HT). Following $d_4$5-HT uptake, the blood was diluted and then incubated with a variety of platelet stimuli. Following stimulation, specimens were centrifuged to obtain releasate. A platelet lysate was used to assess total $d_4$5-HT uptake and calculation of percent release. For sample preparation, methylated-5HT was used as internal standard. Calibrators were prepared by spiking $d_4$5-HT into a matrix of modified Tyrode buffer. Samples were precipitated by perchloric acid and centrifugation. Analytes were detected using a Sciex QTrap 6500 mass spectrometer with an ESI source.

Results: Platelet stimuli being evaluated in this study include ADP, epinephrine, arachidonic acid, U46619, TRAP, and convulxin. The degree of correlation with the release of platelet ATP from stirred platelet-rich plasma varied by agonist. An example of such correlation, in this case following stimulation of platelets from normal volunteers with 10 µM ADP, is shown in Fig. 1.

Conclusions: The deuterated serotonin LC-MS/MS assay developed in this study permits extensive study of platelet dense granule secretion using extremely small blood volumes. This approach offers

promise as a means to overcome serious limitations of current platelet function testing by expanding the availability of such testing both to pediatric patients and to adult or pediatric patients with decreased platelet counts.

PB 1155 | Thromboelastometry can Generate Significant Disagreement in the Management of Patients after Cardiopulmonary Bypass due to Analytical Variability

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Background: Thromboelastometry is widely used after cardiac surgery in patients presenting with bleeding complications. However the role of this test in the management strategy is still subject to debate. One possible explanation for this controversy is that excessive analytical variability modifies the conclusions of the management strategy.

Aims: To test if within-patient variability of ROTEM® results increases the variability in the management of patients.

Methods: We included 85 cardiac surgery patients after cardiopulmonary bypass, during 4 different periods between July 2014 and April 2016. Inclusions were consecutive for each period. The periods reflect different conditions for performing the tests (ROTEM®): point of care, re-localization to lab, single or multiple operators. Measurements were performed within 30 to 60 minutes after blood sampling on two samples obtained from the same patient at the same time and in identical conditions. The primary endpoint was any discrepancy in the conclusion of a standard algorithm applied to both results. The factors influencing the risk of discrepancy were studied using multivariate logistic regression. Odds ratios and 95% confidence intervals (95CI) were verified by bootstrapping. The study was approved by a local research ethics committee (n°13-0215).

Results: The main results are presented in figure 1.

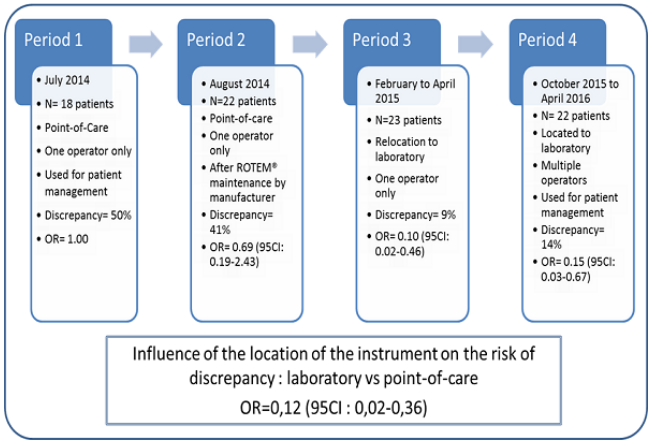

FIGURE 1 Changes in the risk of discrepancy according to study periods

The main factor that reduced discrepancy was location of the ROTEM® to the lab. When measurements were performed by multiple operators, the risk of discrepancy increased by 50% but remained 70% below the risk observed in POC. The model's AUC-ROC was 0.79 (95CI:0.66-0.89).

Conclusions: Significant variability in the management strategy resulting from thromboelastometry can be observed when the ROTEM® is located as point-of-care. Re-localization to the lab considerably reduces this variability. We recommend that within-patient variability of thromboelastometry be assessed in each institution to reduce variability in the management of these patients.

PB 1156 | Establishing of Therapeutic Range for Unfractionated Heparin Therapy: Activated Partial Thromboplastin Time vs. Anti-Xa Measurement

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Background: Activated partial thromboplastin time (aPTT) and anti-Xa activity are currently used to monitor unfractionated heparin (UFH) therapy. However, the aPTT heparin therapeutic range (HTR) is highly dependent on the reagent and analyzer used. So, it is recommended by Guidelines for each laboratory to define its own aPTT HTR using its technical conditions, to correlate with heparin levels between 0.30 and 0.70 IU/mL (anti-Xa).

Aims: To establish the aPTT HTR accordingly in a large cohort of inpatients receiving full dose of UFH, and to compare to the historical aPTT HTR at our institution (ratio=1.5 to 2.5 times the control).

Methods: We retrospectively reviewed the charts of inpatients aged over 18 years who were treated with continuous intravenous infusion of UFH for acute coronary syndrome, venous thromboembolism or other diseases, in Wuhan Asia Heart Hospital between 01/15 and 12/16. Both aPTT and anti-Xa assays were performed simultaneously using HemosIL APTT SynthASil and HemosIL Liquid Anti-Xa reagents respectively. The correlation between aPTT and anti-Xa was analyzed by linear regression, and HTR calculated by best data-fit equation.

Results: A total of 15,311 samples from 4,280 patients were included in our study. aPTT and anti-Xa showed the best data-fit in exponential linear regression analysis (R^2=0.65). The prolongation of aPTT, which corresponded to anti-Xa activity between 0.30 and 0.70 IU/mL, was between 51 and 91 seconds (ratio=1.6-2.9). Historical aPTT HTR between 1.5 and 2.5 times the control corresponded to lower anti-Xa activities between 0.25 and 0.60 IU/mL.

Conclusions: A strong correlation between aPTT and anti-Xa assays was observed in our laboratory. Historical aPTT HTR was lower than that calculated from the correlation curve with anti-Xa activity

between 0.30 and 0.70 IU/mL, further researches are needed to define the optimal HTR in clinical practice.

PB 1157 | Impact of Rivaroxaban and Dabigatran on Clotting Screens as Performed Using the Roche Cobas t 711 Analyser

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Background: Direct Oral Anticoagulants (DOACs) including Rivaroxaban and Dabigatran can cause method specific prolongation of some clotting tests. The cobas t 711 analyser is a new platform likely to be used for analysis of samples from patients taking these drugs. Data on the impact of these drugs on clotting screens performed with this system are therefore needed to facilitate interpretation of such test results in the presence of DOACs.

Aims: To assess the impact of Rivaroxaban and Dabigatran on clotting screens performed using cobas t 711 analyser in comparison to a previously studied instrument/reagent system.

Methods: Pooled normal plasma was spiked with varying amounts of Rivaroxaban or Dabigatran to achieve 6 different concentrations in the range 0-1250 ng/ml. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) were performed on all samples using 2 different methods, Roche cobas t 711 analyser/Roche reagents and Sysmex® CS5100/Siemens reagents. In addition, Clauss Fibrinogen (CFbg) and Thrombin Time (TT) were determined on samples containing Dabigatran.

Results: Results are shown in the table. PT and APTT were prolonged above baseline at >217ng/ml Dabigatran respectively for cobas t 711 analyser and for Sysmex® CS5100. Clauss fibrinogen was unaffected by Dabigatran for the concentrations studied. Thrombin times were unclottable in all samples containing Dabigatran. PT and APTT were prolonged above baseline at concentrations >249ng/ml Rivaroxaban respectively for cobas t 711 analyser and for Sysmex® CS5100.

Conclusions: There was a dose dependent prolongation of PT and APTT as determined using the cobas t 711 analyser by either Rivaroxaban or Dabigatran. The sensitivity of these tests to presence of drug was similar to that observed for a previously studied comparator method. Clauss fibrinogen was unaffected by Dabigatran up to 1250 ng/ml.

PB 1158 | Genotypic Characteristics of Polish Patients with Antithrombin, Protein C and Protein S Deficiency

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Background: Diagnosis of congenital deficiency of antithrombin (AT), protein C (PC), protein S (PS) is hampered by various factors affecting determination of their plasma activity/antigen concentration. The only definite confirmation of congenital deficiency of these proteins is through identification of causative mutations in the encoding genes (*SERPINC1*, *PROC*, *PROS1*, respectively).

Aims: To identify the causative mutations in *SERPINC1*, *PROC1* and *PROS1* in patients with AT, PC or PS deficiency recognized in plasma studies.

Methods: The study comprised 106 Polish patients registered in the local database: 21 with reduced AT activity, 12 with reduced PC activity and 73 with reduced levels of free PS antigen. Patients with clinical and laboratory findings indicative of liver damage were not qualified. *SERPINC1*, *PROC1* and *PROS1* genes analyses were performed by Sanger sequencing of all coding regions and exon/intron splicing sites on ABI 3130XL Genetic Analyzer.

Results: Sequencing data outcome: 11 different mutations in the *SERPINC1* (3 as yet undescribed), 9 in *PROC* (2 not described), 12 in *PROS1* (7 not described). Causative mutations were detected in 44/106 (42%) studied cases, which confirmed the congenital defect in 17/21 (81%) with reduced AT (range 39-66 IU/dl; n.75-125IU/dl); in 10/12 (83%) with reduced PC (range 32-66 IU/dl; n.70-140IU/dl); in 17/73 (23%) with reduced PS (range 15-60 IU/dl; n.70-130 IU/dl). Free PS antigen plasma level (39.9±21.2 IU/dl) was lower in the group of 17/73 patients with identified mutations in the *PROS1* than in 56/73 patients (54.2±10.5 IU/dl) with undetected causative mutations in the *PROS1*.

TABLE 1 Effects of Dabigatran and Rivaroxaban on PT and APTT

	PT	PT	APTT	APTT		PT	PT	APTT	APTT
Dabigatran (ng/ml)	t711 PT Rec (Sec)	CS5100 Innovin (Sec)	T711 APTT L (Sec)	CS5100 AFS (Sec)	Rivaroxaban (ng/ml)	t711 PT Rec (Sec)	CS5100 Innovin (Sec)	T711 APTT L (Sec)	CS5100 AFS (Sec)
0	9.0	10.9	27.3	24.9	0	8.9	10.9	27.8	25.2
217	11.4	12.8	54.0	47.8	249	10.4	12.4	36.4	33.5
522	15.0	15.6	70.0	61.6	488	12.1	14.0	43.4	40.6
732	27.9	20.3	79.7	71.6	639	13.6	15.6	50.6	47.7
1080	31.6	32.3	99.4	83.2	1012	15.4	17.7	56.3	53.7
1248	40.3	44.5	90.1	89.8	1245	17.0	19.2	65.5	59.9

Conclusions: Despite relatively small populations of patients with AT and PC deficiency, we believe that the search for causative mutations in *SERPINC1* and *PROC* is justified even in those symptomatic patients with minimal reduction of AT or PC plasma activity. In contrast, the search for causative mutations in *PROS1* is justified only in patients with significantly reduced levels of free PS antigen.

PB 1159 | Assessment of Overall Coagulation and Fibrinolytic Activity in Hemophilia A Patients by Using Global Hemostatic Laboratory Methods: Overall Hemostasis Potential, aPTT-waveform Analysis and Endogenous Thrombin Potential

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Background: Measurement of FVIII activity allows diagnosis of hemophilia A and categorization of disease severity, but has poor correlation with clinical phenotype. New laboratory methods that assess global hemostasis have been developed, with intention to better diagnose and monitor hemophilia A patients.

Aims: To assess overall coagulation and fibrinolytic activity in hemophilia A patients using non-standard laboratory methods: overall hemostasis potential (OHP), aPTT-waveform analysis (aPTT-WA) and endogenous thrombin potential (ETP).

Methods: Total of 63 hemophilia A patients (30 severe and 33 non-severe) and 27 healthy male subjects as control group were tested. OHP method, based on repeated spectrophotometric registration of the fibrin-aggregation in plasma, after addition of small amounts of exogenous thrombin, tissue-type plasminogen activator and calcium, provides besides OHP parameter (area under the fibrin aggregation curve) 3 supplemetary parameters: overall coagulation potential (OCP), overall fibrinolytic potential (OFP) and clot lysis time (CLT). In-house aPTT-WA was performed on BCS with Actin FS (Siemens Healthcare Diagnostics, Germany), obtaining 3 quantitative waveform parameters from 2 different evaluation modes, drifting baseline (DB) and point of inflexion (PI): DELTA (aPTT-PI minus aPTT-DB), RATIO1 (aPTT-PI/aPTT-DB) and RATIO2 (DELTA/aPTT-DB). ETP method, setting C was performed on BCS-XP (Siemens), giving 4 parameters: area under the thrombin generation curve (AUC), peak thrombin concentration (Cmax), time to peak thrombin concentration (t-max) and time to signal beginning (t-lag).

Results: Obtained results revealed statistically significant difference ($P < 0.05$) for all parameters between analyzed groups, except for CLT between severe and non-severe group (Table 1).

Conclusions: Global assays can serve as a useful laboratory tool for assessing overall coagulation and fibrinolysis activity, providing at the same time additional information about hemophilia A patients.

PB 1160 | Thrombin Generation in Patients with Systemic Lupus Erythematosus (SLE)

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Background: Patients with systemic lupus erythematosus (SLE) are subject to significant morbidity and mortality due to atherosclerotic diseases, which cannot be fully explained by traditional risk factors. Thrombin generation test (TGT) is a global hemostasis test providing information about the speed and amount of generated thrombin in plasma.

Aims: Our aim was to find out whether results of this test might differ in patients with SLE as compared to healthy individuals and to see

TABLE 1 Results of overall hemostasis potential, aPTT-waveform analysis and endogenous thrombin potential in analyzed groups of patients and controls

	Overall hemostasis potential				aPTT-waveform analysis			Endogenous thrombin potential	
	OHP	OCP	OFP %	CLT min	DELTA s	RATIO1	RATIO2	AUC mA	C-max mA/min
Hemophilia A	0.34 (0.00-8.68)	3.61 (0.00-7.35)	79.6±18.7	11.29±4.79	9.7 (4.6-48.7)	1.20 (1.13-1.74)	0.20 (0.13-0.74)	16.4 (3.9-287.7)	6.2 (1.9-39.9)
Controls	5.95±2.03	13.79±4.79	56.0±14.8	15.93±3.10	3.3±0.65	1.13±0.02	0.13±0.02	80.5 (5.6-328.9)	17.7±12.4
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Severe group	0.00 (0.00-5.63)	2.37±2.98	91.7±15.3	10.42±6.17	24.2±12.5	1.37±0.18	0.37±0.18	13.1 (3.9-88.5)	6.3±3.7
Non-severe group	2.79±2.45	9.07±5.21	73.4±17.4	11.75±3.97	7.6±1.6	1.18±0.03	0.18±0.03	30.8 (4.0-287.7)	7.7 (1.9-39.9)
P	<0.001	<0.001	0.002	0.406	<0.001	<0.001	<0.001	0.006	0.042

whether TGT parameters are associated with thrombotic episodes in SLE patients.

Methods: Forty-six patients with SLE not taking anticoagulants and 70 age and sex-matched healthy controls were enrolled. TGT was performed on platelet poor plasma and results were evaluated by the Thrombinoscope software. Lagtime, endogen thrombin potential (ETP), peak thrombin, time-to-peak and velocity index were calculated. Clinical parameters including age, sex, BMI, smoking habit, traditional risk factors, thrombotic history and disease activity were registered.

Results: In SLE patients lagtime and time-to-peak parameters were significantly elevated, while ETP was significantly reduced as compared to controls ($p< 0.0001$). TGT parameters showed significant positive correlation with BMI and CRP in patients and in controls, as well. The presence of lupus anticoagulant increased lagtime and time-to-peak parameters significantly, while the presence of anticardiolipin antibodies was associated with significantly lower ETP. SLE patients with history of thrombotic events had significantly higher ETP values, pregnancy morbidity was associated with elevated peak thrombin levels.

Conclusions: In patients with SLE, the extent of TG was significantly lower as compared to controls, which might be associated with the presence of antiphospholipid antibodies. The history of thrombosis or pregnancy morbidity was associated with increased TG, indicating that the test might be suitable for identifying those with elevated thrombotic risk in this patient population.

PB 1161 | Comparison of Three Different Anti-Xa Assays in Major Orthopedic Surgery Patients Treated with Edoxaban

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Background: The levels of anti-Xa drug cannot be monitored by routine assays; therefore, drug-specific anti-Xa assays use a chromogenic substrate to measure the concentration of anticoagulants that inhibit factor Xa in patients being treated with anti-Xa.

Aims: Measurement of edoxaban plasma concentration in major orthopedic surgery patients receiving edoxaban for the prevention of venous thromboembolism (VTE).

Methods: The anti-Xa activity was measured one hour after edoxaban intake using 3 different assays in 200 patients at three different time points Day 1, Day 4 and Day 8 after major orthopedic surgery.

Results: The anti-Xa activities on Day 8 were significantly higher than those on Day 4 and those on Day 4 were significantly higher than those on Day 1. The anti-Xa activities in assay A closely correlated with those in assay B, but the correlations between assays A and C and assays B and C were not close. The anti-Xa activities as detected in the three Xa assays were significantly higher in the patients without deep vein thrombosis (DVT) than in those with DVT on Day 4. Additionally, there were no significant differences in the anti-Xa activities of assays A, B and C between patients with and without MB on Days 1, 4, 8 and 15.

Conclusions: The three anti-Xa assay kits tested are appropriate for measuring edoxaban plasma concentration in orthopedic surgery patients. The results of this study additionally suggests that anti-Xa level could be predictive of the risk of VTE, but not of the risk of massive bleeding.

PB 1162 | Assessment of Two Chromogenic FVIII Assays In Haemophilia A Patients Infused with B-domain Truncated or Deleted Products: Results from a French Multicentric Study

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TABLE 1 results

			Sysmex CS®			BCS XP®	
		OSA	CSA I	CSA II	OSA	CSA I	CSA II
ReFacto AF (n=28/25)®	Mean(SD) IU/dL Bias (all/>30IU/dL)	46.6 (34.5)	61.7 (47.5) 15.1/24.1%	66.2 (48.8) 19.5/30.8%	44.5 (32.5)	51.4 (41.5) 6.9/7%	60.2 (44) 15.7/19.6%
NovoEight (n=19/21)®	Mean(SD) IU/dL Bias (all/>30IU/dL)	42.3 (32.9)	53.2 (43.4) 10.8/18.6%	59.8 (46.7) 17.5/28.6%	49.9 (47.7)	53.8 (56.1) 4/9.4%	57.3 (52.8) 7.5/12.3%
Nuwiq (n=24/25)®	Mean(SD) IU/dL Bias (all/>30IU/dL)	41.4 (37.8)	58.8 (52.7) 17.5/29.7%	62.7 (55.1) 21.4/35.4%	60.2 (42.2)	71.2 (52) 11/16.7%	77.3 (50.7) 17.1/23.3%
All patients (n=71/71)	Bias		14%	18.8%		6.8%	13.2%
Normal samples (n=56/51)	Mean(SD) IU/dL	89 (24)	88 (24)	97 (24)	107 (21)	100 (23)	96 (19)

Background: The measurement of FVIII activity may be achieved using one-stage clotting (OSA) or chromogenic (CSA) assay. The latter is the reference method for the European Medicine Agency. Discrepant results using both methods in post-infusion samples have been widely reported, especially when B-domain deleted products are used.

Aims: The aim of this French multicentric study was to compare FVIII activity using OSA and CSA on post-infusion plasma samples from haemophilia patients treated by recombinant FVIII B-truncated or deleted products.

Methods: One hundred forty two samples originating from patients receiving ReFacto AF®(Pfizer, n=53), NovoEight® (NovoNordisk, n=40), Nuwiq®(Octapharma, n=49) were gathered by 10 centers, anonymized and randomly distributed to 4 centers for measurement of FVIII activity. OSA was determined using APTT reagent Siemens Actin® FS, CSA was determined using Siemens® Chromogenic FVIII (CSA-I) , and Biophen® FVIII, Hyphen BioMed (CSA-II) on Siemens BCS®XP (2), Sysmex® CS2100 (1), Sysmex®CS2500 (1). Samples from non-haemophiliacs were also tested in each center.

Results:]Based o the good interlaboratory agreement, results of FVIII activity were pooled according to Siemens BCS®XP or Sysmex®CS instrument. Calculation of the bias as difference between CSA and OSA level showed that discrepancies were lower on BCS®XP than on Sysmex®CS whatever the CSA reagent, and more pronounced on both instruments for FVIII levels higher than 30 IU/dL.

Conclusions: Discrepancies between CSA and OSA are confirmed but seem depend on the instrument and on the CSA reagent: they are less extended on BCS®XP with Siemens reagent while wider on Sysmex®CS instruments with Hyphen reagent. Differences between assays may be more acceptable in the Siemens configuration i.e instrument and OSA/CSA reagent.

PB 1163 | Effects of Interfering Substances on Factor Assays Performed Using Q Smart Analyser and DG-PT RecombiLIQ or DG-APTT Synth Reagents

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Background: Guidelines for assay of clotting factors including FVIII consistently recommend analysis of patient samples using at least 3 dilutions of sample to improve both precision and accuracy of results. There are few publications describing the degree of agreement between the 3 results for different dilutions that might be expected in the presence and absence of interfering substances results that would be obtained. We decided to study this using Q Smart analyser which includes assay protocols for analysis of multiple test sample dilutions.

Aims: To assess the effects of interfering substances on intrinsic and extrinsic clotting factor assays.

TABLE 1 Effects of DOACs on FVIII assay results

Patient/Group		FVIII on 1 dilution	FVIII :Mean of 3 dilutions	CV of FVIII results on 3 dilutions	r value for 3 dilutions
Hospital patients	mean of 21 cases	190 IU/dL	183 IU/dL	7% (range 2- 18%)	0.998 (0.994-1.0)
patients with deficiency of FVIII,FIX or FV	mean of 10 cases with different defects	78 IU/dL	73 IU/dL	8% (range 4-19%)	0.998 (0.993-1.0)
Normal plasma	before addition of apixaban	77 IU/dL	76 IU/dL	3%	0.999
same plasma as above with Apixaban added	Apixaban 164 ng/ml	34 IU/dL	48 IU/dL	29%	0.998
Normal plasma	before addition of dabigatran	53 IU/dL	60 IU/dL	11%	0.995
same plasma as above with Dabigatran added	Dabigatran 155 ng/ml	13 IU/dL	21 IU/dL	40%	0.986
Normal plasma	before rivaroxaban added	63 IU/dL	67 IU/dL	7%	1.000
Same plasma as above with Rivaroxaban added	Rivaroxaban 121 ng/ml	26 IU/dL	34 IU/dL	23%	0.988

TABLE 2 Effects of some interfering substances on FVIII assay results

Sample	Relevant test results	FVIII on 1 dilution	FVIII:mean of 3 dilutions	CV of FVIII results on 3 dilutions	r value for 3 dilutions
Lupus anticoagulant	DRVVT >5.0	42 IU/dL	55 IU/dL	20%	0.965
Patient on heparin	Anti Xa- 1.7 IU/ml	23 IU/dL	66 IU/dL	66%	0.5
Patient on Heparin	Anti Xa- 0.87 IU/ml	25 IU/dl	58 IU/dL	55%	0.465
FVIII inhibitor	>120 BU/ml	6 IU/dL	9 IU/dL	29%	0.996

Methods: Factor VIII, FIX and FV assays were performed on 21 samples from hospital cases; 10 subjects with isolated deficiency of either FVIII, FIX or FV; Lupus anticoagulant (n=10); patients on unfractionated heparin therapy (n=10); patients with FVIII inhibitors; and normal plasma before and after addition of apixaban, dabigatran or rivaroxaban. FVIII, FIX and FV assays were performed using 1 or 3 test dilutions using DG-PT RecombiLIQ (liquid human thromboplastin) for FV or DG-APTT Synth (ellagic acid/synthetic phospholipids) for FVIII and FIX in combination with the Q Smart analyser (Diagnostic Grifols) which determines the r value for the line through test sample dilutions and the CV of results on the 3 dilutions.

Results: Factor VIII results of selected cases are shown in tables below. For FIX and FV assays in hospital patients all r values were > 0.994 and the mean and range of CVs for results on 3 different dilutions were 4.7 % (1-11%) and 7.6% (2-16%).

Conclusions: Unfractionated heparin, apixaban, rivaroxaban, dabigatran and lupus anticoagulant can all interfere in factor assays confirming the importance of testing multiple test sample dilutions for accurate results. When 3 dilutions are analysed using the Q Smart analyser protocols a CV of 20% or more for the 3 results indicates that results are inaccurate at some test sample dilutions.

PB 1164 | Comparison of Point-of-Care Instruments I-STAT, ACTPlus and Hemochron Signature Plus for Measurement of Activated Clotting Time (ACT)

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Background: Activated clotting time (ACT) point-of-care testing is frequently used to monitor high-dose heparin therapy.

Aims: We evaluated the performance characteristics of I-STAT (Abott), ACTPlus (Medtronic) and Hemochron Signature Plus (Archiva Diagnostics) point-of care instruments for ACT measurement.

Methods: Three ACTPlus, two I-Stat and two Hemochron Signature Plus were validated. Within-run and between-run imprecision was determined using quality controls of normal (low) and abnormal (high) range. For ACTPlus, intra-assay reproducibility was determined by comparing duplicate channel results for blood samples per instrument, whereas for I-STAT and Hemochron measurements of the same sample performed in parallel on two instruments were evaluated (duplicate measurements). Instruments were compared per type by evaluating measurements simultaneously performed on all instruments (parallel measurements). Acceptance criteria for duplicate (intra-assay) and parallel (inter-assay) measurement were set as < 12% spread error. Linearity (0 - 5IU/ml heparin) in spiked blood samples was evaluated.

Results: Detailed results are displayed in Table 1. Overall, all instrument types performed equally when using normal and high range quality control materials. However, when using real blood samples, I-STAT performed better than Hemochron Plus, which in turn performed better than ACTPlus. On ACTPlus, a significant proportion of duplicate and parallel measurements of real blood samples did not fulfill the acceptance criteria. Linearity was good on all instrument types. Heparin concentrations gave comparable ACT-values on all instruments types in the low range, but values differed in the high range.

Conclusions: This study illustrates that correct evaluation of point-of-care ACT-instruments should not be limited to quality control material, but should also include real blood samples. Overall, I-STAT performed better than Hemochron Signature Plus and ACTPlus.

PB 1165 | Extracellular Proteases of Micromicetes as New Agents for Protein C, Factor X and Plasma Prekallikrein Diagnostics

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Background: Many patients deficient in such hemostatic factors as protein C, factor X and prekallikrein have been described and have an associated thrombotic tendency. Some snake venom proteases are

TABLE 1 Comparison of I-STAT, ACTPlus and Hemochron Signature Plus for measurement of ACT

	ACTPlus 1	ACTPlus 2	ACTPlus 3	I-Stat 1	I-Stat 2	Hemochron 1	Hemochron 2
QC - CV% low range (within-run)	2.2	6.7	2.4	1.9	1.5	4.1	7.2
QC - CV% high range (within-run)	3.0	4.9	3.5	2.2	3.0	1.3	2.0
QC - CV% low range (between-run)	4.0	3.2	8.6	1.8	1.8	8.1	12.5
QC - CV% high range (between-run)	5.6	8.3	10.2	3.2	4.9	1.5	3.2
Blood - duplicate samples (intra-assay reproducibility)							
Measurements that fulfill <12% criteria	7/17	11/17	12/17	30/30		27/30	
Blood - parallel samples (inter-assay reproducibility)							
Measurements that fulfill <12% criteria	12/34	10/34	16/34	30/30		27/30	
Linearity (correlation coefficient)	0.9823			0.9639		0.9409	

used in diagnostics of them. At this time, there are some fungal proteases with activator activity to protein C, factor X and prekallikrein produced by *Aspergillus ochraceus* and *Aspergillus terreus*.

Aims: Study of applicability of proteases of *A. ochraceus* and *A. terreus* for protein C, factor X and prekallikrein determination.

Methods: Proteases were obtained from cultural fluids by FPLC. Activity was studied with the chromogenic peptide substrates after preincubation of proteases with human plasma.

Results: It was demonstrated the same results with specific activity proteinases of *A. ochraceus* with commercial diagnostic preparations Protac® (protein C activator from venom of South American copperhead) and RVV-X® (factor X activator from venom of Russell's viper). Similar experiments were conducted in the presence of factor X deficient plasma and protein C deficient plasma. It was found that concentration of protein C is identical with Protac® (32,7% ± 5%) diagnostic system and specific activity proteinases of *A. ochraceus* (31,5% ± 5%). Uniform diagnostics interval was detected with RVV-X® diagnostic system (36,6% ± 4%) and proteinases of *A. ochraceus* (37,4% ± 4%). *A. ochraceus* protease did not show any activator activity with human plasma, deficient by prothrombin complex factors and plasma deficient by prothrombin complex factors and by contact phase of coagulation factors. *A. terreus* protease retained activator activities with chromogenic substrates of protein C and factor Xa with both of these deficient plasmas. Activity of the *A. terreus* with kallikrein chromogenic substrate showed after preincubation with both plasmas is not reduced.

Conclusions: Proteases of *A. ochraceus* are very perspective for protein C and X factor diagnostics and proteases of *A. terreus* may be useful for prekallikrein determination.

PB 1166 | Laboratory Expert System in the Field of Hemostasis and Thrombosis: A Pilot Project for the Diagnosis of von Willebrand Disease

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Background: A laboratory expert system (LES) in the field of ‚gynecological endocrinology' was recently developed by the Qonsilus company, Germany. The software supports medical doctors in their decision-making process by linking sets of laboratory analyses with data from medical history, signs and symptoms in order to provide individual treatment recommendations. In the field of hemostasis and thrombosis the major parameters in clinical chemistry comprise alterations of hemostatic parameters as well as clinical parameters and specific risk factors.

Aims: The present pilot project aimed to extend the above mentioned LES to the field of ‚hemostasis and thrombosis', specifically to the diagnosis of von Willebrand disease (VWD).

Methods: An iterative process was used to digitize medical expert knowledge into actionable rules with a continuous review of patient cases. Each finding of a knowledge model evaluation had a certainty factor that represents the scoring value reached. Evaluation of the knowledge model was performed by comparing the diagnostic accuracy in 79 patients with suspected or confirmed VWD, with the diagnosis either made by LES or by an expert in the field (EIF).

Results: In 61 (77%) cases we found no differences in the diagnosis assessed by LES versus EIF and 73 (92%) cases evaluated by two different EIF, respectively. Among the deviations there were 6 (8%) non-relevant, 9 (11%) relevant and 3 (4%) critical cases for the evaluation by LES versus EIF and 1 (1%), 3 (4%) and 2 (3%) for EIF versus EIF, respectively.

Conclusions: The automated LES here described may be a promising useful tool for the diagnostic workup of a non-expert in the field of VWD. However, clinical data are essential for detailed diagnostic and therapeutic recommendations. At this stage LES cannot substitute the EIF of hemostasis, but may support the non-specialist to come closer to the diagnosis.

PB 1167 | A New and Improved Euglobulin Clot Lysis Time Assay (iECLT)

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TABLE 1 a) Correlations of iECLT with fibrinolysis proteins in PFP and EF. b) Concentration reduction in the EF compared to PFP

a) CORRELATION WITH iECLT									
	PAI1		tPA		α2antiplasmin		α2macroglobulin		Plasminogen
	PFP	EF	PFP	EF	PFP	EF	PFP	EF	PFP ___ EF
n	17	17	16	16	16	15	15	15	15 _____ 15
Spearman r	0.82	0.72	0.15	-0.68	0.11	0.56	-0.11	-0.03	0.3 ___ 0.29
p value(two-tailed)	0.0001	0.0016	0.58	0.0047	0.68	0.026	0.69	0.90	0.27 ___ 0.3
b) REDUCTION IN EF									
(%, mean±SEM)	97.84±1.24		12.79±29.64		95.25±0.75		99.82±0.06		28.39±3.89

Background: Alterations in balance between coagulation and fibrinolysis may lead to thrombosis or bleeding. Many methods have been proposed for fibrinolytic activity measurement, however the majority have used tPA to initiate fibrinolysis. To assess endogenous fibrinolysis, an assay without tPA addition is desirable. Utilization of the plasma euglobulin fraction (EF) could be a solution. The EF contains significantly reduced amounts of PAI-1 with relatively preserved levels of tPA. This allows intrinsic fibrinolytic activity to be measured without addition of tPA. The original ECLT described 6 decades ago is rarely used now, due to high variability of results and low throughput. We propose a modified ECLT with spectrophotometric readout to address some of these limitations.

Aims: Characterize the iECLT.

Methods: Citrated platelet free plasma (PFP) from 42 healthy individuals was collected after obtaining informed consent.

A] In the iECLT assay, EF was mixed with fibrinogen, ovalbumin and clotted with thrombin. OD changes were read spectrophotometrically for 25 hours. The time needed for half lysis of the clot was measured.

B] ELISA - free PAI-1, free t-PA, plasminogen, α2macroglobulin (α2MG) and α2antiplasmin (α2AP).

Results: iECLT values showed a non-parametric bimodal distribution, with normal range from 11.06 to 21.56 hours. iECLT intra-assay and inter-assay variabilities were 2.45% and 9.85%, respectively.

A strong correlation was found between iECLT and PAI-1 (both in PFP and EF), tPA (in EF) and α2AP (in EF) (Table 1a).

In addition, we have confirmed that levels of endogenous inhibitors are significantly reduced, while activators are partially preserved in EF (Table 1b).

Conclusions: iECLT allows global analysis of fibrinolysis without addition of exogenous plasminogen activators. iECLT has low variability of results and high throughput, compared with the original ECLT.

PB 1168 | Platelet Estimation from Peripheral Blood Smear: Does it Really Works?

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Background: Assessment of platelet count is an important diagnostic parameter in haematology. Platelet counts can be done manually or with an automated analyzer. Counts can also be estimated during blood smear examination.

Aims: The objective of our study is to compare 03 manual methods using peripheral blood smear with the automatic method and to determine their effectiveness in the estimation of platelet count.

Methods: Blood samples were obtained from 220 patients.

Automated method : Sysmex XT4000i

Manual methods :

1st methode : blood smears were stained and examined under light microscopy. The red cell: platelet ratio was calculated : The number of erythrocytes observed in a quarter of the oil-immersion field was multiplied by four. Then all the platelets in the same field were counted. Other fields were examined until we reached a number of 1000 erythrocytes. The number of platelets per 1000 erythrocytes was multiplied by the automated Red Blood Count to give an approximate count.

2nd method :we counted the numbers of platelets per 10 oil immersion fields and divided by 10 to find the average number of platelets per field and then multiply by 14 (field factor)

3rd method: we multiply the average number of platelets per 10 fields by the patient's hemoglobin and then by 1,000 mm3 to obtain the number of platelets/mm3.

Results: The results showed positive correlation between the fours methods .The ICC was calculated in order to identify the reliability of the manual techniques in comparison to the automated method. In our study, the ICC was equal to 0.946 ,0.937 ,0.888 for method 1, 2 and 3 respectively. The paired t-test showed no significant difference between the four methods ($p>0.05$).

Conclusions: We conclude there is a no difference between the automatic method and the manual methods.The results of our study proove that manual estimation is a reliable method and can be used in rural areas where automatic methods are not available and when there is discordance between clinical and biological results.

PB 1169 | Comparison of Rotational Thromboelastometry Reference Ranges with Normative Ranges across Trimesters in Pregnancy

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Background: In pregnancy and postpartum, women exhibit hypercoagulability and physiologic changes of coagulation factors Rotational thromboelastometry (ROTEM) is increasingly used as a point of care coagulation monitoring device in patients with massive haemorrhage; however, there are limited data on reference ranges in pregnancy across different gestational age.

Aims: We hypothesized that normative ranges of rotational thromboelastometry (ROTEM) may differ across trimesters of pregnancy and differ in compare to the non-pregnant values recommended by the manufacture.

Methods: After IRB approval, citrated blood was collected from 72 healthy pregnant women and grouped by trimester: 1st (11±3 weeks gestation, n= 20), 2nd (21±5, n=23), and 3rd (33+3, n= 29). EXTEM, INTEM, and FIBTEM were performed on ROTEM (IL, USA) to establish reference ranges. Reference ranges defined by the 5th and 95th percentiles, and were compared to the manufacturer's recommended ranges. ANOVA with Tukey test were used (SPSS v23, 2016, IBM, USA; significance = $P < 0.05$).

Results: After excluding outliers, only maximum lysis (ML) in INTEM, EXTEM, and FIBTEM differed significantly across trimesters (Table 1)

TABLE 1 Maximum Lysis (ML) difference across the trimesters

Table-1	1st trimester, range	2nd trimester, range	3rd trimester, range
ML INTEM	3-16%	3-18%	2-9%
ML EXTEM	3-16%	2-16%	3-11%
ML FIBTEM	0-16%	0-4%	0-2%

Our population reference ranges overlap with those from the manufacturer, however all variables showed a narrower range in pregnancy, especially in 3rd trimester. (Table 2).

TABLE 2 reference ranges in different trimester and comparing to reference ranges provided by manufacture

Table-2	Manufacture	1st trimester	2nd trimester	3rd trimester
INTEM CT(sec)	122-208	176-187	169-183	163-177
INTEM CFT(sec)	44-110	62-73	62-72	61-69
INTEM MCF(mm)	51-71	64-68	64-68	67-71
EXTEM CT(sec)	43-82	58-65	56-59	53-59
EXTEM CFT(sec)	48-127	63-74	64-73	59-70
EXTEM MCF(mm)	52-70	67-70	67-70	69-72
FIBTEM MCF(mm)	7-24	21-25	21-25	24-28

Conclusions: Majority of ROTEM reference ranges remain similar across pregnancy, with the exception of ML. Normal ranges in all trimesters are significantly narrower than those suggested by the manufacturer. Upper limits of CT and CFT are significantly shorter, consistent with physiologic hypercoagulability. Use of pregnancy and trimester-specific ranges may prevent clinical over- or underestimation of function of coagulation function.

PB 1170 | Enhanced Coagulation Decreases Probability of Positive Outcome during in vitro Fertilization

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Background: Infertility in women is often accompanied with hypercoagulable state such as thrombophilia, antiphospholipid syndrome and etc. In vitro fertilization (IVF), which is widely used for infertile women, increases the coagulation potential futher due to controlled ovarian stimulation (COS).

Aims: To investigate the coagulation system state with standard assays (clotting times, fibrinogen and D-dimers levels) and global coagulation assay (thrombodynamics) in women with infertility prior to the IVF cycle and during the treatment to reveal the association between coagulation imbalance and outcome of the IVF procedure.

Methods: A total of 114 patients undergone fresh IVF cycles were included in the prospective cohort study. All gave their written informed consent. Blood samples for monitoring of coagulation were collected before IVF, one week after COS starting, on the day of follicles puncture, on the day of embryo transfer and one week after embryo transfer. The main outcome was clinical pregnancy rate.

Results: In total, 28 women (24.6%) became pregnant after IVF. Women with higher platelet count (>260x10^9/L) and higher clot growth rate (>29.9 μm/min, detected with thrombodynamics) before IVF demonstrated a higher percentage of unsuccessful IVF outcomes (odds ratio (OR) 5.98, 95% confidence interval (CI) 1.64-21.73, p=0.007 and OR 4.47, 95% CI 0.98-20.38, p=0.053, respectively). Clotting times, fibrinogen and D-dimers levels before and during IVF did not differ significantly between groups with successful/unsuccessful outcomes. Hemostasis assays demonstrated shift towards hypercoagulation in response to COS with subsequent normalization in response to low molecular weight heparin prophylaxis after the embryo transfer.

Conclusions: High platelets level and hypercoagulation detected with thrombodynamics in infertile women independently predict higher risk of unsuccessful IVF outcomes. The results of current research may be of relevance in counseling and monitoring women undergoing IVF.

PB 1171 | Development and Implementation of a Coagulation Factor Testing Method Utilizing Autoverification in a High Volume Clinical Reference Laboratory Environment

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Background: Testing coagulation factor activities requires that multiple dilutions be assayed and analyzed to produce a single result. The slope of the line created by plotting measured factor concentration against sample dilution is evaluated to discern the presence of inhibitors giving rise to nonparallelism. Moreover, samples that produce results on initial dilution that fall outside the analytic measurement range of the assay must be tested at additional dilutions to produce reportable results.

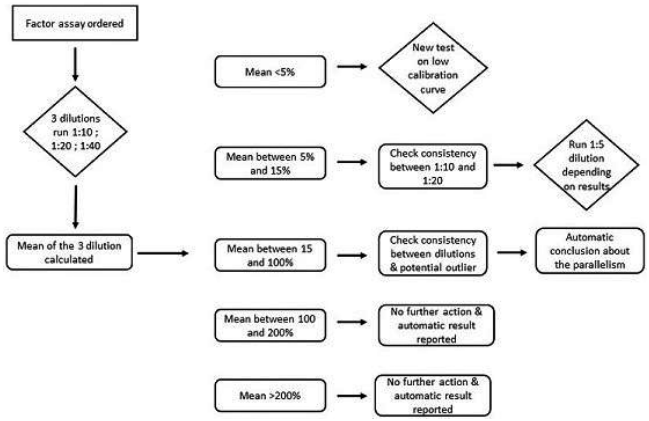

FIGURE 1 Factor Testing Expert Rules Flow Chart

Aims: The complexity of the coagulation factor testing process motivated a large clinical reference laboratory to develop advanced computer algorithms with automated reflex testing rules to complete coagulation factor analysis.

Methods: Prospective validation of the expert rules was accomplished using 552 different anonymized FVIII deficient plasma samples each tested at 1:10, 1:20, and 1:40 dilutions giving a total of 1,656 individual results on an automated coagulation analyzer using the using the STA Coag Expert data manager system (Diagnostica Stago Inc., Parsippany, NJ).

Results: The testing cascade and rules for factor activity assays programmed into the STA Coag Expert data manager are displayed in the figure included. Starting from the top left point of the flow chart, where the test is ordered through the LIS (Factor assay ordered); initial testing at three different dilutions (1:10, 1:20 and 1:40) is performed. The mean of these three dilutions is used to determine the next step, followed by subsequent steps as shown.

Conclusions: Expert rules utilizing autoverification procedures can be applied on the instrument, in the data manager software, or in the LIS. When properly implemented, the expert rules can

1) handle the mundane and error prone task of result verification, allowing medical technologists to focus on the true problem samples,

2) improve the consistency in the quality of test results, and

3) reduce staff fatigue, improving the work environment in the process.

PB 1172 | Factors VII and VIII Assays: Analytical Performance and Reference Ranges for Argentinian Population

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Background: Laboratory scientists are responsible for providing both accurate and precise test results as well as valid reference intervals (RI) in order to allow clinicians to differentiate between health and disease to properly manage patients.

Aims: To verify the analytical performance of one stage determination of Factor VII (FVII) and Factor VIII (FVIII) and to determine the RI for local population.

Methods: FVII and FVIII activity were determined on a STA®-Compact Max analyzer using STA® Deficient FVII and FVIII. Precision and trueness were determined with the CLSI EP15-A3 Protocol with STA®-System Control N+P. Linearity was determined using CLSI EP6-A protocol with serial dilutions of a patient sample, covering the working range of products (15-150% for FVII and 5-150% for FVIII). RI was determined with 65 blood donors plasma (ages between 24 and 64). Informed consent was obtained prior to blood being drawn. Outliers were eliminated using Tukey´s method.

Results: Precision was evaluated at normal (N) and Patological (P) levels: intra-assay coefficient of variation (CV) was 4.42% (N) and 3.95% (P) for FVII; 4.17% (N) and 5.20% (P) for FVIII. Inter-assay CV was 5.79% (N) and 5.93% (P) for FVII; 6.60% (N) and 9.87% (P) for FVIII. With respect to trueness, FVII average was 95.5% (77.5-104.5) and 44.7% (37-49); FVIII average 101.1% (82.4-115.6) and 49.4% (34.4-57.6) for N and P respectively. Both factors showed a Gaussian distribution and proved to be linear in the working range. The RI for FVII was 51 % (43-60) to 142% (134-150) and 67% (54-80) to 206 % (193-218) for FVIII. FVIII was 18% lower in O blood carriers compared with non O blood group.

Conclusions: The performance for measuring both factors showed accuracy and trueness according to manufacter‘s report. The RI obtained in our laboratory was quite different from the manufacture‘s information, so it´s important to establish local RI to better classify the patients at risk of bleeding or thrombosis.

PB 1173 | Can Thromboelastography Accurately Assess Coagulation in Anemic Patients?

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Background: Thromelastography (TEG) is a method performed on whole blood, examining the physical properties of blood clots. TEG is often performed in bleeding patients so it is essentially important to examine its significance when hematocrit (Hct) decreases.

Aims: Assessing the effect of ex-vivo Hct reduction (by erythrocyte removal) on TEG parameters in healthy volunteers.

Methods: Fifty one healthy volunteers aged 18-50 years, were studied. Blood drawn into EDTA tube served as a baseline measure. Four citrate tubes were used for measuring TEG, PT, PTT and derived fibrinogen. Two of the citrate tubes retained Hct of 35-55%, and served as reference. The other tubes were used to produce a variable Hct around 20-25% by removal of red blood cells pellet. Two methods of TEG were used: citrate kaolin TEG on all 51 blood samples, and FFR (functional fibrinogen reagent) TEG used to determine the functional

fibrinogen contribution on the TEG MA (maximal amplitude) of 38 blood samples.

Results: Using the citrate kaolin TEG, there was a significant elevation of MA correlated with Hct reduction (Pearson correlation, R = -0.551, p< 0.001, n=51), which may imply a pattern of hypercoagulation. Using the FFR TEG there was a statistically significant correlation between Hct reduction and elevation of functional fibrinogen levels (Spearman correlation, R = -0.390, p=0.016, n=38), indicating its contribution to the MA changes. Fibrinogen levels measured in all tubes were not significantly changed by Hct reduction. The changes in platelet numbers were in all cases in normal range, and no statistical correlation was found between platelets number changes and the Hct changes (Spearman correlation, R = 0.027, p=0.850, n=51).

Conclusions:

1. Ex-vivo reduction of Hct is strongly associated with MA elevation.
2. The high MA is explained by elevation of functional fibrinogen levels.
3. It is not clear whether these findings reflect physiology or an artifact of the method, and further in-vivo studies are needed.

PB 1174 | Standardization of a Cytometry Test to Measure the von Willebrand Factor Collagen Binding Activity

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder characterized by von Willebrand factor (VWF) deficiency. Diagnoses of different types and subtypes of the disease require expensive tests based on immune and functional assays.

Aims: This study aimed to standardize a cytometry test to measure the VWF binding to collagen.

Methods: Plasma samples from VWD patients (n=27) were tested for collagen binding assay using a commercial vWF:CBA ELISA kit. A cytometry assay based on collagen-coated beads was developed and all samples were tested. Median Fluorescence Intensity (MFI) obtained in cytometry for tested samples was transformed in percentage based on normal control MFI. Pooled plasma collected from six healthy blood donors constituted the normal control. Spearman test was used to compare the results of the two tests. ROC curve analysis was generated to test to define a cut-off point (distinction between low and normal binding to collagen) for cytometry assay. This study was approved by Brazilian ethics committees and patients signed an informed consent.

Results: Collected samples presented a wide range of results in vWF:CBA kit (median of 0.34 U/mL; range of 0.01-0.89 U/mL; normal range: 0.4-2.5 U/mL). Results from cytometry test also presented a wide range of results (median of 59.8%; range of 17.1-99.4%). Spearman test revealed a strong correlation between the two tests (r=0.93; p< 0.01; 95% CI 0.85-0.97). The ROC curve defined (AUC=0.97; 95% CI 0.92-1.02; p< 0.01) identified 59.2% as the best cut-off point for cytometry test (sensitivity=0.92, 95% CI 0.64-0.99; specificity=0.86, 95% CI 0.57-0.98).

Conclusions: We initiate to standardize a low cost method based on flow cytometry that can be useful in VWD treatment centers. This is an ongoing study and additional molecular defects and clinical aspects will be reported upon completion of the work. Financial support: FAPEMIG and HEMOMINAS.

PB 1175 | Implementing Autoverification of Coagulation Screen Tests in Universitary Hospital Laboratory in Brazil

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Background: Autoverification (AV) has been widely used on clinical laboratories. However, few studies related to AV of coagulation tests (CT) were published.

Aims: Implementing AV of coagulation screen tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), fribrinogen (FIB) aiming: reduce turnaround time (TAT) and technician intervention, report more than 80,0% of CT in an interval shorter than 2 hours (general laboratory goal).

Methods:

1) Definition AV limits: Were calculated using percentile 90 from CT between 2011 and 2015. Intervals defined: for PT: 10,5 - 31,5 seconds; aPTT: 23,0 - 45,0 sec; FIB: 1,15 - 6,00 g/L.

2)Delta check (DC) calculation: From coefficient of variation analytical (CVa) and coefficient of variation individual (CVi, biological variation), using the Reference Change Values formula :

RCV=2(1/2) x Z x(CV(A)(2) +CV(I)(2))(1/2), where Z is the number of standard deviations appropriate to the probability. In our study, Z= 2, 58 (corresponding 99,0%). DC PT:18.9% (35 days); DC aPTT: 12.6% (1 day); DC FIB: 47.0% (1 day).

3) Evaluation ofTAT 3 months before (25.933 tests) and after (25.066 tests) AV implementation, using Wilcoxon test.

Results:

1) TAT reduction: PT (median before and after AV: 114 to 50 minutes, p< 0,001), aPTT (median before and after AV: 111 to 49 minutes, p< 0,001), FIB (median before and after AV: 101 to 44 minutes, p< 0,001).

2) Passing rate results < 2 hours before and after AV (median, 95% CI):PT= 53,0% (52,2-53,8%) to 91,0% (90,5-91,5%); aPTT= 54,9% (54,0- 55,9%) to 92,0% (91,4- 94,6%); FIB= 60,9% (56,8- 64,9%) to 92,7% (90,1- 94,6%).

Conclusions:

1) The general **turnaround time** of coagulation screen tests was shortened by 56,0% (109 minutes to 48 minutes).

2) Incorrect results were not reported by clinicians neither laboratory supervisors, since autoverification implementation.

3) Our laboratory reached passing rate >90,0% of coagulation screen tests in less than 2 hours.

PB 1176 | Assessment of a Pre-analytical Check System on ACL TOP 750 CTS®

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Background: ISO15189 requires us to indicate in the report whether the quality of the primary sample is unsuitable for examination or not. ACL TOP 750 CTS® has a pre-analytical check system that detects incorrect tube filling volumes and clog-clot, and identifies optical interferences (Hemoglobin, Icterus, Lipemia ; HIL) by means of a new multi-wavelength module.

Aims: We assessed the pre-analytical check system on the detection accuracy of ACL TOP 750 CTS® (Instrumentation laboratory, Bedford, MA, USA) by comparing with visual inspection.

Methods: We measured general coagulation parameters including PT, APTT, fibrinogen, antithrombin, FDP, and D-dimer on ACL TOP 750 CTS® in 2,360 blood samples collected in the Yamagata University Hospital from June to July 2016. Then we compared the prevalence of rejection between ACL TOP 750 CTS® and visual inspection.

Results: Seventeen of 2,360 samples were rejected on ACL TOP 750 CTS® by the reason of HIL, incorrect tube filling volumes, or clog-clot (Table 1). Eleven of these 17 samples were not rejected in visual inspection, even if they should have been rejected as they consisted of 8 incorrectly filled tubes and 3 samples with clots. There was no significant difference in the incidence of rejection between the two groups (0.72% vs 0.64%, *P*= 0.86). In the analysis using only HIL, the prevalence of rejection in visual inspection was significantly higher than that in ACL TOP 750 CTS® (0.47% vs 0.09%, *P*= 0.02).

Conclusions: We compared the prevalence of rejection in the ACL TOP 750 CTS® with visual inspection to assess the pre-analytical check system on ACL TOP 750 CTS®. This system might be useful not only for detection of the incorrect tube filling volumes and clog-clot, but also for decrease of the number of rejection limited in HIL.

TABLE 1 Statistical analysis between ACL TOP 750 CTS® and visual inspection

HIT+clog-clot+tube-filling		Visual inspection			P value
		rejcted	good		
ACL TOP 750 CTS	rejcted	6	11	17	0.8596
	good	9	2,334	2,343	
		15	2,345	2,360	
HIL		Visual inspection			P value
		rejcted	good		
ACL TOP 750 CTS	rejcted	2	0	2	0.0223
	good	9	2,334	2,343	
		11	2,334	2,345	

PB 1177 | Results of Implemented Postanalytical Quality Indicator - Turnaround Time (TAT) Monitoring for Urgent Coagulation Tests

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Background: Turnaround time (TAT) represents an important postanalytical quality indicator of laboratory service.

Aims: The aim was to present the results of TAT monitoring for urgent coagulation tests during the year 2016.

Methods: As accredited to ISO15189 norm, our laboratory has implemented daily TAT monitoring for urgent tests measured as the difference between sample receipt time and the result sending time.

For the purpose of long term TAT monitoring, we defined two measures: ratio (%) of reports delivered outside the specified time (60 mins) with sigma metric evaluation included (minimal acceptable sigma 3.0; desirable sigma 5.0) and time (mins) for International Normalized Ratio (INR) value reporting at 90th percentile with predefined eligibility criteria (minimal 67.5 mins, desirable 54.0 mins and optimal 40.5 mins). The results of TAT monitoring data were compared between the six-month periods.

Results: Total ratios (%) of reports with exceeded TAT were 1328/26946 (4.9%) for the first six months and 1150/26501 (4.4%) for the second six months with almost equal and minimal acceptable sigma values for both periods i.e. 3.2 and 3.3, respectively. In the first 6 months 90th percentile for urgent INR deviated from 3.4 to 7.4% (range for monthly obtained values was 56 to 58 mins) of the desired criteria (54.0 mins) and only a minimum criterion (67.5 mins) of eligibility was obtained. For the second six months 90th percentile for urgent INR deviated from -7.4 to + 5.6% (range for monthly obtained values was 50-57 mins) and desirable criteria was obtained.

Conclusions: Our results showed the minimal acceptable rather than desirable values of both TAT measures for urgent coagulation tests during the observed period. The implemented system for TAT monitoring is useful tool and we assumed that auto-validation implementation could be the key factor for further TAT improvement.

PB 1178 | Is it Helpful for Physicians the Laboratory Post Analytical Comments on a Prolonged APTT?

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Background: Partial Activated Thromboplastin Time (APTT) evaluates globally the intrinsic and common pathway of coagulation. When an APTT is prolonged, Thrombin Time (TT) is normal, and APTT fail to correct in mixtures with pooled normal plasma (NP), it is suggestive of

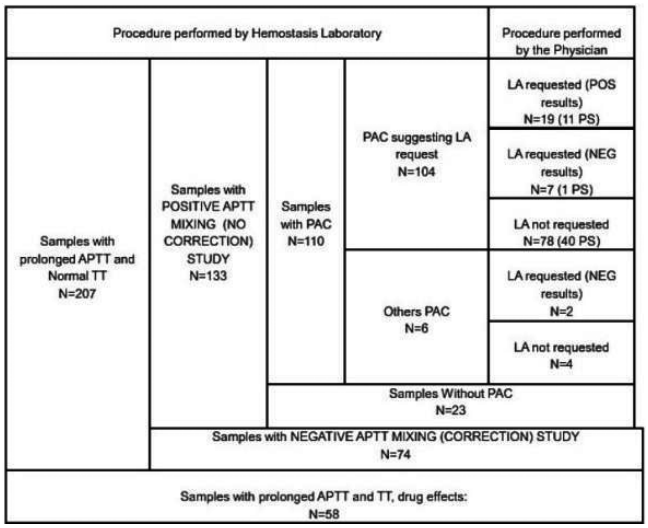

FIGURE 1 Results of the procedure performed by the laboratory and by the physician

the presence of a lupus anticoagulant (LA), particularly in the absence of bleeding manifestations. The prevalence of LA in elderly patients is increased.

Aims: To analyze the samples of elderly patients with prolonged APTT in routine laboratory setting, like presurgery evaluation (PS), and to evaluate the request for LA testing in those patients with prolonged APTT without correction with NP in which a post analytical comment (PAC) suggesting LA search was made by laboratory (lab).

Methods: 265 samples of asymptomatic patients (60-93 years) with normal prothrombin time and prolonged APTT were analyzed during a period of 15 months. APTT-SP IL (reference range 24 - 38 sec) in an ACL TOP300 was performed, and mixing studies were performed in 1: 1 mixtures with NP. Samples were divided in groups according to mixing studies results and post analytical comment (PAC) or suggestion made by lab. AL was performed according to the ISTH guidelines.

Results: See figure1

Conclusions: The physician´s response to the laboratory´s PAC suggesting LA request was only 25% (26/104). Of these studies, 19 had results compatible with the presence of LA (73%). Even when more than 50% of APTT studies were in the setting of a PS evaluation, the physician response to PAC suggestion was surprisingly low. Although the search for LA in asymptomatic patients is questioned, the prevalence of LA positive results in the study was high, probable related to the population age. It seems to be important to do PAC suggesting for LA searching to avoid postponements or additional treatments in elderly PS patients.

PB 1179 | Sensitive Method for Determination of Blood Hypercoagulation

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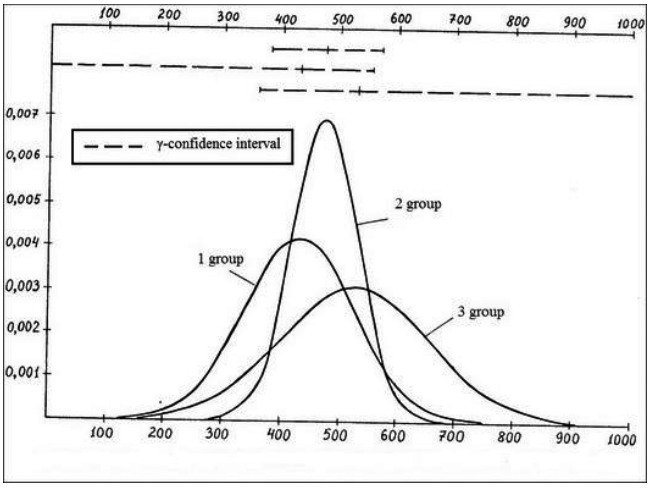

FIGURE 1 The probability density function of blood clotting time, sec. (after recalcification 0.98 mg/0.66 ml blood)

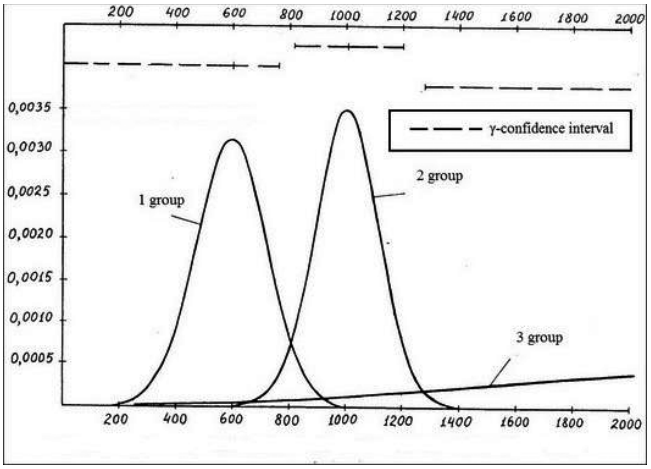

FIGURE 2 The probability d*ensity function of blood clotting time, sec. (after recalcification 0.55 mg/0.66 ml blood)*

Background: Hypercoagulation of blood (HPRB) is one of the main predictors of thrombosis. One of the methods of diagnosis of HPRB is recalcification technique. The principle of this method is the recovery of ionized calcium initial concentration in citrated blood (balanced recalcification technique - BRT).

Aims: The aim of our study was to investigate the influence of lesser amounts of calcium (LBRT) than in BRT.

Methods: We investigated the influence of different concentrations of ionized calcium (less than in BRT) on recalcification time. As a basis for our investigation we selected instructions for thromboelastograph ROTEM, where the final concentration of calcium in the mixture with blood after recalcification was 0.98 mg/0.66 ml blood. We chose the reaction time of thromboelastography as the end of obtained results. Empirically we determined that the most convenient final concentration of calcium in the mixture with blood was ~0.55 mg/0.66 ml blood. We selected 3 groups for the study: 1 group - 350 patients (pts) with thrombosis, 2 group - 60 healthy donors and 3 group - 215 pts from the 1st group during anticoagulant therapy (the blood was

investigated after 3-4 hours after injection of therapeutic dose of low molecular weight heparin (LMWH)).

Results: The results of our study are shown on Figure 1 and 2. BRT coincided with the shortest time of blood coagulation. When we used BRT, the γ-confidence intervals of all three groups were essentially surpassed each other for any confidence coefficient that didn't allow pattern recognition (Fig. 1). In contrast, in LBRT confidence intervals were separated from each other (Fig. 2).

Conclusions: Use of LBRT in patients' study has demonstrated its higher diagnostic potential in the determination of HPRB and possibly monitoring of anticoagulant therapy with LMWH.

PB 1180 | Variants of FVII Deficiency at an Elderly Care Hospital

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Background: FVII deficiency (FVIId) is an autosomal recessive disorder. There are two types: Type I (FVII-T1) with decreased FVII:C activity and antigen (FVII:Ag) and Type II (FVII-T2), with decreased FVII:C, variable with different thromboplastin (THR) origin, and normal FVII:Ag.

Aims: To report 5 cases of FVIId at an Elderly care Hospital.

Methods: Patients (P) and initial diagnosis (ID) performed with rabbit brain THR (rBT):

P 1: woman age 64, severe (S) FVIId

P 2: daughter of P1 age 44

P 3: woman age 71, S FVIId

P 4: man age 78, S FVIId

P 5: man age 75, mild FVIId

PT and FVII:C were performed with at least two THR of different origin:

* rBT: PT-Fibrinogen HS Plus; Instrumentation Laboratory (IL), THR S; Hemo Medica, STA Neoplastin Plus; Diagnostica Stago.

* Recombinant human: Dade Innovin; Siemens, Recombiplastin 2G; IL

* Human placental: Thromborel; Siemens

* Ox Brain: Thrombotest; Nyagaard Laboratories.

APTT (APTT-SP, IL), Factor deficient plasmas (IL), Coagulometer ACL TOP 300 (IL). Genetic Analysis (GA): ABI PRISM BigDye and ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, Ca, U.S.A.)

Results: PT and FVII:C results are in tables 1 and 2

APTT and factors within the reference range.

P1, P2 and P4 presented lower PT % of activity and levels of FVII:C when tested with rBT compared to THR from human origin, possible FVII-T2.

GA of P1 and P2 reveled Homozygous Mother, Heterozygous Daughter for p.Arg364Gln mutation in exon 8 of FVII gene.

Conclusions: P1, P2 and P4 showed variable results with different THR. P1 and P2 confirmation of FVII Padua variant by GA became the first report of the mutation in Argentina. GA of P4 is pending. P3 and P5 did not show significant results variation with different THR (possible FVII-T1), maintaining the ID. It is important to study patients with at least two THR of different origin to determine which type of variant is present, because this could have therapeutic implications.

TABLE 1 PT (% of activity) with different THRs - (reference range: 70 - 120%)

	HUMAN RECOMBINANT	HUMAN RECOMBINANT	HUMAN PLACENTA	RABBIT BRAIN	RABBIT BRAIN	RABBIT BRAIN	OX BRAIN
Patients	Recombiplastin 2G	Dade Innovin	Thromborel	PT-Fibrinogen HS plus	STA Neoplastine Plus	Tromboplastin S	Thrombotest
P1	65	55	49	18	21	15	96
P2	81	80	89	67	75	73	*
P3	20	*	*	27	*	*	
P4	82	*	*	15	*	*	*
P5	81	*	*	*	67	*	*

TABLE 2 FVII:C with different THRs (Reference range 70 - 120%)

Patients	Recombiplastin 2G	Dade Innovin	Thromborel	PT-Fibrinogen HS plus	STA Neoplastine Plus	Tromboplastina S	FVII:Ag (Elisa) Asserachrom
P1	53	39	29	<1	<1	<1	105
P2	71	70	63	48	45	37	*
P3	5	*	*	5	*	*	*
P4	43	*	*	1	*	*	*
P5	34	*	*	*	40	*	*

PB 1181 | Towards Harmonization of Critical International Normalized Ratio Values Reporting: The Croatian Survey

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Background: Development and implementation of international normalized ratio (INR), reflecting patient´s prothrombin time (PT) adjusted for the reagent/instrument combination, corrected by International Sensitivity Index (ISI) and Mean Normal Prothrombin Time (MNPT), has clearly improved oral anticoagulation therapy (OAT) monitoring. However, critical INR values are variable due to lack of published guidelines for appropriate critical value usage.

Aims: The study aimed to determine how laboratories obtain and report critical INR values.

Methods: Responses related to obtaining and reporting INR results received from 104 hospital and outpatient laboratories participating in a survey conducted in 2015, were analyzed.

Results: An estimated 56.7% respondents use PT reagents from human placenta and rabbit brain, whereas 39.4% respondents use recombinant reagents. ISI value of reagent in use provided 90.4% laboratories (median:0.99, range: 0.84-1.40). Majority of laboratories (72.1%) determine INR values from a direct INR calibration curve, while 26.9% laboratories calculate INR using manufacturer-provided ISI values.Only 12/28 respondents stated how they determine MNPT, mostly using plasma pool from healthy individuals (9/12), and only 1 participant stated how MNPT is calculated (the arithmetic mean). Of all respondents, 50% report INR as a numerical value, regardless the obtained result, whereas 48% report INR as a numerical value greater than certain critical limit (4.0 to 13.0: median >6.7).

Conclusions: Laboratory policies and procedures for obtaining and reporting INR results are highly variable, although almost all laboratories follow recommendation provided by the Croatian Chamber of Medical Biochemists, related to the use of PT reagents with desireable ISI value. As reporting of obtained critical INR value could be confusing to physicians, further efforts should be directed towards harmonization and development of national and international recommendations.

PB 1182 | Improving Laboratory Operational Efficiency with the Implementation of a Specialized Hemostasis Lab Automation

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Background: In 2013 Vall d'Hebron University Hospital Laboratories (VHLabs) merged its operations with two primary care hospital laboratories: Manso and Bon Pastor. In the case of hemostasis, this resulted in an increase in the number of samples in VHLabs while they had to maintain specialization as a reference center and meet demanding turnaround time (TAT) goals.

Aims: To evaluate the impact of a specialized hemostasis lab automation on the hemostasis testing efficiency in the recently consolidated VHLabs.

Methods: The study is a comparison of the operational data from pre-merger VHLabs in 2013 versus data from one consolidated VHLabs following implementation of HemoCell specialized lab automation (Instrumentation Laboratory) from 2013-16. Data was extracted from the LIS and complemented with inputs from the personnel involved in the laboratory. Information was gathered on number of samples analyzed, TAT of each sample, number of analyzers, number of personnel involved in testing, analyzers' maintenance activities and reagent efficiency.

Results: Three years after implementation of specialized hemostasis lab automation (integrated specialty, routine and stat testing), the total number of samples analyzed in VHLabs increased by 47% while TAT decreased by 40%.

In the network, automation also led to a 36% decrease in hemostasis personnel, who were reallocated to other areas of the lab. This led to a 72% increase in samples analyzed/hemostasis laboratorian. In addition, the total number of hemostasis instruments reduced from 8 on different platforms to 3 on one platform, which led to an increase of 206% in samples analyzed/instrument, an estimated 25% increase in reagent efficiency and 89% decrease in maintenance hours/year.

Conclusions: With the installation of the a specialized hemostasis lab automation system, VHLabs was able to absorb the increase in workload due to the merger of three laboratories, meet the TAT goals and maintain specialization as a reference center.

PB 1183 | In Search of a Sensitive and Reliable Laboratory Tool to Assess Hypercoagulability

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Background: Obesity is considered a prothrombotic state, which would increase the risk for thrombotic events: stroke (VTE) and myocardial infarction (MI). The ability of conventional coagulation assays to detect hypercoagulation and to evaluate thrombotic risk is poor. Additionally, traditional coagulation tests give only a partial picture of coagulation. A promising strategy to assess hypercoagulation could be the use of global hemostasis tests.

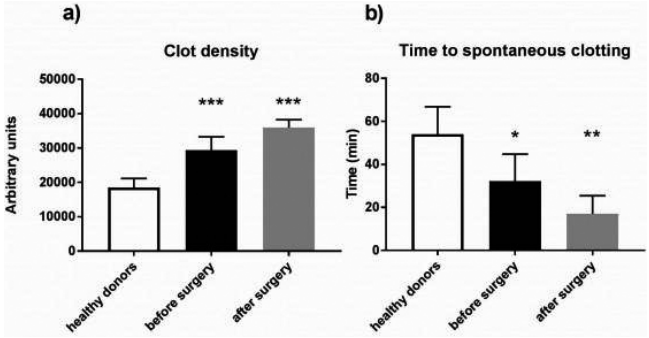

FIGURE 1A and 1b Clot density and time to spontaneous clotting in obese patients versus healthy donors.

Aims: The aim of this study was, using two different global hemostasis tests, to assess the hypercoagulable state of obese patients, before and after bariatric surgery (early postsurgical phase), and to compare it to healthy individuals.

Methods: Whole blood was collected from 12 obese patients with a body mass index ≥ 35 kg/m^2 before and after bariatric surgery and from 5 healthy donors. Propagation of clot growth from a tissue factor (TF)-coated surface and TG were monitored in platelet-poor-plasma (PPP) with a Thrombodynamics analyzer. TF induced thrombin generation (TG) was measured with calibrated automated thrombogram (CAT) in PPP.

Results: All Thrombodynamics parameters describing clot growth formation were significantly increased in obese individuals already at baseline compared to controls, and they further increased after surgery. Clot density increase in obese patients was particularly significant compared to healthy controls ($p < 0.001$); time to the appearance of spontaneous clot was significantly faster in obese patient (Figure 1a and 1b).

Maximum concentration of TG (peak) was significantly increased in obese patients compared to controls. Peak and velocity index were higher post-surgery with both systems tested.

Conclusions: Our preliminary results show that Thrombodynamics has a promising potential as a tool to evaluate hypercoagulability and thrombotic risk. CAT assay confirmed TG Thrombodynamics results in patients before and after surgery. Further investigations are needed in order to correlate assays parameters with clinical phenotypes.

PB 1184 | HIL Interferences in Coagulation Testing: Evaluation of Automatic Check with the New ACL TOP 550 CTS Analyzer

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Background: Preanalytical issues are the most common part of errors in coagulation testing. Spurious hemolysis, lipemia or icterus can jeopardize the quality of results in coagulation testing.

Aims: This study was to evaluate automatic check for HIL interferences offered by pre-analytical module of ACL TOP 550 CTS analyzer (Instrumentation Laboratory, Bedford, USA) in comparison to standard visual inspection of samples.

Methods: 5293 samples were examined during 2 months of routine laboratory work . All samples prior to analysis were visually inspected and noticeable abnormalities in sample color (due to excessive HIL interferences) were recorded. Based on the inspection samples were classified as “rejected” - questionable due to HIL issues or “accepted” - suitable for testing. Samples with clots were not included in the study. Assays included: PT, APTT, Fib, D-Dimer, AT, FVIII, LMWH. Whole set of samples was analyzed with ACL TOP 550 CTS which detected and flagged samples with HIL interferences. Configuration of the analyzer was with HIL limits (reagent and parameter specific) provided by producer.

Results: Of the 5293 samples analyzed 162 were rejected because of HIL visual inspection only. In 43 samples HIL interferences were confirmed by automatic check. Of the 5131 samples qualified visually as acceptable 5 were flagged by analyzer as having HIL interferences for at least one of the assay´s tested. ACL TOP 550 CTS system reduced sample rejection rate because of HIL issues by 70,3% (% of total samples rejected by visual inspection 3.06% (2.6% - 3.6%) vs automatic inspection 0.91% (0.7% - 1.2%);CI95%;p< 0,0001 Fisher X^2 test). Moreover analyzer detected some samples and tests with HIL issues which were not noticed visually.

Conclusions: Automatic check for plasma indices provided by ACL TOP 550 CTS system is objective tool which helps to improve the accuracy of HIL interferences management. It significantly reduces the number of HIL questionable results, unnecessary sample redraws saving both costs and staff time.

PB 1185 | Comparison of Clot Waveform Analysis from Sepsis Patients and Healthy Control Group

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Background: PT and aPTT are parameters mostly asked by physicians for patient management. But in these measurements, the results only show time of plasma to clot. No information about how the clot is form. Clot Waveform Analysis (CWA) will gives us information about the clot formation include the acceleration and deceleration of clotting which may can be used to predict patient‘s condition in severely ill such as sepsis.

Aims: To compare CWA from patients with sepsis and healthy control group.

Methods: Subjects include in control groups are people without signs and symptoms of hemostasis disorders, no drugs consumed include vitamins and contraceptive within last 10 days, and female not in perimenstrual periode. All control subjects should showed normal CBC, ALT, AST, negative CRP and normal hemostasis measured with thrombelastography. Measurement of PT, aPTT and CWA were using

TABLE 1 PT, aPTT and CWA results

	Normal Subjects	Normal Subjects	Sepsis Subjects	Sepsis Subjects	Mann-Whitney p
	Median	Range	Median	Range	
PT(sec)	10.4	9.3 - 11.3	13	10.4 - 30.5	<0,001
Min1	1.62 (mean)	0.342 (SD)	2.19 (mean)	1.15 (SD)	0.0477 *t-test
Max2	0.3	0,2 - 0,4	0.373	0.04 - 0.90	0.018
APTT(sec)	24.3	21.5 - 35.8	33.9	23.4 - 82.1	<0.001
Min1	4.18 (mean)	0.786 (SD)	4.22 (mean)	1.63 (SD)	0.928 *t-test
Min2	0.7	0.5 - 1.0	0.621	0.07 - 1.18	0.234
Max2	0.6	0.4 - 0.9	0.471	0.05 - 0.92	0.004

automated coagulation analyzer CS 2100i. Statistical methods used: normality test, independent t-test, or Mann-Whitney U test. Study was done in Dr Hasan Sadikin Bandung during August until November 2016. This study approved by ethical committee.

Results: There are 33 healthy people recruit for this study and 18 patients diagnosis as sepsis. PT, aPTT and CWA results can be seen in Table 1.

For PT: max coagulation velocity and maximum coagulation deceleration in sepsis significantly longer compare to normal subjects; while for aPTT: maximum coagulation velocity is not significantly different, and for maximum coagulation acceleration and maximum deceleration in normal subjects significantly longer compare to sepsis.

Conclusions: This study showed that from CWA we can observed that PT (extrinsic pathway) is more affected in sepsis patients.

PB 1186 | Impact of New Oral Anticoagulants on Routine Coagulation Assays

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Background: New oral anticoagulants (NOAC), like dabigatran and rivaroxaban, have been recently approved in many countries for primary and secondary prophylaxis of thromboembolic conditions. Monitoring of coagulation function is not routinely required with these drugs but may be useful in emergencies. Routine coagulation tests do not provide enough information on the anticoagulant effect of NOAC and the interpretation of the results obtained in everyday practice is still unsolved.

Aims: The aim of this study was to assess the effect of dabigatran and rivaroxaban on routine coagulation assays: Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT) and the Fibrinogen assay.

Methods: Samples collected at Traumatology Clinic from 43 patients treated with dabigatran (N = 24) and rivaroxaban (N=19) during 2015 were analysed and obtained results on PT, aPTT and fibrinogen were reviewed.

Results: In 24 patients on dabigatran treatment, PT and aPTT values ranged from 0.29-1.11 and 21.9-67.8 s, respectively. A prolonged PT was obtained in 9 (median 0.64; range 0.36-0.66) and aPTT in 12 patients (median 38.3 s range 30.4-67.8). Obtained median INR in patients with prolonged PT was 1.2 (range 1.0-1.8). Low levels of fibrinogen (< 1.8 g/L) were obtained in 4 patients. In 19 patients treated with rivaroxaban PT and aPTT values ranged from 0.45-1.28 and 21.9-39.9 s, respectively. A prolonged PT was obtained in 8 (median 0.53, range 0.36-0.66,) and aPTT in 11 patients (median 34.5s, range 30.4-67.8) whereas low levels of fibrinogen were not obtained in these patients. Obtained INR median value in patients with prolonged PT was 1.1, range 1.0-1.3.

Conclusions: Most of the routine coagulation assays are affected by the application of dabigatran and rivaroxaban, which makes their interpretation a challenge. As an increasing number of patients who use NOACs could be expected, appropriate educational strategies and policies to avoid misinterpretation of routine coagulation test results should be established.

PB 1384 | Evaluation of a New D-dimer Concept for Venous Thrombosis Exclusion: A Prospective Study (FSET Study) ClinicalTrials.gov ID: NCT02523937

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Background: D-dimer (DDi) is the reference test to rule out venous thromboembolism (VTE) in patients with a low or medium probability, due to its high negative predictive value (NPV). However, it cannot be used for its positive predictive value (PPV) since it is also detected in patients with inflammation and hypercoagulability. A new DDi-based assay insensitive to these processes was described to improve VTE diagnosis specificity (ISTH 2017 submitted).

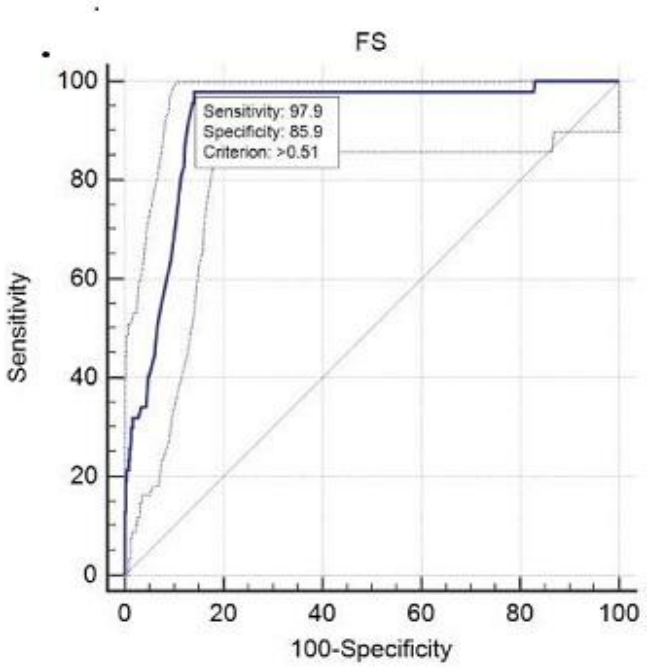

FIGURE 1 ROC curve of the new assay in FSET study

Aims: Evaluate in patients with clinically suspected VTE the diagnostic performances of this assay (sensitivity (Se), specificity (Sp), NPV and PPV) from calculation of the area under the curve in comparison with DDi. Determine the cut off.

Methods: Patients with suspected VTE were prospectively included in Emergency and Internal Medicine Units of one center. VTE was excluded by DDi test in patients with a non-high clinical probability, and confirmed or excluded on the basis of imaging tests and follow up. Blood was taken in patients with no anticoagulant treatment. Citrated plasma was frozen until use. The assay was blindly performed with regard to the diagnosis. An algorithm based on DDi and Fibrin polymerization curve variables was used for calculation and result. Receiver operating characteristic (ROC) curves were used to determine the performances and the cut-off value according to VTE diagnosis.

Results: 628 patients were enrolled (48%>50 years, 19%>75 and 7.5% with cancer). VTE was confirmed in 47 (7.5%). One false negative case was observed in a patient with an isolated subsegmental pulmonary embolism. Test clinical performances from ROC analysis (Se 100%, Sp 85.9%, NPV 99.8% vs 100% for DDi, PPV 35.8% vs 15.6% for DDi at 0.51 µg/mL FEU) are compared to DDi and age-adjusted DDi ones.

Conclusions: In this prospective study in patients with suspected VTE, the new assay demonstrated an increased specificity and PPV with a similar sensitivity and NPV as DDi, resulting in excluding more VTE than DDi and reducing imaging testing.

PB 1385 | A New D-dimer concept for More Specific Detection of Venous Thromboembolism

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Background: D-dimer (DDi) used with pretest probability score (PTP) has excellent negative predictive value to rule out venous thromboembolism (VTE). However due to low specificity, many VTE-suspected patients undergo imaging tests, mainly because of inflammation or hypercoagulability.

We designed a new assay insensitive to inflammation and hypercoagulability to improve VTE diagnosis.

Aims: Evaluate method analytical (reproducibility, sensitivity (Se), specificity (Sp)) and clinical performances without PTP, compared with DDi in a preliminary prospective study on patients with suspected VTE.

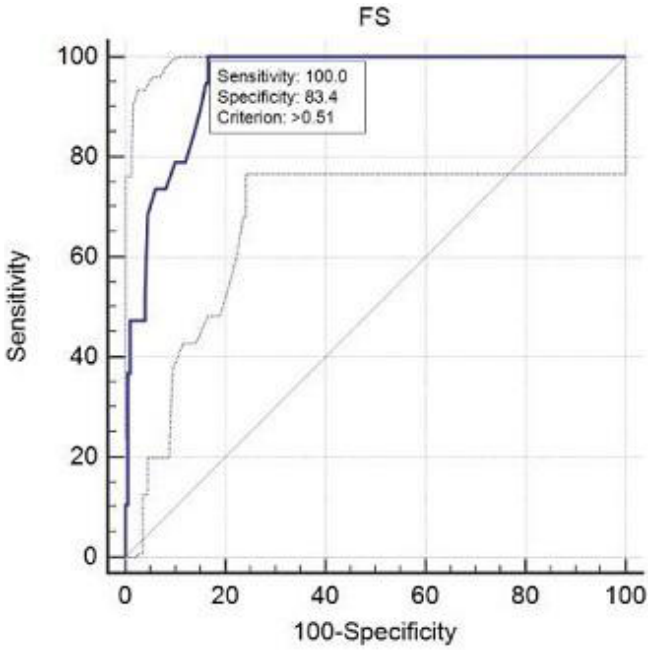

FIGURE 1 New assay ROC curve

TABLE I Clinical performances of the new assay in FSET study (*1 isolated SSPE)

N=628	Number of patients excluded for VTE diagnosis	Sensitivity % [95% CI]	Specificity % [95% CI]	Positive likelihood ratio	Negative likelihood ratio
New assay	500/628 (79.6%)	97.9%* [88.7 - 99.9%]	85.9% [82.8 - 88.6%]	6.93	0.025
D-dimer	329/628 (52.4%)	100% [92.5 - 100%]	56.6% [52.5 - 60.7%]	2.31	0.00
Age-adjusted D-dimer	366/628 (58.3%)	100% [92.5 - 100%]	63.0% [58.9 - 66.9%]	2.70	0.00

TABLE I Clinical performances of the new assay versus regular D-dimer cut-off or D-dimer with age-adjusted cut-off

N=218	Number of patients excluded for VTE diagnosis	Sensitivity % [95% CI]	Specificity % [95% CI]	Positive likelihood ratio	Negative likelihood ratio
New assay	166/218 (76.2%)	100% [82.4 - 100%]	83.2% [77.5 - 88.3%]	6.03	0.00
D-dimer	115/218 (52.8%)	100% [82.4 - 100%]	57.8% [50.6 - 64.7%]	2.37	0.00
Age-adjusted D-dimer	123/218 (56.4%)	100% [82.4 - 100%]	61.8% [54.7 - 68.6%]	2.62	0.00

Methods: Patients with suspected VTE were included prospectively in one center. VTE was excluded by D-dimer testing and confirmed or excluded by compression ultrasonography, lung scanning or spiral computed tomography angiography. The assay, run using frozen citrated plasma on STA® prototypes, combines use of DDi and fibrin polymerization curve variables: Fibrin Formation Time (FFT), Time to reach fibrin polymerization Plateau (TP), Clot Polymerization Rate (CPR), Optical Density variation (OD). Assay result was calculated by an algorithm built in partnership with clinicians using assay parameters that were faced with clinical data in an open clinical pre-study.

Intra/inter-assay reproducibility was studied using normal and hypercoagulable control plasmas (3 prototypes, 5 consecutive days). Receiver operating characteristic (ROC) curves were used to define cut-off value according to VTE status.

Results: 218 patients with VTE suspicion (47% > 50 years, 17% > 75 and 15% with cancer) were tested. VTE was confirmed in 19 (9.4%). Result is available in 10 min. Coefficients of variation (CV) for FFT, TP and CPR were below 6% and below 3% for OD; inter-instrument CV was below 2.5 %. Test clinical performances (Se 100%, Sp 83.4%) are in Fig 1 and Table I.

Conclusions: In this preliminary study, proof of concept was demonstrated. The test looks promising with improved VTE diagnosis specificity leading to imaging testing reduction.

PB 1386 | GDF-15 Alters the expression of Actin Associated Protein, Increases Migration and the Phosphorylation of Smad2 is Mediated via TGFβRII

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Background: The stress induced cytokine GDF-15 is strongly associated with cardiovascular disease.

Aims: Our aim was to investigate GDF-15´s function and the more precise identity of the receptor.

Methods: We have performed a proteomic array, 2-D DIGE, with purified human monocytes after GDF-15 stimulation. GDF-15´s function was investigated with a transwell migration assay. Protein regulation was verified by using Western blot and for receptor studies we used inhibitory chemicals and siRNA together with Western blot.

Results: When human monocytes were stimulated with GDF-15 and oxidative stress the expression of several actin associated proteins but also chaperones and glycolysis proteins were affected. We could confirm the upregulation of WD repeat 1 (WDR1, actin regulator) and T complex protein 1 (TCP-1 ε, chaperone) by Western blot. When performing a migration assay we found an increased migration when THP-1 cells are stimulated with GDF-15 alone, together with oxidative stress and oxidative stress alone. The oxidative stress in itself increases the expression of GDF-15, shown by Western blot, which in turn amplifies the migration. We detected an increased phosphorylation of Smad2 after 15 minutes of GDF-15 stimulation in human monocytes, which was blocked in THP-1 cells with the inhibitors ITD 1 and A 83-01 and by siRNA treatment, indicating that TGFβRII is involved in the binding of GDF-15. When analyzing migration after ITD 1 and A 83-01 treatment the increasing effect seen by GDF-15 is prohibited. The phosphorylation of Smad2 was decreased when stimulating monocytes with the natural existing variant of GDF-15 (H6D).

Conclusions: GDF-15 upregulates the actin associated protein WDR1, the chaperone TCP-1 ε and affects migration. The GDF-15 initiated Smad2 phosphorylation transmits via TGFβRII as shown by inhibitor studies. The natural existing variant of GDF-15 (H6D) decreased the phosphorylation of Smad2 indicating that this mutation would give GDF-15 less potency.

PB 1387 | Validation of Plasma Marker Candidates for VTE Using Affinity and Mass Spectrometry-based Proteomics Indicate a Link to Alternative Complement Pathway

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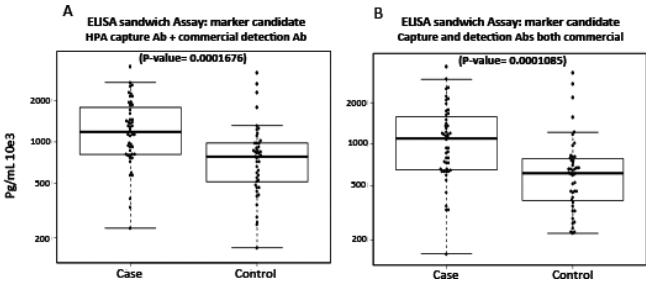

FIGURE 1 Quantitative ELISA-sandwich assay (in-house developed) for the marker candidate using Human Protein Atlas and commercial antibodies

Background: Venous thromboembolism (VTE), has an incidence of 1-2/1,000 year, high mortality and 25% recurrence rate. There is a clear need for improvement in the clinical tools for diagnosis and risk prediction of VTE; D-Dimer, the only plasma marker currently used, has low specificity.

Aims: To identify novel plasma markers for diagnosis and risk prediction of incident and recurrent VTE and to study their relevance to disease development.

Methods: The project is based on the VEBIOS study that includes both patients with acute VTE and patients sampled after discontinuation of anticoagulant treatment following a first-time thrombosis. Plasma samples (n=270) were screened for 760 proteins, using multiplex suspension bead arrays with antibody (Ab) reagents from the Human Protein Atlas Project (HPA). Immunocapture-mass spectrometry (IC-MS) and ELISA-sandwich assays were used to verify and validate the selected targets with significant association with VTE.

Results: 29 proteins were found to be significantly associated with VTE in the cohort that included the patients sampled after discontinuation of anticoagulants (P< 0.01); and 3 proteins in the acute-VTE cohort (P< 0.01). One of these proteins was found to overlap both cohorts. By IC-MS we verified the binding of this target candidate to the Ab used in the discovery. IC-MS analysis also revealed a component of the alternative complement pathway. In plasma the latter protein was found to be associated with acute VTE when measured using two independent quantitative ELISA sandwich assays (48 cases vs 48 controls P=0.0001, Figure1).

Conclusions: This study may yield clinically relevant markers for VTE diagnosis and risk prediction. The results suggest a possible role of the alternative complement pathway in VTE pathobiology.

PB 1388 | Proteomic Profiling to Find Candidate Biomarkers Associated with the Risk of First and Recurrent Venous Thrombosis

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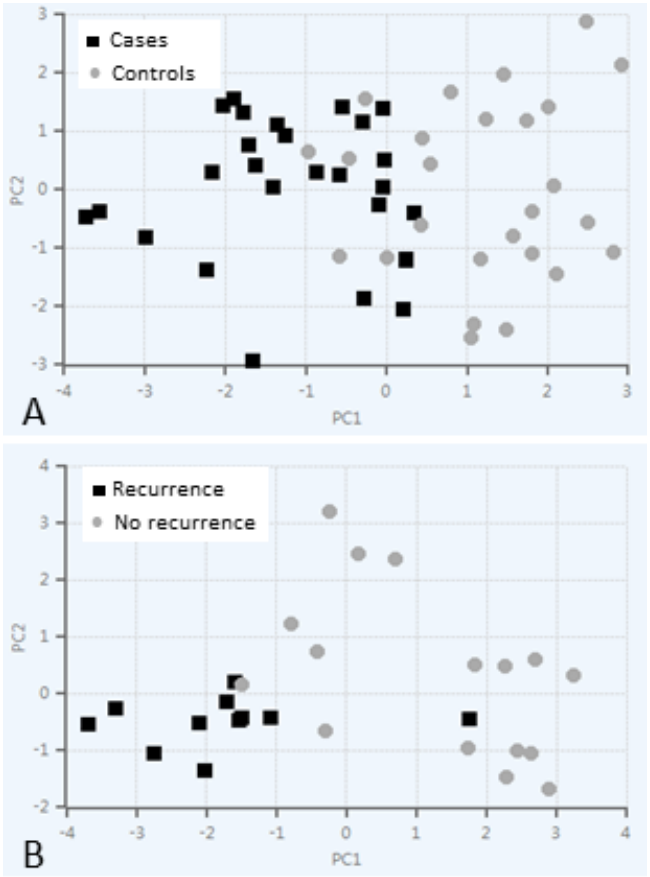

Figure Principal component analysis. Panel A: patients (squares) and controls (circles); Panel B: patients with recurrence (squares) and patients without recurrence (circles)

FIGURE 1

Background: Large-scale proteomic profiling has the potential to identify novel biomarkers that can aid in the assessment of venous thrombosis risk.

Aims: To assess the role of proteomic profiling to find candidate biomarkers associated with the risk of first and recurrent venous thrombosis.

Methods: As a proof-of-principle study, we analysed a sample of THE VTE case-control study on risk factors for venous thrombosis. We selected 27 random control subjects and 27 patients with a first idiopathic venous thrombosis, i.e., no surgery, plaster cast, injury, immobilisation, hormone use, pregnancy, or post-partum period in the three months prior to the event. Furthermore, individuals had no active cancer, normal levels of protein C, protein S and antithrombin (>80 U/dL), and no factor V Leiden or prothrombin 20210A mutation. Citrated plasma samples were obtained 2-3 months after discontinuation of anticoagulation therapy in the cases. Out of the 27 patients, 11 had a recurrent event during subsequent follow-up. We applied an aptamer-based proteomic profiling platform (SOMAscan) that currently measures 1310 proteins.

Results: 119 Proteins were associated with a first venous thrombosis at a significance level of p< 0.05 but due to the small sample size, no tests were significant after correction for multiple testing. Four

proteins were associated with a q-value of 0.05: Immunoglobulin superfamily containing leucine-rich repeat protein 2 (ISLR2), tissue factor pathway inhibitor (TFPI), antithrombin, and Histone-lysine N-methyltransferase EHMT2. Using principal component analysis (PCA) using the top proteins (p< 0.05), we were able to separate the patients and controls and also patients with and without a recurrent event (figure).

Conclusions: This pilot study shows the potential value of using large-scale proteomic profiling to find candidate biomarkers associated with the risk of first and recurrent venous thrombosis.

Supported by: The Merel Foundation and Leiden University Fund/ Nypels van der Zee Fund

PB 1389 | Clinical Determinants of Thrombin Generation Measured in Presence and Absence of Platelets - Results from the Gutenberg Health Study

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Background: The tendency of a plasma sample to generate thrombin, a central enzyme in blood coagulation, might be an important indicator of prothrombotic and haemorrhagic risk.

Aims: To investigate the clinical and laboratory determinants of thrombin generation (TG), measured in platelet rich plasma (PRP) and platelet free plasma (PFP), in individuals from the adult population-based Gutenberg Health Study (GHS).

Methods: Clinical data, standard laboratory markers and TG, investigated in both PRP and PFP at 1pM TF, were available in 407 individuals from the GHS. Endogenous thrombin potential (ETP) and TG_peak were the investigated parameters of a TG curve.

Results: No differences were observed in TG parameters both in PRP and PFP between males and females. The multivariable linear regression analysis, adjusted for age, sex, laboratory markers and antithrombotic treatment, showed that ETP_{PRP} was positively associated to C-reactive protein (CRP,β:64.6[28.2;101],p=0.00056) and negatively to leukocyte count (β:-15.0[-27.0;-3.01],p=0.015). The TG_peak_{PRP} was positively associated to mean platelet volume, MPV, (β:4.97[0.529;9.41],p=0.029) and platelet count (β:0.106[0.04;0.17],p=0.0017). Further, ETP_{PFP} was positively associated to erythrocyte count (β:88.7[13.9;164],p=0.021) and CRP (β:31.5[4.96;58.1], p=0.021) and negatively to cholesterol (p=0.021). TG_peak_{PFP} was negatively associated to cholesterol only (β:-0.182[-0.299;-0.065],p=0.0024). Of traditional cardiovascular risk factors (CVRFs) obesity only was positively associated to ETP_{PRP} (β:218;44.3]131],p=0.0033) in a fully adjusted multivariable model. Antithrombotic agents were strongly and negatively associated to TG in both PRP and PFP (p< 0.0001).

Conclusions: This is the first adult population-based study comprehensively investigating TG from both PRP and PFP samples. Our findings support that TG, particularly in PRP, relates to cardiovascular risk in an apparently healthy population.

PB 1390 | Metabolomic Study in Plasma Samples to Identify a Metabolite Profile Associated to Venous Thromboembolism

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Background: Current laboratory techniques do not identify all individuals at high risk of venous thromboembolism (VTE), therefore there must be unknown thrombotic risk factors that interact with those already known. Metabolomics could be a good method for detecting these new molecules.

Aims: To identify a characteristic metabolomic profile of patients diagnosed with VTE.

Methods: Citrated plasma samples from 20 VTE patients and 20 healthy volunteers from our VTE cohort were selected. Informed consent was obtained from all subjects. Four methods, based on acetonitrile protein precipitation, were compared to establish which of them were able to extract the highest number of metabolites. Metabolites were analyzed by UHPLC-QqTOF-MS/MS, since it offers high separation power and high sensitivity for metabolomic research. In addition, a homemade MS/MS Library was developed to improve the identification of the metabolites. The results were analyzed using an elastic net penalized logistic regression model (R version 3.3.1), to focus the study in the variables that have the discriminant information.

Results: We obtained a list of 33 variables able to distinguish between patients and controls. A prediction graph of class probability including these variables was drawn. This graph showed how both populations are perfectly classified. We identified each variable using our

homemade library. The results showed that various metabolites, such as L-carnitine, are responsible for distinguishing between patients and controls. Consistently, previous studies associated plasma levels of acylcarnitines and the risk of VTE.

Conclusions: Our results suggest that untargeted metabolomic analyses may be useful for identifying new molecules involved in VTE. Further studies in larger cohorts are needed to corroborate these results and to elucidate the implication of these metabolites in the blood clotting system. ISCIII-FEDER (PI14/00512, PI12/00027, RD12/0042/0029, PI14/00079, FI14/00269, CPII15/00002), GVA (PROMETEOII/2015/017), IIS La Fe.

PB 1391 | Prevalence of Deep Vein Thrombosis and Wells Score Accuracy for Inpatient with Suspected Deep Vein Thrombosis

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Background: The Wells score to determine the pretest probability of deep vein thrombosis (DVT) was validated in outpatient settings, but its utility for risk stratification of inpatients it is unclear.

Aims: To establish the prevalence of DVT in inpatients and whether the Wells score applies for risk stratification in inpatients with suspected DVT

Methods: A single centre cross-sectional study has been performed. All the patients from our academic hospital with suspected lower-extremity DVT were evaluated using Wells score and underwent whole leg ultrasound to confirm the presence of DVT. Padua score was also calculated for patients in medical wards.

Results: We enrolled 402 inpatients (males 40%): 302 (75.1%) were hospitalized in medical wards, 100 (24.9%) in surgical wards. Age was 76.9±14.4 y and mean duration of hospitalization was 5.6 days. We found 82 (20.4%) DVTs: 31 proximal DVTs (7.7%) and 51 (12.7%) isolated distal DVTs. DVT incidence in low, moderate, and high pretest probability groups was 10.1%, 24.1%, and 37.7%, respectively (P≤0.001). The area under the receiver operating characteristics curve (AUC) for the discriminatory accuracy of the Wells score for risk of DVT identified on whole leg ultrasound ultrasound was 0.65±0.03. When only proximal DVTs were considered the AUC was 0.69±0.05. The percentage of subjects receiving heparin thromboprophylaxis was similar in patients with a high and a low Padua score (53.4% vs. 58.9% p=0.526). DVT incidence in low and high probability groups according to Padua score was 10.2% and 27.3%, respectively (P≤0.001), and did not change after excluding patients receiving thromboprophylaxis (9.1% vs. 31.4%, respectively, p=0.001).

Conclusions: The accuracy of the Wells score for inpatients was lower compared with that reported in the outpatient literature. Therefore, the Wells score risk stratification is not sufficient to rule out DVT in the inpatient setting. In our series, DVT incidence was not negligible in patients with low Padua Score.

PB 1392 | Impact of Chronic Inflammation, Assessed by C-reactive Protein, on the Association between Red Cell Distribution Width and Risk of Incident Venous Thromboembolism

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Background: Red cell distribution width (RDW), a quantitative measure of variability in size of erythrocytes, is associated with risk of venous thromboembolism (VTE). The mechanism(s) underlying the association remains unsettled. RDW is a marker of pro-inflammatory conditions, and it is suggested that the association between RDW and VTE risk is mediated by inflammation.

Aims: To investigate whether inflammation, assessed by C-reactive protein (CRP), is the underlying link between RDW and VTE in a nested case-control study.

Methods: The study included 202 cases with incident VTE and 496 age- and sex-matched controls sampled from a general population-based study (the Tromsø Study 1994-2012). Baseline information, including RDW, was obtained in 1994/95. The plasma concentration of high-sensitivity CRP was determined by ELISA. RDW was categorized into tertiles (≤12.5, 12.6-13.1 and ≥13.2%), and CRP levels were categorized as low (< 1 ml/L), intermediate (1-3 mg/L) or high (≥3 mg/L). Multivariable logistic regression was performed to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE by categories of RDW and CRP, and for combined exposures (high RDW and high CRP).

Results: RDW was associated with VTE, and the OR was 1.58 (95% CI 1.02-2.45) for the highest versus the lowest tertile of RDW. The risk estimate remained unchanged after adjustment for CRP. CRP correlated with RDW (Pearson's correlation coefficient: 0.15, p< 0.001), and subjects with a high CRP-concentration (≥3 mg/L) had a greater risk of VTE than subjects with low CRP-concentration (< 1 ml/L) (OR 1.46, 95% CI 0.92-2.29). The risk of VTE increased further for subjects with both high RDW and CRP (OR 3.06, 95% CI 1.48-6.30) when compared to those with low RDW and CRP. The combined estimate did not exceed the sum of the individual components.

Conclusions: Our findings suggest that chronic inflammation is not a mediator of the association between RDW and VTE risk. A combination of high RDW and CRP had an additive effect on VTE risk.

PB 1393 | Improved Exclusion of the Pulmonary Embolism Diagnosis in the Emergency Department Using a New D-dimer-Based Assay

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Background: D-dimer (DDi) testing excludes pulmonary embolism (PE) in patients with non-high clinical probability but its specificity is limited. A new assay is proposed with improved specificity (ISTH 2017 submitted).

Aims: To assess diagnostic performances of a new DDi-based concept in patients with suspected PE.

Methods: Consecutive outpatients admitted to the emergency department of a single center for a clinically suspected PE were included provided they did not receive anticoagulant treatment. The reference test was performed as follows. PE was excluded on the basis of a negative DDi test or of a negative computed tomography pulmonary angiography (CTPA) according to clinical probability and a 3-month follow-up without venous thromboembolism (VTE). PE was diagnosed on the basis of a positive CTPA or of an objectively confirmed VTE during follow-up. The new test was performed on citrated frozen plasma from blood taken on admission. Test diagnostic value was evaluated based on the 0.51 µg/mL cut-off value determined in another patients´ sample. The assay was performed blindly from clinical context, results of diagnostic tests and follow-up data. Patients were managed without knowledge of the new test result.

Results: As of January 27th 2017, 448 of the 500 planned patients were included: 186 men (41.5%), mean age 53.3 ± 19.3 years. Clinical probability was low in 43.3%, intermediate in 54.1% and high in 2.6%. PE was confirmed in 41 (9.8%) patients. Results of the assay were available for 387 patients. Sensitivity was 100% (95% confidence interval (CI): 90.6-100.0), specificity, 82.9% (78.6 - 86.4%), positive predictive value, 38.1% (29.1 - 48.1%) and negative predictive value, 100% (98.7 - 100.0%). Results among the 500 planned patients will be presented at the meeting.

Conclusions: If these results are confirmed in a multicenter setting, this assay could help to safely exclude PE in a large number of patients.

PB 1394 | Plasma Leptin is Not a Mediator of the Association between Obesity and Risk of Venous Thromboembolism

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Background: The underlying mechanism(s) for the strong and consistent association between obesity and risk of venous thromboembolism (VTE) is unknown. Leptin, a hormone synthesized and released by adipose tissue, is increased in obesity and demonstrated to have prothrombotic properties. No previous study has investigated whether plasma leptin is associated with future VTE risk or modulates the VTE risk by obesity.

Aims: To investigate whether plasma leptin is associated with future risk of VTE or a mediator of VTE risk by obesity in a nested case-control study recruited from the general population.

Methods: We performed a nested case-control study of 205 VTE cases and 502 age- and sex-matched controls recruited from a general population-based study (The Tromsø study). Body mass index (BMI) was measured at baseline, and leptin concentration was measured in thawed samples using ELISA. Logistic regression models were used to calculate ORs according to tertiles of leptin (< 10.6, 10.6-23.5, >23.5 ng/mL) and BMI (< 24.2, 24.2-27.2, >27.2 kg/m^2).

Results: Leptin levels correlated significantly with BMI (r=0.59, p< 0.001), but were not associated with risk of VTE (OR upper versus lower tertile: 1.22, 95% CI 0.73-2.06). Subjects with BMI in the upper tertile had a 1.9-fold (95% CI 1.2-2.9) higher risk of VTE than those in the lower tertile. This risk estimate increased to 2.1 (95% 1.3-3.5) after adjustment for leptin levels. A combination of high BMI and low leptin levels was associated with the highest risk of VTE. Subjects within the upper tertile of BMI and low- or mid-tertiles of leptin had a 2.7-fold (95% CI 1.2-6.1) and 3.1-fold (95% CI 1.6-6.0) increased risk of VTE, respectively, when compared to those within the lowest tertiles of BMI and leptin.

TABLE 1 Odds ratios with 95 % CIs for VTE according to tertiles of BMI and plasma leptin

BMI (tertiles, kg/m2)	Leptin (tertiles, ng/mL)	Controls	Cases	OR (95% CI)
<24.2	<10.6	87	21	Reference
<24.2	10.6-23.5	59	23	1.59 (0.76-3.34)
<24.2	>23.5	16	3	0.75 (0.20-2.93)
24.2-27.2	<10.6	55	21	1.59 (0.79-3.20)
24.2-27.2	10.6-23.5	68	29	1.73 (0.89-3.34)
24.2-27.2	>23.5	44	13	1.19 (0.51-2.74)
>27.2	<10.6	23	15	2.71 (1.20-6.08)
>27.2	10.6-23.5	44	33	3.08 (1.59-5.96)
>27.2	>23.5	104	46	1.76 (0.91-3.41)

TABLE 2 Odds ratios for VTE according to the presence of obesity (yes/no) and plasma leption below/above the median

Obesity (BMI> 30 kg/m2)	Leptin (>15.2 ng/mL)	Controls	Cases	OR (95% CI)
No	No	246	88	Reference
No	Yes	193	71	1.15 (0.74-1.81)
Yes	No	5	9	5.04 (1.64-15.46)
Yes	Yes	56	36	1.99 (1.15-3.42)

Conclusions: Our findings suggest that plasma levels of leptin neither was associated with VTE, nor mediated the VTE risk by obesity. However, subjects with dysregulation of leptin, i.e. high BMI and low leptin levels, appeared to have the highest VTE risk.

PB 1395 | Identifying a Common Biochemical Background for Thrombotic Events in Patients with Deep Vein Thrombosis (DVT) and Peripheral Artery Disease (PAD)

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Background: There is an increased risk for atherothrombosis following DVT. The role of neutrophil activation as contributor to arterial thrombosis in non-acute DVT is unknown.

Aims: Elucidate the role of neutrophils in patients with non-acute DVT in comparison with patients with PAD, a high risk population for atherothrombosis .

Methods: We studied 115 patients from two cohorts (75 DVT, 40 PAD). For PAD 20 patients with progressive disease, for DVT 25 patients with recurrence and 25 with Post thrombotic syndrome (PTS). Control patients were age and sex matched. Markers of neutrophil recruitment (P-selectin), activation (nucleosomes,human neutrophil elastase- **α1**anti-trypsin (HNE-AT)) and anti-inflammation (Lipoxin A4)

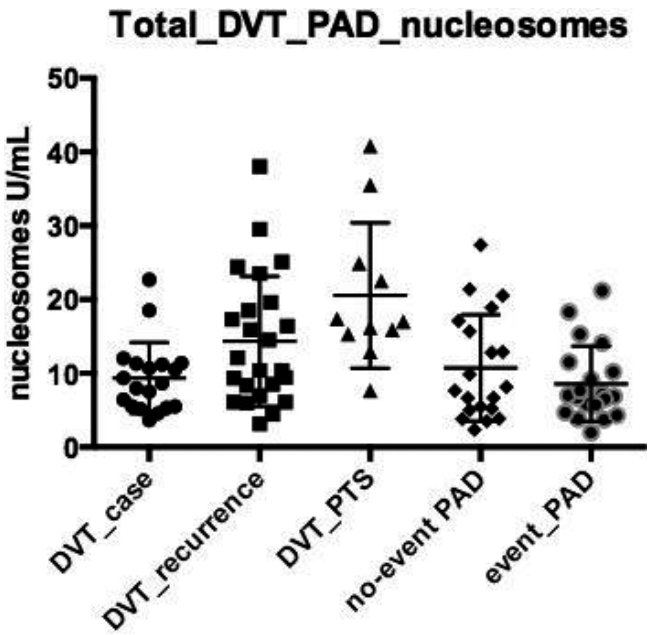

FIGURE 1 Nucleosomes

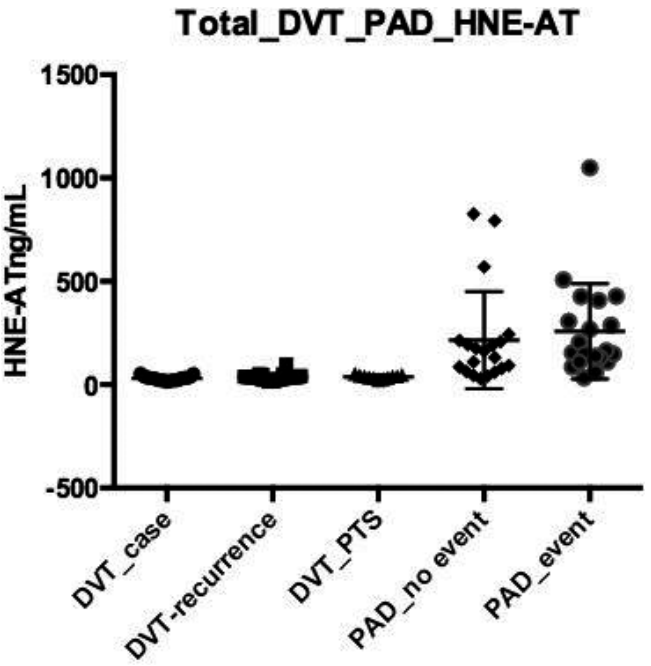

FIGURE 2 HNE-AT

were measured with ELISA. Coagulation was analysed by thrombin generation (CAT method) and D-dimer.

Results: P selectin was not different in PAD vs.DVT (p0.08). Nucleosomes differed between PAD and DVT: 7.1 (5.1-13.8) vs. 11.3 (7.4-17.7), p0.008, and were borderline significant in recurrent DVT (11.3 (7.3-19.3) vs. no recurrence 8.7(5.5-11.3), p0.07 and higher in PTS 17(15.3-14.9), p0.0002. HNE-AT was higher in PAD compared to DVT: 158(88.1-283) vs. 33.4(23.5-40.5), p< 0.0001; in PTS levels were higher: 40.4(31.3-44.6) vs. DVT without recurrence 31.1 (20.6-35.9), p=0.046. Lipoxin A4 was higher in PAD vs DVT: 35.6 (16.6-80.1) vs. 2.4(1.7-4.8), p< 0.001, but not different within DVT. TG was significantly higher for PAD compared to DVT: p=0.003. TG peak was higher in recurrent DVT: 174.5±26.0 vs. 259.6±35.3, p0.059. D-dimer levels were higher in PAD vs. DVT 550 (369-959) vs. 330 (220-550), p0.003. D-dimer was higher in both recurrent DVT (580(330-1950), p 0.008 and PTS (380(257-500), p0.0013 vs DVT without recurrence 225(199-297). D-dimer was not correlated with nucleosomes or HNE-AT.

Conclusions: Neutrophil activity is 5-fold higher in PAD and is hardly detectable in DVT. This suggests that mechanisms for atherothrombosis in DVT are unlikely to depend on neutrophil activity.

PB 1396 | Plasma Levels of miRNAs Are Associated with Risk of Cancer-related Venous Thromboembolism

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Background: Venous thromboembolism (VTE) is a serious complication in patients with malignancies. Current markers do not allow accurate prediction of the majority of VTEs in cancer patients. Thus, there is a need to identify novel biomarkers for the risk of cancer-associated VTE.

Aims: To investigate whether the plasma miRNAs are differentially expressed in subjects who develop cancer with or without concurrent VTE during follow-up.

Methods: Participants were recruited from the Tromsø VI study. We performed a pilot nested case-control study including six subjects who developed cancer and eight subjects who developed both cancer and VTE within the first year after blood sampling. Total RNA was extracted from plasma using the miRNeasy Mini kit (Qiagen, USA) with modifications. Profiling of 179 miRNAs was conducted on the Serum/Plasma Focus MicroRNA PCR Panel (Exiqon, Denmark). miRNAs expression levels were normalized by the expression of miR-425-5p as suggested by BestRef. Statistical analyses were performed using R software (v3.2.3). Logistic regression analysis with backward selection was used to build a predictive model of risk of cancer-related VTE. The study was approved by the regional ethical committee and all participants gave their informed written consent.

Results: High quality signals of 90 miRNAs were found in all samples in both study groups. We determined three microRNAs (miR-133a-3p, miR-324-3p, and miR-421) as possible predictors of VTE in patients with malignancies. We identified the target proteins of these miRNAs.

Conclusions: In this pilot nested case-control study, we found that three plasma miRNAs could be promising markers of future risk of VTE in cancer patients. Our findings will be validated in a larger cohort.

PB 1397 | A Global Metabolomic Analysis Comparing Low-risk and High-Risk Pulmonary Embolism

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Background: To improve our understanding of the physiology of PE, we performed the first-ever global metabolomic study comparing low- and high-risk PE.

Aims: To identify metabolomic markers associated with high risk PE.

Methods: We studied 92 patients diagnosed with acute PE in an academic emergency department from 2009-12. We compared 46 high-risk PE patients with 46 age, sex and cancer-status matched low-risk PE patients. High-risk PE were lobar or more proximal, with right heart strain or a positive troponin. Low-risk PE had segmental or smaller PE, no right heart strain, negative troponin, no clinical deterioration within 5 days and no 30-day death. Blood samples were drawn within 24 hrs of PE diagnosis and processed within 60 min. We performed global metabolomic analysis using the Metabolon® DiscoveryHD4™ platform. We used Welch's two-sample *t*-test to identify biochemicals that differed across groups and Fisher's Exact Test for Metabolite Set Enrichment Analysis (MSEA) to determine pathways/metabolite categories driving differences. We calculated false discovery rates (FDR) to account for multiple comparisons.

Results: 42 metabolites differed significantly between low- and high-risk PE at p-values ≤0.05, after FDR correction. The top three are: picolinate, xanthosine and alpha-ketoglutarate. We performed a first MSEA at a global level, identifying the categories Nucleotide and Energy to be significantly enriched (FDR ≤0.05). The Amino Acid category had an FDR = 0.1. A second MSEA, focused on sub-categories, identified Fatty Acid Metabolism (Acyl Carnitine), Purine Metabolism, (Hypo)Xanthine/Inosine containing and TCA cycle at FDR ≤0.05. Methionine, Cysteine, SAM and Taurine Metabolism and Polyamine Metabolism had a FDR = 0.09.

Conclusions: Our results are the first to show that metabolites linked to energy homeostasis, polyamine synthesis, purine turnover, transsulfuration, and protein turnover differ between low- and high-risk PE patients. These findings may be useful in risk-stratifying PE.

PB 1398 | Changes in the Fibrinolytic Status and Cardiovascular Profile in Obese Patients Undergoing Bariatric Surgery

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Background: Obesity is characterized by chronic inflammation, impaired fibrinolysis and the presence of cardiovascular risk factors (CVRF). Proinflammatory cytokines contribute to endothelial dysfunction and an increase in the expression of plasminogen activator inhibitor-1 (PAI-1) and thrombin activatable fibrinolysis inhibitor (TAFI). Bariatric surgery induces and improvement in the haemostasis and the CVRF. However, it is not well-known how these markers are modified after the bariatric surgery.

Aims:

- To assess the changes in PAI-1 and functional TAFI once completing the fasting phase and after the bariatric surgery
- To evaluate the impact in the prothrombotic state induced by the obesity.

Methods: We designed a prospective study including obese patients undergoing bariatric surgery in our institution from May 2014 to July 2015. All patients have signed the informed consent and the study was approved by the local ethics committee. We have obtained blood samples for PAI-1 and TAFI study at enrolment, once completing the fasting phase, 1 month and 6 months after the bariatric surgery. In addition, we have evaluated the main CVRF at inclusion and 6 months after the surgery. All the patients received antithrombotic prophylaxis with pneumatic compression and enoxaparin 60 mg once daily

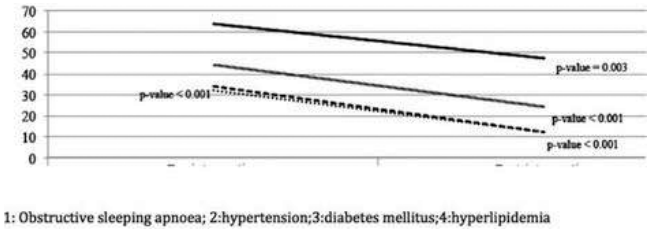

FIGURE 1 Changes in CVRF after the bariatric surgery

for 1 month following the operation. A p value< 0.05 had statistical significance.

Results: We studied 91 patients with a mean age of 45±10.7 years (72% women). The mean body mass index was 49.7 ±7.2kg/m^2. We have observed a reduction in PAI-1 along the visits (p=0.009). In the same way, a TAFI reduction not only after the fasting phase but after the surgery has been detected (p< 0.001). No thrombosis has been documented. The CVRF strongly improved after the surgery (p< 0,001) (see figure).

Conclusions: The massive loose weight implies an improvement in the fibrinolytic balance and a correction of CVRF in a high number of patients. These changes contribute to a reduction in the thrombotic risk development.

PB 1399 | Evaluation of Risk Factors for Postpartum Hemorrhage after Vaginal Delivery in Uncomplicated Pregnancy

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Background: Postpartum hemorrhage (PPH) is the leading cause of maternal morbidity after a vaginal delivery. As most coagulation factors increase during normal pregnancy, the prothrombin time (PT) and the activated partial thromboplastin time may be decreased, but the association between PPH and the coagulation profile is unclear.

Aims: The present study aimed to identify risk factors for PPH in uncomplicated pregnancies.

Methods: Our retrospective cohort study included 396 pregnant women without complications from two institutions between Jan 1, 2015 and Dec 31, 2016. The logistic regression model was used to evaluate univariate and independent multivariate associations of the clinical parameters with PPH
(blood loss >800 ml).

Results: The incidence of PPH > 800ml was 11.6% (46/396). Univariate analysis using the logistic regression model revealed that PT/INR at 36-37 weeks of gestation, multipara, birth weight > 3000g, and episiotomy or laceration were significantly associated with PPH. Multivariate analysis revealed that independent risk factors for PPH were PT/INR, episiotomy or laceration, and birth weight (p= 0.0022, 0.0032, and 0.0132, respectively). Based on receiver operating characteristic analysis, the cut-off value of PT/INR level to predict PPH >800ml was 1.03.

Conclusions: The findings of the present study indicated that risk factors for PPH > 800ml were PT/INR at 36-37 weeks of gestation, episiotomy or laceration, and birth weight > 3000 g. A PT/INR value at 36-37gestational weeks > 1.03 is an independent predictor of severe PPH.

PB 1400 | Analysis of the Substrate Specificity of Factor VII Activating Protease (FSAP) and Design of a Specific and Sensitive Peptide Substrate

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Background: Factor VII (FVII) activating protease (FSAP) is a circulating serine protease which is likely to be involved in a number of disease conditions such as stroke, atherosclerosis, liver fibrosis, thrombosis and cancer. To date, no systematic information is available about the substrate specificity of FSAP.

Aims: To characterize the substrate specificity of FSAP using multiple approaches and to design specific and sensitive FSAP probes to measure its activity in plasma.

Methods: We have characterized the specificity of FSAP towards small peptides using phage display and positional scanning substrate combinatorial library (PS-SCL) approach. Results were compared with known protein substrates and molecular modelling of the peptides in the active site of FSAP was performed. Novel fluorescent substrates were tested for sensitivity, specificity and suitability for the measurement of endogenous FSAP activity in plasma.

Results: The representative FSAP-cleaved sequence obtained from the phage display method was Val-Leu-Lys-Arg-Ser (P4-P1'). The sequence X-Lys-Nle-Arg (P4-P1) was derived from the PS-SCL method. These results show a predilection for cleavage at a cluster of basic amino acids on the nonprime side. Molecular modelling studies showed a preference for Arg over Lys at the P1 site. Based on these data a fluorescent substrate (Ala-Lys-Nle-Arg-AMC) was synthesized with a 10-fold higher selectivity for FSAP compared to other proteases from hemostasis. This substrate could be used to measure FSAP activity in plasma. In histone-treated plasma FSAP was strongly activated as validated by the use of an inhibitory antibody.

Conclusions: Natural FSAP substrates as well as small peptide substrates are preferentially cleaved in regions rich in basic amino acids. Development of FSAP activity probes shows a specific activation by histones that may contribute to the latter's hemostasis-related effects. These novel data will help to elucidate the role of FSAP in vivo.

PB 1401 | Von Willebrand Factor Propeptide May Identify the Patients Susceptible for Renal Dysfunction in Collagen Disease

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Background: VWF propeptide（VWF pp）is cleaved from mature von Willebrand factor (VWF) just before secretion and released from endothelial cell. Since VWF pp has a shorter half-life and is less involved in maintaining physiological homeostasis than VWF, it has been proposed to be a better predictor of acute vascular endothelial dysfunction.

Aims: To investigate whether VWF pp levels discriminate among collagen diseases and predict subsequent renal dysfunction.

Methods: The patients diagnosed as collagen disease from January 2007 to March 2015 were investigated. We evaluated the plasma levels of VWF pp, VWF antigen (VWF), ADAMTS13 antigen (ADAMTS13) at diagnosis using ELISA, and subsequent renal function including eGFR and urine findings (protein and hematuria).

Results: Sixty eight patients were diagnosed as collagen diseased; systemic lupus erythematosus (SLE, n=10), Systemic scleroderma (SSc, n=12), Sjögren´s syndrome (SS, n=19), rheumatoid arthritis (RA, n=27). Mean value of VWF pp was 329±85% (mean ± SD) in SLE, 195±63% in SSc, 179±60% in SS, 186±120% in RA. VWF pp in SLE was significantly higher than that in others, but there were no differences among groups in VWF and ADAMTS13. Also, higher levels of VWF pp were observed in patients who developed renal dysfunction including reduced eGFR by over 25%, proteinuria, or hematuria.

Conclusions: We speculated that increased plasma VWF pp levels in SLE may reflect vascular endothelial dysfunction due to vasculitis, and found that VWF pp may be a more sensitive early indicator of renal dysfunction than VWF and ADAMTS13. VWF pp may identify the patients with vasculitis and susceptible for renal dysfunction in collagen disease.

PB 1402 | Age-related Diagnostic Value of D-dimer Testing and the Role of Inflammation in Patients with Suspected Deep Vein Thrombosis - Results from the VTEval Study

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Background: While previous reports have focused on the impact of age on D-Dimer testing in elderly individuals with suspected deep vein thrombosis (DVT), data on the age-related diagnostic value of D-dimer in a sample covering a broad age range are limited.

Aims: To assess the age-dependent diagnostic performance of D-dimer in patients with suspected DVT.

Methods: In the VTEval project (NCT02156401), patients ≥18 years with suspected DVT underwent a standardized investigation plan including assessment of biomarker concentrations and compression duplex ultrasound. In the present study, the diagnostic accuracy of D-dimer was investigated in young and elderly patients and compared to C-reactive protein (CRP).

Results: In 231 of 500 patients, proximal DVT (N=111) and isolated distal DVT (N=120) were diagnosed. Sensitivity of D-dimer was lower in patients < 60 years in comparison to patients ≥60 years (Δ-16.8%), whereas specificity was 27.9% higher. In patients < 60, lowest sensitivity was detected for female sex (69.0%, 95% confidence interval (CI) 52.9%/82.4%), unprovoked DVT (69.0%, 95%CI 52.9%/82.4%) and low thrombotic burden (61.1%, 95%CI 46.9%/74.1%), and distal DVT (60.9%, 95%CI 45.4%/74.9%). In contrast to the established cut-off (0.5mg/L FEU) and an age-dependent threshold (age/100mg/L FEU), a fixed D-dimer threshold of 0.25mg/L FEU resulted in elevated sensitivity for patients < 60 with a reduction of false negatives by 40.0% for proximal DVT and by 50.0% for distal DVT. D-dimer and CRP demonstrated comparable diagnostic performance for proximal ($AUC_{D\text{-}dimer}$=0.843 vs. AUC_{CRP}=0.832) and distal DVT ($AUC_{D\text{-}dimer}$=0.674 vs. AUC_{CRP}=0.667) in patients < 60 years.

Conclusions: This study highlights clinically-relevant limitations of D-dimer testing in young patients with suspected DVT and the role of inflammation in venous thrombosis.

PB 1404 | Characterization of a Transient Procoagulant Phenotype Induced by Recombinant Activated Factor VII (rFVIIa)

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Background: In a significant proportion of patients with unprovoked venous thromboembolism (VTE) no laboratory risk factors are found. Monitoring a standardized and limited activation of coagulation in these patients could presumably help to identify intermediary phenotypes in VTE. Promising biomarkers to detect subclinical changes of the hemostatic activation status are plasma levels of active enzymes including thrombin and activated protein C (APC).

Aims: To characterize a physiological phenotype of coagulation activation in healthy individuals as a prerequisite for the characterization of a thrombophilic phenotype.

Methods: Limited activation of extrinsic hemostasis was induced by i.v. injection of rFVIIa (15 µg/kg) into 12 healthy volunteers (6 males). rFVIIa-induced hemostatic activation was monitored by measuring plasma levels of free thrombin and APC at baseline and repeatedly during a 24 h lasting follow-up period using oligonucleotide-based enzyme capture assays (OECA). In addition, prothrombin fragment F1+2 (F1+2), thrombin-antithrombin complex, plasmin-α2-antiplasmin complex, soluble fibrin monomer (sFM), and D-dimer were determined.

Results: rFVIIa was well tolerated, and its elimination kinetics showed an expected course in all probands. Median APC plasma levels of 35 (IQR: 28-68) pg/ml were measured at baseline. Following rFVIIa injection, a consistent and highly significant increase of APC was observed in samples taken between t=10 min and t=5h with a peak of 164 (139-194) pg/ml at t=1h (p=0.0003). Among the other biomarkers, only F1+2 showed an increase from baseline (125, 87-188 pmol/l) that became statistically significant (p=0.03) at t=2h (179, 111-200 pmol/l). Levels of D-dimer and sFM remained unchanged and within normal ranges at all sampling points.

Conclusions: APC appears to be a promising candidate to discriminate between a physiological and a thrombophilic response to rFVIIa-induced hemostatic activation, which we currently evaluate in carriers of thrombophilic mutations.

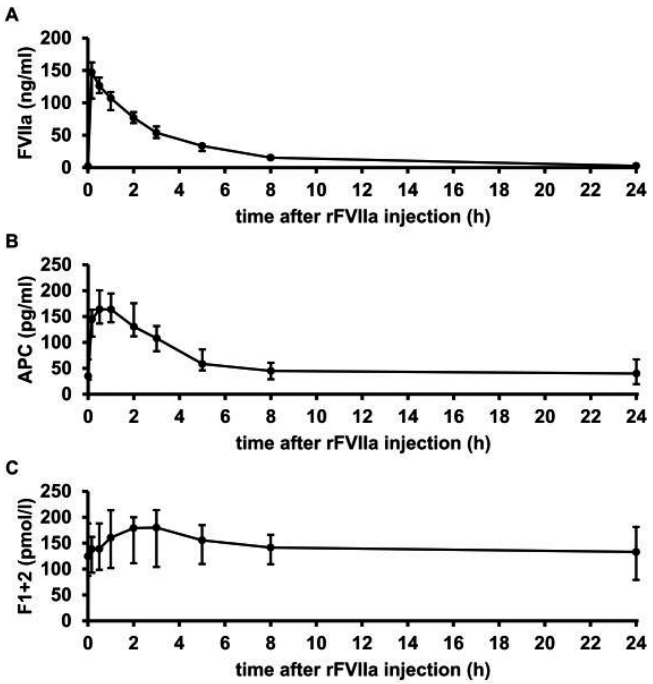

Fig.1: Plasma levels (median, IQR) of (**A**) FVIIa, (**B**) APC, and (**C**) F1+2 in 12 healthy subjects before (t = 0) and after injection of 15 µg/kg rFVIIa.

FIGURE 1 Plasma levels of FVIIa, APC, and F1+2 in healthy subjects before and after injection of rFVIIa

PB 1405 | The Effects of Shear Stress on Platelet Activation and Incipient Clot Characteristics: Exploring the Mechanisms of Shear Induced Clot Formation

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Background: Shear flow is known to modulate the kinetics of fibrin polymerization leading to changes in clot microstructure. It has been widely reported that clot microstructure plays a pivotal role in a variety of diseased states and dictates susceptibility to fibrinolysis. A possible mechanism for altered clot microstructure as a consequence of shearing is its direct effect on individual platelets that provide a surface for enhanced thrombin generation.

Aims: The objective of the study was to investigate the links between platelet activation and resultant clot microstructure in blood that has been exposed (in vitro) to shear stresses, the latter being assessed by a novel rheological biomarker. This aims to provide insight into the mechanisms of clotting during its earliest stage of formation.

Methods: Samples of human blood, Platelet Rich Plasma and Platelet Poor Plasma were subjected to different levels of shear stress for fixed periods using an AR-G2 Rheometer. Flow cytometry was used to measure platelet activation. Rheometry was further utilised to measure incipient clot formation, in terms of a fractal dimension (a biomarker of clot complexity) in order to quantify the microstructural characteristics of the underlying fibrin network.

Results: The results show that subjecting blood to relatively high levels of shear rate (>1000 s-1) leads to a decrease in the time to incipient clot formation, an increase in fractal dimension and an increase in platelet activation. However, at intermediate levels of shear rate (*ca* 100 s-1 to 1000 s-1), an increase in fractal dimension is observed in the absence of any increase in platelet activation.

Conclusions: Alternative mechanisms other than enhanced activation of platelets may be responsible for differences in incipient clot formation as a result of exposure of blood to shear. The results have importance for understanding clotting in blood that has been subjected to elevated levels of shear such as in diseased states associated with atherosclerosis and in stenotic vessels.

PB 1406 | VWF and ADAMTS13 Levels in Early Onset Preeclampsia: Prothrombotic Mechanisms in Mothers with Elevated Risk of Venous Thromboembolism

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Background: Preeclampsia (PE) is a serious complication of pregnancy with potentially life-threatening consequences for both mother and baby. Early onset preeclampsia (EOPE; onset < 34 gestational weeks), is associated with higher maternal and fetal risks than late onset PE. The risk of venous thromboembolism is increased, particularly in severe PE. Consequently, mothers may be considered for thromboprophylaxis. However, risk assessment may be challenging due to complicated by competing bleeding risks such as placental abruption, post-partum haemorrhage, or renal impairment.

Aims: To characterise the relationship between VWF and ADAMTS13 levels in EOPE patients in order to better understand parameters that may modulate thrombotic and bleeding risk.

Methods: Plasma samples obtained from healthy pregnancy (HP) or PE were compared to healthy control (HC) females. Plasma VWF and ADAMTS13 levels were determined by VWF and ADAMTS13 ELISA. ADAMTS13 activity was measured using FRETS73.

Results: Mean plasma VWF levels were significantly (p=0.0001) elevated in pregnancy and further significantly (p=0.0008) increased in PE (HC = 110%, HP = 240%, PE = 380%). In contrast, plasma ADAMTS13 levels were significantly decreased in pregnancy (p=0.0019) and PE (p=0.05) when compared to HCs. (HC = 100%, HP = 36%, PE = 20%). While severity of PE did not correlate with VWF levels, the lowest ADAMTS13 levels and activity was observed in patients with severe PE. Moreover the VWF/ADAMTS13 was calculated to be lowest in patients with severe PE.

Conclusions: PE is associated with highly elevated plasma VWF and decreased ADAMTS13 levels and activity. In combination with other parameters including thrombin generation and platelet function, these data are of relevance to understanding overall haemostatic balance in high-risk pregnant mothers with EOPE.

PB 1407 | Changes in Thrombin Generation in Obese Patients Undergoing Bariatric Surgery: Improvement in the Hypercoagulable Status

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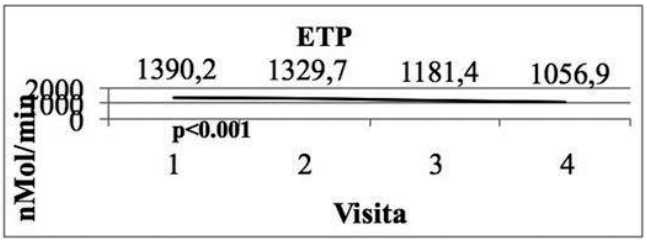

FIGURE 1 Changes in ETP

Background: Obesity modifies the haemostasis, leading to a hypercoagulable state. Bariatric surgery induces a massive loose weight, improves cardiovascular risk factors and the haemostatic parameters. These changes imply an improvement in the inflammation status, and haemodinamic parameters as well, reducing the cardiovascular events.

Aims: The aim of this study is to evaluate the changes in the thrombin generation parameters and F1+2 prothrombin fragment once completing the fasting phase and after the bariatric surgery.

Methods: We have designed a prospective study including obese patients candidates to bariatric surgery enrolled in our institution from May 2014 to July 2015. All patients have signed the informed consent and the study was approved by the local ethics committee. The following parameters were measured: 1) F1+2 (µmol/l), 2) Lag time (LT, seconds), 3) Peak of thrombin (PMAX, nmol), 4) Time to peak (TPEAK, seconds), 5) Endogenous thrombin potential (ETP, nmol x minute), 6) Start tale (ST, seconds). All the patients received antithrombotic prophylaxis with pneumatic compression and enoxaparin 60 mg once daily for 1 month following the operation. A p value< 0.05 had statistical significance.

Results: We have studied 91 patients with a mean age of 45±10.7 years, 72% of them were women. The mean body mass index was 49.7 ±7.2 kg/m^2. We have observed a reduction in ETP, PMAX, TPEAK and ST throughout the scheduled visits (p< 0.001). However, no LT reduction has been documented, but it had no clinical impact (p:0.412). Regarding F1+2, in the same way, we have also documented a progressive reduction not only following the bariatric surgery but also once completing the fasting phase.

Conclusions: The weight loose induced by bariatric surgery has evidenced a clear reduction in thrombin generation. These changes contribute to an improvement of the hypercoagulable status of the obesity. This improvement together with a favourable cardiovascular risk profile minimizes the thrombus development.

PB 1408 | Combined Detection of Factor XIII and D-Dimer Is Helpful for Differential Diagnosis in Patients with Suspected Pulmonary Embolism

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Background: D-dimer has been used to rule out pulmonary embolism (PE). Previous authors have reported decreased concentration

of coagulation factor XIII (FXIII) in venous thromboembolism, and no change in the FXIII concentration in patients with acute cardiovascular disease. we speculated that combined detection of the FXIII and D-dimer concentrations might be useful to distinguish PE from cardiovascular disease, such as acute coronary syndrome (ACS) and aortic dissection (AD).

Aims: We evaluated the contribution of measurement of the combination of the D-dimer and FXIII concentrations to the diagnosis of PE.

Methods: In this prospective single-center study, 209 patients initially suspected to have PE were enrolled, D-dimer and FXIII concentrations of each patient were detected. Forty-oneof them were diagnosed with PE and 168 with other final diagnoses, including ACS; AD; spontaneous pneumothorax (SP); other respiratory, heart, digestive and nervous diseases; and uncertain diagnoses.

Results: Patients with PE had significantly higher D-dimer and lower FXIII concentrations than patients without PE. At the best-fit cut-off value of 65.0%, the sensitivity and specificity of FXIII concentrations for PE were 88.2% and 81.9%, respectively. Specifically, patients with AD or ACS showed a higher FXIII concentration and mean platelet volume than did patients with PE or SP, and patients with PE and AD had higher D-dimer concentrations than did other patients. At the thresholds of 69% for FXIII and 1.10 μg/mL for D-Dimer, 123 of 151 patients (81.5%) with serious diseases (PE, AD, ACS and SP) were correctly distinguished.

Conclusions: The combination of D-dimer and FXIII measurement seems to be helpful in distinguishing PE from serious diseases with similar symptoms, likely because of increased FXIII release from active platelets in cardiovascular disease.

PB 1409 | Clinical Significance of Circulating Endothelial-derived Microparticles and Coagulation Profiles in Patients with Advanced Solid Tumors

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Background: Venous thromboembolism (VTE) is a frequent and clinically important issue in patients with cancer. Early and proper risk estimation and diagnosis are important for the prevention and treatment of VTE. Circulating microparticles (MP) are known to provide a catalytic surface for the assembly of blood coagulation factors, and are therefore associated with thrombotic conditions.

Aims: This study was aimed to investigate the clinical significance of endothelial-derived MP (EMP) and platelet-derived MP (PMP) in patients with advanced cancer.

Methods: Patients newly diagnosed with cancer, who were scheduled to receive chemotherapy, were enrolled in this study. D-dimer, fibrinogen, fibrin degradation product (FDP), prothrombin time (PT), activated partial thromboplastin time (aPTT), protein C, and protein S levels were defined as CD41+/Annexin V+ and CD31+/Annexin V+ particles, respectively. Development of VTE was monitored for one year after diagnosis.

Results: 67 cancer patients were enrolled. In the cancer stages, 2 were stage II , 8 were stage III and 57 were stage IV of enrolled population. 10 of 67 (14.9%) patients developed VTE during the first year period after diagnosis. D-dimer levels were significantly higher in cancer with VTE (7.1±10.4 mg/L) than those of cancer without DVT (2.4±2.7 mg/L), (P=0.005). EMP (%) were significantly higher in cancer with VTE (33.1±17.2%) than in cancer without VTE (22.3±15.4%), (p=0.04). PMP (%) were higher in cancer with VTE (36.6±15.3%) than in cancer without VTE (31.3±17.9%); however, statistical significance was not shown (p=0.38). Both EMP and PMP were significantly associated with levels of D-dimer (p=0.002 and p=0.04) and FDP (p=0.0007 and p=0.03), respectively.

Conclusions: EMP are increased in cancer with VTE. Both EMP and PMP were significantly associated with levels of D-dimer and FDP. EMP and PMP are procoagulant markers and may help to predict VTE in patients with tumors.

PB 1410 | Markers of Neutrophil Extracellular Traps in Patients with Thrombosis and Sepsis

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Background: Neutrophil extracellular traps (NETs) are networks of extracellular fibres produced from neutrophil DNA with a pathogenic role in infection and thrombosis. Reliable assays for measuring NETs are desirable as novel treatments targeting NETs are being explored for the treatment of these conditions.

Aims: To quantitate serum markers of NETs in septic and thrombotic patients for potential diagnostic and monitoring purposes.

Methods: Serum markers were measured in 21 healthy controls, 18 patients fulfilling the sepsis criteria and 23 patients with radiologically confirmed thrombosis within 3 days of the diagnosis. Informed consent was received from all participants and the study was approved by local Research Ethics.

Serum double stranded (ds) DNA and DNAse were measured by fluorometric assay. Myeloperoxidase, myeloid-related protein (MRP), nucleosomes and elastase were measured by ELISA. Production of reactive oxygen species (ROS) by donor platelets incubated with patient serum was quantified by the 2',7'dichlorofluorescein diacetate fluorometric assay. Serum markers were compared by Mann-Whitney test.

Results: There is a significant increase in dsDNA, MRP, DNase and ROS production in patients with sepsis and thrombosis compared with healthy controls (p< 0.005). Serum myeloperoxidase is significantly increased in patients with thrombosis compared with healthy controls

but not in sepsis patients. Neutrophil elastase is significantly increased in patients with sepsis (545 +/- 141 ng/mL) compared with healthy controls (198 +/- 37 ng/mL) but not in thrombosis (291 +/- 59 ng/mL). Serum nucleosomes did not show a difference between patient groups and controls.

Conclusions: Serum markers of NET are a simple method of quantifying NETotic burden in patients with thrombosis and sepsis. The differential expression of myeloperoxidase in thrombosis patients and neutrophil elastase in sepsis patients may reflect the difference in pathogenesis of NETs in these two conditions.

PB 1411 | A Quantitative Clot-based Assay for Antithrombin Activity

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Background: Antithrombin (AT) is the primary physiologic inhibitor of the active coagulation enzymes thrombin and activated Factor X (FXa). The inhibitory effect of AT is enhanced in the presence of glycosoaminoglycans such as heparin. To date assays for AT are chromogenic based on the use of either a FXa or thrombin specific chromogen.

Aims: We report here on a simple clot based assay that involves both FXa and thrombin.

Methods: Test plasma is diluted with buffer, mixed with antithrombin deficient plasma and a silica based activated partial thromboplastin time reagent with added exogenous heparin, and incubated at 37C. The reaction is initiated with 0.025 M calcium chloride, and the time to clot formation is recorded. The time to clot formation is proportional to the amount of AT in the patient sample.

Results: The reagents are stable for 24hr at 2-8°C. The assay is linear from 0% AT to 150% AT with all r^2 greater than 0.990. The limit of detection is 11%. No interference was seen in icteric, lipaemic or haemolysed samples up to 20mg/dl of unconjugated bilirubin, 2,000 mg/dl of triglyceride or 500mg/dl of bilirubin. Unfractionated heparin in the patient sample up to 1IU/mL showed no interference in the assay. Samples from a normal patient (n=40) and samples from patients suspected of having an AT deficiency (n=50) were assayed using this assay and the results were compared with results obtained using a commercial AT assay. Good comparison was obtained with this assay (y=1.054x + 0.886). No deviation in result was observed with any of the samples tested.

Conclusions: We conclude that we have a sensitive clot based assay that can be used for AT assays. The assay can be performed on any coagulation instrument even the simplest clot only instruments. We have successfully performed the assay on a range of instruments of different complexity. We can conclude that this assay can be performed in even the most basic of coagulation laboratories.

PB 1412 | Identification of microRNAs as Biomarkers for Predicting the Risk of Venous Thromboembolism

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Background: Venous thromboembolism (VTE) is a multicausal disease with a high risk of recurrence. Currently available risk models are not predictive enough to assess either the risk of a first or a recurrent VTE in an individual patient. To improve risk assessment of VTE, more appropriate biomarkers are needed. Recently, circulating microRNAs (miRs) have attracted attention in their disease predictive value and emerged as a promising class of biomarkers.

Aims: Up to now, information on VTE-related miRs is limited which hampers our understanding of the role of these short RNAs and options to use them as tools in the management of patients with VTE. Therefore, our purpose was to identify miRs which are clinically useful biomarkers of VTE.

Methods: To generate an important and convincing miR database, a plasma pool from 20 patients with a high thrombotic risk (male, history of two unprovoked VTE, mean age 48) and one pool of age-matched healthy men were subjected to miR expression analysis. The Affymetrix miRNA Microarray includes 2.578 human miR samples, which cover the majority of all thus far known human miRs

Results: By miR expression analysis we identified 5 miRs which were exclusively detected in the plasma pool of VTE patients and 23 in the control pool, whereas 55 miRs were detected in all samples (Fig. 1). miRs which were exclusively detected in the plasma pool of VTE patients or the controls were just moderately expressed. The miR expression analysis also showed that the levels of miR-3613-3p and miR-6126 of VTE patients were higher (≥2-fold) than those of controls, whereas nine miRs showed lower levels (≤2-fold).

Conclusions: Plasma miRs could be reliable candidates for the development of minimally invasive biomarkers for predicting the risk of VTE. Moreover, these VTE-related miRs could provide a basis for further research of the molecular mechanism underlying the pathomechanism of VTE.

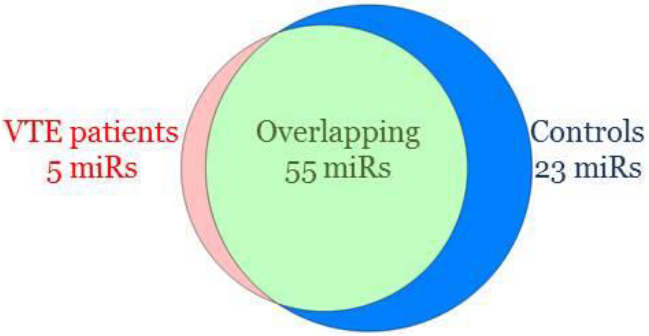

FIGURE 1 Venn diagram of differential and overlapping expression of miRs in patients with a high thrombotic risk and matched controls

PB 1413 | Platelet Counts and Plateletcrit are Associated with an Increased Risk of Venous Thrombosis in Females. Results from the RETROVE Study

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Background: Platelets play a role in the pathophysiology of arterial and venous thrombosis (VTE). Several studies have associated cardiovascular risk with high platelet counts. However, the association of these platelet parameters with VTE remains unclear.

Aims: The aim of our study was to evaluate the thrombotic risk in patients with VTE related to the platelet counts (PLT, PLTOP) and plateletcrit (PCT).

Methods: RETROVE (Riesgo de Enfermedad TROmboembólica VEnosa) is a Spanish case-control study which include 400 adult patients (over 18 years old) with VTE and 400 healthy volunteers. The platelet parameters were determined with an analyser Sysmex XE-2100® (Roche Diagnostics, Switzerland). The Mann-Whitney and Chi-square tests were used to evaluate differences. The platelet values over the 90th percentile were considered risk factors. To evaluate the *odds ratio* (OR) and 95% confidence interval (CI) for the risk of VTE, we used an unconditional logistic regression analysis for the platelet parameters (over 90th percentile). We analysed three OR models: not adjusted model (model 1); age and gender adjusted model (model 2) and; age, gender, BMI, hypertension and the risk factor VIII and von Willebrand factor (over 215.6% and 183%) levels (model 3).

Results: We did not find any significant difference between patients and controls for the platelet parmeters. However, in females, the platelet counts over 326·10^{9}/L showed an OR of 2.27 (95% CI, 1.22–4.29). Plateletcrit over 0.34% showed an OR of 2.24 (95% CI, 1.22–4.16).

Notably, no risk of VTE was found for elevated the platelet counts in males.

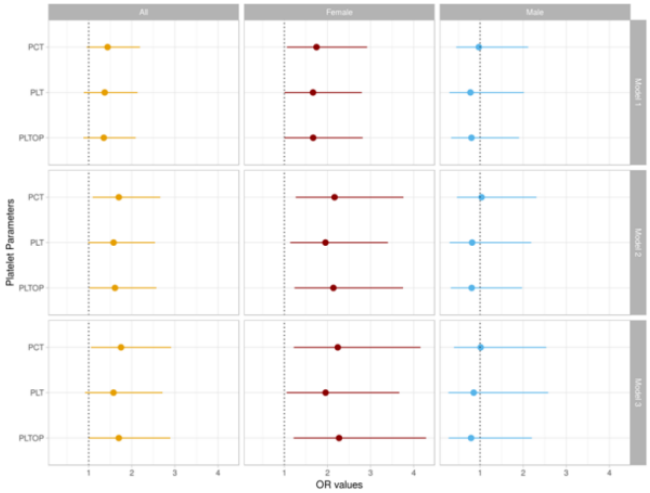

FIGURE 1 Risk of venous thromboembolism (VTE) for 3 platelet parameters in the sample, females and males following the three models.

Conclusions: In females, high platelet counts and plateletcrit (even within the reference clinical range) were associated with a double risk of VTE.

We believe that our results provide a firm foundation for additional studies which might be confirm our observations that high platelet counts are predictors of VTE risk in females.

Grants: FIS PI12/00612, RIC RD12/0042/0032 and FIS PI 15/0269.

PB 1414 | Plasma Levels of miRNAs Are Associated with Future Risk of Incident Venous Thromboembolism

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TABLE 1 Odds ratios of VTE events (90th percentile) for the platelet parameters.

	Parameters	Interval	Patients	Controls	Model 1 OR (95% CI) p-value	Model 2 OR (95% CI) p-value	Model 3 OR (95% CI) p-value
Total (800)	PLT (×109/L)	[302-657)	54	41	1.35 (0.88–2.09) NS	1.61 (1.02-2.57) 0.044	1.70 (1.00–2.89) NS
	PLTOP (×109/L)	[313-725)	53	40	1.37 (0.89–2.13) NS	1.58 (0.99-2.54) NS	1.57 (0.92–2.71) NS
	PCT (%)	[0.32-0.64)	61	44	1.44 (0.95–2.19) 0.048	1.70 (1.09-2.66) 0.019	1.75 (1.06–2.91) 0.03
Females (410)	PLT (×109/L)	[326-480)	44	29	1.67 (1.00–2.82) NS	2.14 (1.24-3.75) 0.007	2.27 (1.22–4.29) 0.011
	PLTOP (×109/L)	[335-474)	45	30	1.67 (1.00–2.80) NS	1.95 (1.14-3.40) 0.016	1.96 (1.05–3.67) 0.034
	PCT (%)	[0.34-0.47)	47	30	1.75 (1.06–2.92) 0.031	2.16 (1.26-3.76) 0.005	2.24 (1.22–4.16) 0.01
Males (390)	PLT (×109/L)	[276-657)	10	12	0.80 (0.33–1.90) NS	0.81 (0.32-1.98) NS	0.79 (0.27–2.20) NS
	PLTOP (×109/L)	[286-725)	8	10	0.78 (0.29–2.02) NS	0.82 (0.30-2.19) NS	0.85 (0.25–2.58) NS
	PCT (%)	[0.3-0.64)	14	14	0.97 (0.45–2.11) NS	1.04 (0.46-2.31) NS	1.01 (0.40–2.54) NS

Background: Circulating miRNAs are emerging as a prominent class of biomarkers in many diseases. Previous studies reported differential expression patterns of certain miRNAs in venous thromboembolism (VTE). In these reports, differentially expressed miRNAs could be a consequence of the disease rather than a cause. No study has prospectively evaluated the association between the expression of plasma miRNAs and risk of future VTE.

Aims: To investigate whether plasma levels of miRNAs are differentially expressed in subjects with and without incident VTE during follow-up.

Methods: We conducted a pilot nested case-control study including 19 subjects who developed VTE within 3 years after blood sampling and 19 age- and sex-matched healthy controls recruited from the general population (Tromsø VI survey). Total RNA was extracted from plasma using the miRNeasy Mini kit (Qiagen, USA) with modifications. Screening of 179 miRNAs was performed on a Serum/Plasma Focus microRNA PCR panel (Exiqon, Denmark). miRNAs expression levels were normalized by the expression of miR-425-5p as suggested by BestRef. Statistical analyses were performed in R software (v3.2.3). We used logistic regression with backward selection to build a predictive model of incident VTE. The study was approved by the regional ethical committee and all participants gave their informed written consent.

Results: High quality signals from 61 miRNAs were identified in all 38 plasma samples. Plasma levels of nine miRNAs, namely, miR-328-3p, miR-208a-3p, miR-629-5p, miR-15a-5p, miR-451a, miR-199a-3p, miR-363-3p, miR-20b-5p, miR-223-5p were found to be associated with future risk of incident VTE.

Conclusions: In this pilot nested case-control study, we found nine plasma miRNAs that could be important markers of future risk of incident VTE in the general population. Currently, these miRNAs are included in a larger-scale validation study.

PB 1415 | The Blood Components Strongly Affect Thrombus Structure Formed in a Novel Microfluidic Device

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Background: Venous thromboembolism (VTE) is a major cause of sudden death. Prevention is still hard for lacking understanding of mechanical property of thrombus, that should be important because fatal VTE is caused by breakdown of thrombus. We reported that shear rate strongly affects thrombus structures in the ISTH SSC 2016 Meeting (BR06). In this study, we tried to clarified the effect of some kinds of blood cell on thrombus structure.

Aims: Our aim is to extract mechanical property of venous thrombus to predict the breakdown of venous thrombus preemptively, using multiscale mechanics simulation model and mathematical model of biological structure.

Methods: Human venous blood was obtained from healthy volunteers. We separated red blood cells (RBCs), washed platelets and platelet free plasma by centrifugation. We mixed them with various ratios of platelets or RBCs. Plasma was fluorescently-stained with FITC-conjugated dextran to visualize RBCs. Platelets were fluorescently-stained with quinacrine. Alexa fluor 546-conjugated fibrinogen was added to visualize fibrin formation.

The blood sample was introduced to a home build microfluidic channels that ware fully siliconized and partly coated with collagen. The thrombus imaged was observed by a fluorescence microscope.

Results: The structures of thrombus with various ratios of blood components were much different from each other.

1. Thrombus formed by platelet poor blood traps more RBCs compared with control.

2. Fibrin fibers of thrombus formed using very low hematocrit blood appeared to anisotropic in orientation and also fibers are thinner and unclear than control.

3. The size of platelet clusters adhered on collagen surface formed by very low hematocrit blood is much bigger compared with that of normal hematocrit blood.

Conclusions: RBCs and platelets play pivotal roles to determine thrombus structure. Our findings are very informative to construct mathematical model of venous thrombus to elucidate mechanical property of thrombus in various conditions.

PB 1416 | D-dimer in Acute Medically Ill Adults: A Multicenter Observational Study Evaluating the Prevalence of Elevated D-dimer in Acute Medically Ill, Hospitalized Adults: The DAMIACT Study, Initial Results

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Background: Recent guidelines for the diagnosis of venous thromboembolism (VTE) in hospitalized non-surgical patients use D-dimer for risk stratification. The prevalence of an elevated D-dimer at the time of hospitalization in this cohort is unknown.

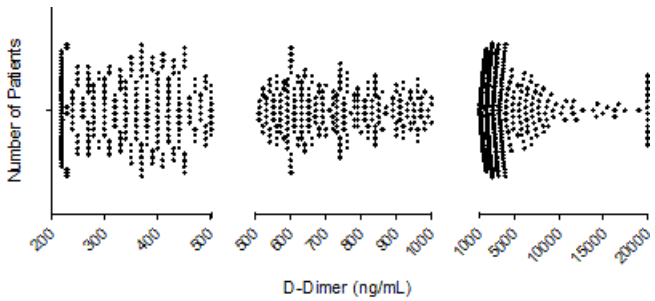
Aims: We aimed to evaluate the prevalence of an elevated D-dimer in older, hospitalized, acute medically ill patients.

Methods: This was a prospective observational study of acute medically ill patients admitted Feb 14-Nov 15, 2016, across 9 US hospitals. Patients were eligible if they were ≥60 y and admitted for an acute medical illness; excluded if they were admitted for suspected or diagnosed VTE, were anticoagulated prior to enrollment, or had surgery within 30 d of presentation. After consent, a single D-dimer sample was drawn within 24 h of admission and before receiving anticoagulation. Samples were analyzed at a central laboratory using the STA®Compact, Diagnostica Stago analyzer (range 0.22-0.50 µg/mL). Results were stratified using 3 cut-offs: 1) ≥the upper limit of normal (ULN) ng/mL; 2) ≥2xULN; 3) ≥age x 10. Data were analyzed using SAS for Windows®, v9.3.

Results: There were 995 patients included; mean age was 70±8 y; 491(49.3%) were male; 707(72%) were Caucasian, 260(26%) African American, 61(6.1%) Hispanic. Elevated D-dimer was found in 74.4% of patients (Table 1). D-dimer values ranged from < 220 ng/mL to >20,000 ng/mL (Figure 1). The most common diagnoses were chest pain [127(12.8%)], pneumonia [91(9.2%)], and CHF [88(8.8%)]. ICU admissions were 10%, mortality 1%. The median D-dimer level in patients with VTE (n=15) was 1100 ng/mL (IQR 600-3210). VTEs included deep venous thrombus (n=9), pulmonary embolus (n=3), superficial thrombus (n=2), and cardiac thrombus (n=1).

TABLE 1 The prevalence of an elevated D-dimer in acute medically ill patients

	Number of Patients (N=995)
D-dimer ≥ULN (500 ng/mL)	740 (74.4%)
D-dimer ≥2xULN (1000 ng/mL)	486 (48.8%)
D-dimer ≥the age adjusted D-dimer (≥age x 10)	619 (62.2%)



*33 patients had D-dimer levels <220 ng/mL, and 12 patients had D-dimer levels >20,000 ng/mL

FIGURE 1 Distribution of D-dimer values*

Conclusions: D-dimer above both the ULN and the age adjusted D-dimer were found in the majority of older acute medically ill patients, with almost half of the patients with levels ≥2xULN. This may have important implications for clinical risk assessment and research studies.

PB 1417 | Down-regulation of Protein C Pathway Associated with Increased Inflammation in Patients with Implanted Ventricular Assist Devices as a Potential Cause of Pump Thrombosis

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Background: Left ventricular assist devices (LVADs), increasingly common for heart failure management, have associated thrombosis as a major cause of mortality. We hypothesize that inflammation in LVAD patients dysregulates the protein C pathway, creating a hypercoagulable state leading to thrombosis.

Aims: To identify changes in the protein C pathway in LVAD implanted patients who experienced a thrombotic event.

Methods: Citrated plasma samples, collected from patients implanted with a Thoratec HeartMate II LVAD, were analyzed by commercial ELISAs. Retrospective sample selection included near time of adverse event and 1-3 months prior. LVAD-associated thrombosis (n = 51 samples, 17 patients) was characterized as cerebrovascular accident, rise in LDH or plasma free hemoglobin, pump dysfunction identified by pump parameters, clot imaging or surgical pump exchange. A comparator group consisted of patients with LVAD-associated bleeding (n = 20 samples, 6 patients) characterized as anemia or bleeding.

Results: The median level of C-reactive protein (CRP) was higher and total and free protein S (PrS) was lower in patients with thrombosis compared to bleeding. Median levels of protein C (PrC) and soluble thrombomodulin (TM) were comparable in patients with thrombosis or bleeding. Total PrS, free PrS, and PrC (but not TM or CRP) were further decreased at time of thrombosis. No changes were observed in these parameters at time of bleeding. Pre-event levels of PrC and PrS were consistent with low-dose warfarin (INR 1.5-2.0); TM levels were near normal (2.8 ng/mL). Soluble endothelial cell protein C receptor (EPCR) level was lower than normal (828 ng/mL) in all groups with no change at time of event. Compared to normal (0.8 µg/mL) CRP levels were more elevated in thrombosis than bleeding patients.

TABLE 1

	Median Levels			
	Pre-Bleeding	Bleeding	Pre-Thrombosis	Thrombosis
Total PrS; %	47.5	55.6	40.0	34.3
Free PrS; %	50.3	47.3	42.9	34.7
PrC; %	51.5	56.8	57.5	50.5
CRP; ug/mL	8.0	9.9	27.5	25.4
TM; ng/mL	3.3	3.3	3.5	2.9
EPCR; ng/mL	444	496	513	437

Conclusions: This study suggests that protein S, influenced by the inflammatory state, is a gatekeeper for the function of protein C in patients with LVAD-associated thrombosis.

PB 1418 | Impact of Iron Status, Assessed by Ferritin Light Chain and Soluble Transferrin Receptor, on the Association between Red Cell Distribution Width and VTE. The Tromsø Study

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Background: Red cell distribution width (RDW), a measure of the variability in size of circulating erythrocytes, is associated with incident venous thromboembolism (VTE). The mechanism underlying the association remains unclear, but iron deficiency has been proposed as a potential mediator.

Aims: To investigate whether markers of iron deficiency may explain the relationship between RDW and VTE.

Methods: A nested case-control study was performed with 202 VTE cases and 496 age- and sex-matched controls randomly selected from a general population-based study (The Tromsø Study 1994-2012). Baseline characteristics, including RDW, and blood samples were collected in 1994/95. To assess iron status, plasma samples were thawed and analyzed for levels of ferritin light chain (FtL) and soluble transferrin receptor (sTfR). Logistic regression models were used to calculate odds ratios (OR) with 95% confidence intervals (CI) for VTE by tertiles of RDW (≤12.5, 12.6-13.1 and ≥13.2%), FtL (≤4.8, 4.9-9.9 and ≥10.0 ng/mL), sTfR (≤407, 407.5-565.5 and ≥567 ng/mL), and for the combined exposures of RDW and FtL.

Results: Subjects with RDW in the upper tertile had a 54% increased risk of VTE compared to subjects in the lower tertile (OR 1.54, 95% CI: 1.00-2.38). After adjustments for FtL and sTfR, the risk estimate increased to 61% (OR 1.61, 95% CI: 1.04-2.50). Moreover, the risk of VTE increased across tertiles of FtL (OR for upper vs. lower tertile: 1.39, 95% CI: 0.90-2.13). No relationship was observed between sTfR and VTE. For subjects with upper tertile of both RDW and FtL the OR was 3.32 (95% CI: 1.43-7.70) when compared to those with lowest tertile for both variables. The relative excess risk caused by interaction was 0.59, indicating a modest synergistic effect.

Conclusions: Our findings suggest that the link between RDW and VTE is not explained by iron status. However, there was a modest synergistic effect of high RDW and high FtL on the risk of VTE.

PB 1419 | Utility of Novel Biomarkers of DIC—TAT (Thrombin-antithrombinIII Complex), PIC (alpha2plasmin Inhibitor-plasmin Complex), tPAIc (Tissue Plasminogen Activator-plasminogen Activator Inhibitor Complex) and TM (Thrombomodulin) in Predicting the Worsening of Coagulopathy and/or Mortality in Acute Promyelocytic Leukaemia (APL) and Sepsis: A Prospective Observational Study

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Background: DIC characterised by consumptive coagulopathy with initial thrombotic phase in sepsis patients and predominantly fibrinolysis in APL patients.

Aims: To test the significance of novel biomarkers in predicting worsening of DIC and/or mortality.

Methods: Study was conducted from May 2014-September 2015. Patients were recruited based on clinical suspicion of DIC in Sepsis and lab diagnosis of APL. ISTH DIC score was calculated daily and serial sampling done. Endpoints studied were worsening of DIC score and death.

Novel Markers were analysed using HISCL5000(CLEIA tech.,Sysmex,Kobe,Japan). Differences in biomarkers between the outcome groups were analysed using Wilcoxon rank sum test. Kruskal-Wallis test was used to study the statistical difference between DIC types(APL, sepsis and control). AUROC was computed for different biomarkers at baseline.

Results: Of 24 and 36 patients with Sepsis and APL respectively 15 and 5 died. 15 of sepsis and 27 of APL patients had worsening DIC. Comparison of biomarkers between the DIC types and control is depicted in Fig1.

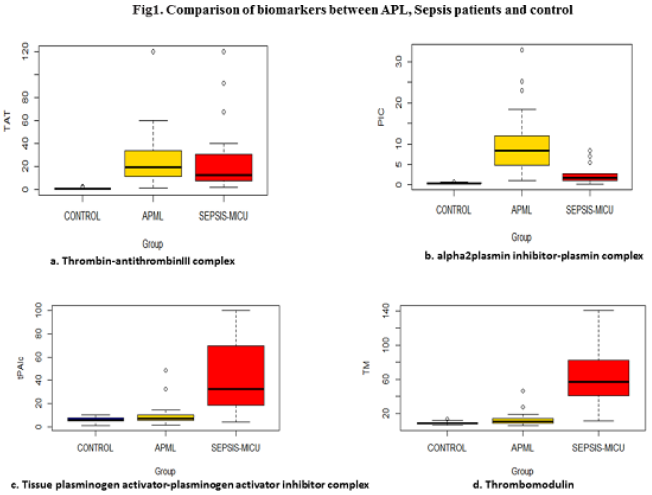

FIGURE 1 Comparison of novel biomarkers in APL, sepsis-induced DIC and controls

APL

TAT, PIC and TM(p=0.003,0.01,0.001 respectively) were showing significant difference between worsening and recovery groups. There was a significant difference in t-PAIc and TM(p=0.002,0.001 respectively) between the two outcome groups-Alive and Dead.

Sepsis

Among the sepsis patients, none of the novel biomarkers or the routine parameters were significantly different between worsening and recovery groups. TM and t-PAIc had comparatively better AUROC. Between the alive and dead, only ATIII was significantly different(p=0.011).

AUROCs are shown in Tab1. Combined ROC was not done because there were very less number of patients in each group.

Conclusions: The main findings of this study are - the patients of APL in whom DIC worsened had a higher PIC while it was a higher tPAIc in sepsis type. This study helps us in predicting worsening DIC with novel biomarkers using a method with potentially short turnaround time.

PB 1420 | Measurement of Thrombin Generation in Conditions of Low Antithrombin

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Background: As a global assay Thrombin Generation (TG) has proven useful in evaluating the coagulation system. The Calibrated Automated Thrombography (CAT) method allows for the measurement of thrombin generation in clotting plasma using a fluorogenic substrate and a thrombin calibrator. Fitusiran is an investigational RNAi therapeutic targeting antithrombin (AT), designed to promote thrombin generation, and therefore hemostasis, in persons with hemophilia and other rare bleeding disorders. The CAT assay has the potential to be a useful tool for demonstrating the effect of fitusiran on coagulation parameters. AT is the main inhibitor of thrombin, and conditions of low AT can potentially result in substrate consumption prior to completion of the assay run. Therefore, it is important to recognize conditions in which the Thrombogram can be properly calculated.

Aims: To demonstrate the ability to accurately measure thrombin generation in plasma samples with low AT levels.

Methods: The CAT assay was run on samples from patients with hemophilia A and B, with and without inhibitors, from the Phase 1 and open label extension study with fitusiran (NCT02035605, NCT02554773).

Results: Under standardized conditions (platelet poor plasma, 1 pM tissue factor, 4 µM phospholipids), the CAT assay demonstrated an average increase in peak thrombin by 290% at AT lowering ≥75%, approaching the lower end of the normal range in healthy volunteers in the Phase 1 study. The CAT analysis software was used to ensure absence of substrate consumption in the analyzed values. The addition of thrombomodulin to activate the Protein C system, a second anticoagulant pathway, was tested as an approach to modify the CAT assay for low AT conditions. Addition of thrombomodulin (0.5-5 nM) led to dose dependent reductions in peak thrombin levels up to 75%.

TABLE 1 AUROCs for DIC worsening and Mortality in APL and Sepsis induced DIC patients

Biomarkers (Day 1)	DIC Worsening: Cut-off values	DIC Worsening: AUC	DIC Worsening: Sensitivity/ Specificity(%)	DIC Worsening: PPV/NPV(%)	Mortality: Cut-off values	Mortality: AUC	Mortality: Sensitivity/ Specificity(%)	Mortality: PPV/NPV(%)
APL: TAT	18	0.84	76.9/77.8	90.9/53.8	20	0.70	80.0/56.7	23.5/94.4
APL: PIC	6.5	0.79	76.9/77.8	90.9/5.38	11	0.61	60.0/70.0	25.0/91.3
APL: tPAIc	6.5	0.71	69.2/66.7	85.7/42.9	12.5	0.94	80.0/93.3	66.7/96.6
APL: TM	13	0.87	50.0/100.0	100.0/40.9	14	0.96	100.0/83.3	50.0/100.0
Sepsis: TAT	11.5	0.63	60.0/55.6	69.2/45.5	11.5	0.66	60.0/55.6	69.2/45.5
Sepsis: PIC	1.5	0.49	60.0/33.3	40.0/33.3	1.5	0.36	53.3/22.2	53.3/22.2
Sepsis: tPAIc	24	0.66	80.0/55.6	75.0/62.5	28	0.58	66.7/55.6	71.4/50.0
Sepsis: TM	58.5	0.69	66.7/77.8	83.3/58.3	55	0.64	66.7/66.7	76.9/54.5

Conclusions: The CAT assay may be a method to reliably measure thrombin generation in hemophilia patients treated with fitusiran; modified conditions may be used to further optimize the assay for low AT.

PB 1421 | Sensitivity of Different D-dimer Cut-off Levels In Unselected Patients with Confirmed Acute Pulmonary Embolism: FOCUS, a Prospective Multicenter Cohort Study

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Background: The ongoing multicenter ´Follow-up after acute pulmonary embolism´ (FOCUS) observational study has set out to prospectively follow, over a 2-year period at predetermined intervals with standardized follow-up (FUP) protocols, the clinical and hemodynamic course of 1,000 unselected patients with confirmed acute symptomatic pulmonary embolism (PE).

Aims: To estimate the sensitivity of D-dimer levels at PE diagnosis and evaluate the course of D-dimer levels over FUP.

Methods: Quantitative D-dimer measurement was performed at each recruiting site as part of the PE diagnostic algorithm. For this analysis, a negative D-dimer test was defined by using a fixed (500 ng/mL), or an age-adjusted (age*10 ng/mL in patients >50 years) cut-off. We calculated the diagnostic sensitivity (95% Confidence Interval [CI]) of D-dimer testing measured upon admission and also began to determine the proportion of patients with negative values at 3-month FUP visit. FOCUS was approved by ethics committee and patient informed consent obtained.

Results: A total of 611 consecutive acute PE patients were included in FOCUS as of December 2016. Mean age was 62.0 years, 323 (52.9%) were males, 103 (16.7%) had symptoms of concomitant deep vein thrombosis, and 58 (9.5%) had active cancer. A negative D-dimer was demonstrated in 12 (1.96%) and in 14 (2.29%) patients using the fixed or age-adjusted cutoff, respectively, leading to calculated maximum sensitivities of 98.0 (95% CI: 96.6-99.0) and 97.7 (95% CI: 96.2-98.7) (*Table 1*).

TABLE 1 Sensitivity of D-dimer at different cutoffs measured at the time of pulmonary embolism diagnosis

	Fixed cutoff of 500 ng/mL	Age-adjusted cutoff (age*10 ng/mL) in patients >50 years
Negative D-dimer, n	12	14
Whole study population (n=611)	98.0 (95% CI: 96.6-99.0)	97.7 (95% CI: 96.2-98.7)
Patients tested (n=499)	97.6 (95% CI: 95.8-98.8)	97.2 (95% CI: 95.3-98.5)

A total of 383 patients was retested for D-dimer at 3 months and values remained higher than 500 ng/mL in more than 20% (*Table 2*).

TABLE 2 Course of D-dimer measurements over early follow-up

Visit number	Timepoint	D-dimer >0.500, n (%)	D-dimer ≤0.500, n (%)	Total
Visit 1	PE diagnosis	487 (97.6)	12 (2.4)	499
Visit 2	Discharge	51 (96.2)	2 (3.8)	53
Visit 3	Month 3	85 (22.2)	298 (77.8)	383

Conclusions: The sensitivity of D-dimer test measured at presentation appears lower than previously described in patients with suspected acute PE and low clinical probability, but consistent with that observed in patients with high clinical probability.

PB 1422 | Assessment of Endothelial and Inflammatory Markers in Brazilian Renal Transplant Recipients

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Background: Chronic kidney disease (CKD) and kidney transplantation(KTx) are associated to endothelial dysfunction, which contributes to a hypercoagulable state, with a consequent increase cardiovascular risk. Hypertension and diabetes mellitus, common causes of CKD, can result in endothelial dysfunction, vascular fragility and hypoxia, contributing to cardiovascular mortality in KTx

Aims: The aim of this study was to evaluate endothelial markers, thrombomodulin (TM) and vWF, in KTx and correlate them to the primary cause of pre-transplantation CKD. Additionally, the correlation of these parameters with the pro-inflammatory cytokine interleukin-6(IL-6) was also investigated

Methods: One hundred and sixty KTx were studied. Patients were distributed into 4 groups, according to the primary cause of pre-transplantation CKD, G1: glomerulopathies -N=27, G2: hypertensive nephrosclerosis -N=38, G3: diabetic nephropathy -N=18 and G4: other causes /unknown etiology -N=76. The patients were also assessed according to creatinine levels (C1≤1.4; C2>1.4mg/dL) and estimated glomerular filtration rate(eGFR, R1≤60; R2< 60 mL/min/1.73m$^{2)}$. TM and vWF levels were determined by ELISA and IL-6 was performed by Flow Cytometry.

Results: The median TM levels were significantly higher (8.38ng/mL; P=0.001) in G1 group compared to the others (G2: 5.51ng/mL; G3: 5.88ng/mL; G4:6.33ng/mL).(Fig.1A) There was no difference among the groups comparing vWF and IL-6 levels (Fig.1A and B). TM levels were significantly higher (P=0.028) in patients with low eGFR(R2) compared to patients with eGFR>60 (Fig.1C). In subsequent correlation analysis, the levels of TM were positively correlated with IL-6 (r=0.23, P=0.012), serum creatinine (r=0.20; P=0.012) and negatively with eGFR (r=-0.17; P=0.028). IL-6 levels were positively correlated with vWF(r=0.23; P=0.012). However, these correlations were weak.

Conclusions: Our data showed that TM was the most promising endothelial marker in Brazilian KTx with glomerulopathies and low eGFR.

Support: Capes, CNPq, FAPEMIG

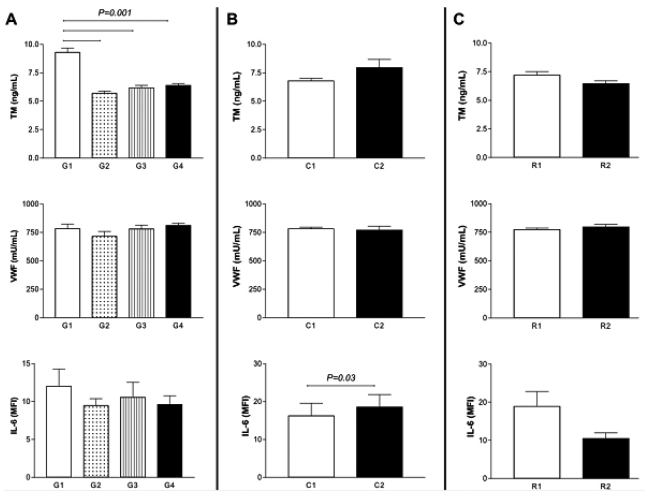

FIGURE 1 TM, VWF and IL-6 plasma levels in kidney transplated recipients. (A: Type of glomerulopathies, B: Creatinine, C: eGFR)

PB 1423 | Remote Platelet Function Testing Using Platelet-Bound P-selectin as a Marker of Platelet Activation

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Background: Platelet function is often limited by access to specialist equipment and expertise. Measurement of P-selectin on activated platelets by flow cytometry following fixation of blood from a patient offers the opportunity of testing in remote locations, with samples transferred to a central laboratory for analysis.

Aims: To describe our experience of the use of P-selectin in several patient groups.

Methods: Kits are provided to enable platelet activation in whole blood from patients in remote locations. Activating agents include arachidonic acid (AA), adenosine diphosphate (ADP) and thrombin receptor activation peptide. The fixing agent is PAMFix (Platelet Solutions, Nottingham, UK). Activated/fixed samples are stable for up to 9 days. Platelet P-selectin is measured using flow cytometry.

Results: Studies in patients with acute coronary syndromes (ACS), acute stroke and other neurological syndromes, and enhanced bruising and bleeding are either complete or on-going. Studies to assess platelet viability prior to using platelet-rich plasma in wound healing are ongoing. Successful outcomes include: 1) demonstration of a significant relation between high platelet reactivity to ADP in patients with ACS taking clopidogrel who go on to experience a further thrombotic event; 2) demonstration of good inhibition of AA-induced platelet reactivity in patients with acute stroke taking aspirin, but variable ADP-induced platelet reactivity in the same patients taking clopidogrel; 3) detection of drug non-compliance and/or reduced effectiveness in patients scheduled for a neurological stent insertion; 4) identification of secretory defects in patients who bleed; 5) reassurance on platelet viability during wound healing procedures.

Conclusions: Measurements of platelet-bound P-selectin as an approach to platelet function testing without access to specialist equipment or expertise is proving of value in a variety of patient groups.

PB 1424 | Endothelial Microparticles, Thrombomodulin and D dimer in Women with Systemic Lupus Erythematosus under treatment

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Background: Systemic Lupus Erythematosus (SLE) is an inflammatory and autoimmune multisystem disease. Microparticles (MPs) are cell

TABLE 1 Endothelial Microparticles, Thrombomodulin and D Dimer expressed as medians in women with Systemic Lupus Erythematosus under treatment

Parameter	Controls (n=30)	Patients with SLE (n=60)	SLE Patients with Low Activity (n=30)	SLE Patients with Moderate/ High Activity (n=30)	P value
EMPs(EMPs/μL)	a,b,c 7.40	a 12.13	b,d 10.33	c,d 10.70	a0.019 b0.088 c0.0056 d0.2732
TM(ng/mL)	a,b,c 0.21	a 0.29	b,d 0.25	c,d 0.34	a0.0341 b0.0001 c0.0007 d0.0002
DDi(ng/mL)	a,b,c 374	a 1155	b,d 930	c,d 1563	a0.0133 b0.0001 c0.0198 d0.0004

membrane-shedded fragments released during apoptosis and cell activation. Endothelial microparticles (EMPs) are derived from activated endothelial cells and may reflect endothelial dysfunction in response to the inflammatory process. As EMPs can stimulate coagulation by exposure of negatively charged phospholipids and tissue factor can be hypothesized that the higher the number of EMPs the greater the state of hypercoagulability.

Aims: This study has evaluated EMPs, endothelial lesion, and hypercoagulable status, and their relationship to disease activity in SLE women under treatment, compared to controls.

Methods: Disease activity was defined by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI 2K). This study included women with similar age distributed into 3 groups: Group 1: healthy women (control, n=30); Group 2: SLE women with low disease activity (SLEDAI 2K ≤ 4, n=30); Group 3: SLE patients with / moderate high disease activity (SLEDAI 2K > 4, n=30). EMPs were purified by ultracentrifugation, labeled with antibody anti-CD51 / 61 and annexin V and then analysed flow cytometry. Endothelial lesion was assessed by thrombomodulin and hypercoagulable status by D-dimermeasurements in citrated plasma samples. The results were analyzed with Mini Tab program and median values were compared.

Results: The results are shown in Table 1.

Conclusions: The data from this study suggest that increasing the number of EMPs may partially support the state of hypercoagulability found in patients with SLE, particularly those with active disease, which is reinforced by increased plasma levels of thrombomodulin and D-dimer. These tools may be useful in stratifying and monitoring patients.

Support: CNPq,CAPES and FAPEMIG

PB 1425 | Chronic Lymphocytic Leukemia: Is there a Role for Circulating Microparticles or Trombin Generation in Clinical Heterogeneity?

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Background: CLL prognosis is variable and some patients progress with short survival. It is unclear whether hemostatic changes, variable by tumor type, play a role in clinical heterogeneity in patients with CLL.

Aims: To evaluate parameters of the hemostatic profile in patients with CLL, compared to controls and their correlation with the progression of the disease.

Methods: Thirty-five CLL patients classified according to the BINET criteria of Hospital das Clínicas-UFMG-Brazil and 35 apparently healthy individuals (Controls) were evaluated, from whom informed consent was obtained. Hemostatic profile was evaluated by quantification of MPs derived from endothelial cells (EMP), B lymphocytes (LMP) and platelets (PMP) using flow cytometry and by Thrombin Generation Test using CAT® method.

Results: Significantly increased levels (/μL) of EMPs were observed in patients with CLL, [132.1 (78.6 to 199.1)] compared with the control group [81.6 (53.1 to 121, 0), p = 0.002]. The same was observed for LMP [142.1 (93.7 to 203.1)] compared to controls [85.2 (63.4 to 115.9), p < 0.001] and PMPs [134.8 (85.7 to 183.1)], control group = [81.2 (52.8 to 118.9), p = 0.003]. When patients were stratified according to Binet staging, significant differences were observed (p < 0.05) between groups Binet A versus controls for EMPs [149.8 (75.3 to 212.5)] LMP [145.2 (91.9 to 235.6)] and PMPs [148.1 (82.2 to 202.1)]. No significant difference was observed between Binet A and Binet B+C. On the other hand, endogen thrombin potential (ETP) was lower in CLL patients [1453.00 (1176.33 to 1602.57)] compared to control group [1577.38 (1326.20 to 1816.95), p = 0.031] especially in severe cases classified as Binet B + C [1216.54 (1130.47 to 1540.15), p = 0.009].

Conclusions: Increased number of MPs in CLL and decreased thrombin generation are not necessarily related to disease progression. So, it seems that such variables do not play a role in clinical heterogeneity in patients with CLL.

Supported by Brazilian agencies CAPES, CNPq and FAPEMIG

PB 1426 | Determinants of Clot Strength in Polycythemia Vera and Primary Myelofibrosis using Rotational Thromboelastometry

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Background: The Myeloproliferative Neoplasias (MPNs) Polycythemia Vera (PV) and Primary Myelofibrosis (PMF) are clonal disorders of hematopoietic stem cells associated with thrombosis. Rotational

Thromboelastometry (ROTEM) is a point of care global coagulation assay, which has showed a hypercoagulable state in MPNs. Little is known about the contribution of platelets to clot strength in MPN.

Aims: We aimed to identify determinants of whole blood clot strength in PV and PMF using ROTEM and to assess the contribution of platelets to clot strength.

Methods: Venous blood samples were collected from 23 PV and 17 PMF patients. ROTEM analysis was performed using EXTEM reagents, which provides a measure of extrinsic activation of coagulation and FIBTEM reagents where platelet contribution to clot strength is inhibited. Maximum Clot Firmness (MCF, [mm]) was obtained, and Shear Modulus (G, [dyne/cm^2]) and the platelet component of clot strength were calculated. The local medical ethics committee approved the study and informed consent was obtained from all patients.

Results: For PV and PMF EXTEM MCF was 69±5 and 69±6 (reference range 50-72) and FIBTEM MCF was 25±10 and 26±9 (reference range 9-25). For PV EXTEM MCF correlated with platelets, r=0.61 (0.27-0.82, p=0.002) and fibrinogen, r=0.48 (0.09-0.75, p=0.02) and FIBTEM MCF correlated with platelets, r=0.72 (0.43-0.87, p=0.0001) and fibrinogen, r=0.52 (0.13-0.77, p=0.01). For PMF EXTEM MCF correlated with platelets, r=0.49 (0.01-0.78, p=0.04) and FIBTEM MCF correlated with fibrinogen, r=0.64 (0.23-0.86, p=0.01). For PV and PMF platelet contribution to clot strength was 0.85±0.06 and 0.84±0.05.

Conclusions: ROTEM analysis confirmed hypercoagulability in PV and PMF. The contribution of platelets to clot strength was comparable to what others have reported for pregnancy, which is also a hypercoagulable state with increased fibrinogen concentration and lower than reported for patients undergoing cardiac surgery.

PB 1427 | Identification & Characterization of Novel Hemostatic Biomarkers of Adverse Clinical Events in Patients with Continuous Flow Left Ventricular Assist Device Implants

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Background: Increased incidence of heart failure and lack of donor hearts has led to increased use of continuous flow left ventricular assist devices (CF-VAD). CF-VAD patients are at risk for non-surgical bleeding and thrombotic complications.

Aims: To identify biomarkers to improve risk assessment for CF-VAD patients.

Methods: Blood samples collected from 16 patients implanted with a Thoratec HeartMate II CF-VAD (25 normal individuals as controls) were analyzed by commercial ELISAs and SELDI mass spectrometry. Patients experienced 12 thrombotic, 8 hemorrhagic and 2 septic events. CF-VAD thrombosis included: cerebrovascular accident, rise in LDH or plasma free hemoglobin, hemolysis, pump dysfunction consistent with thrombus, evidence of clot upon imaging or pump exchange. CF-VAD bleeding events included anemia or overt bleeding.

Results: Annexin V levels increased 3-fold compared to normal (9.2±0.9 *vs.* 2.8±0.2 nM). Levels of C-reactive protein (CRP) (14.5±2.7 *vs.* 2.2±0.6 µg/ml; p=0.034) and TF(+)microparticles (3.7±1.1 *vs.* 0.5±0.1 pg/ml; p=0.038) were significantly higher in CF-VAD patients. SELDI-MS analysis indicated distinct peaks, not found in normals, at 8.1, 11.7 and 15.2/16.2 kDa in CF-VAD patients. The 8.1 kDa biomarker was found in 10/12 patients with thrombosis, 2/6 patients with bleeding, 0/2 patients with sepsis and 0/2 event-free patients. The 8.1 kDa peak was associated with elevated annexin V (p=0.01) and the presence of annexin V(+) microparticles (p=0.005); the 11.7 kDa peak was associated with elevated CRP (p=0.01); the 15.2/16.2 kDa peaks were associated with the presence of TF(+)microparticles (p=0.002), annexin V(+) microparticles (p<0.001) and elevated annexin V (p=0.03).

Conclusions: Despite treatment with low-dose aspirin and warfarin, CF-VAD patients exhibit signs of hemostatic activation. The SELDI-MS identified biomarkers of CF-VAD-associated adverse events may provide a feasible approach to facilitate management of this patient population.

PB 1428 | Haemostasis in Children with Acute Lymphoblastic Leukemia

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Background: The incidence of thrombosis in children with Acute Lymphoblastic Leukemia(ALL) is nearly 40%. Fast and accurate assessment of the haemostasis in children with ALL is still important and actual problem.

Aims: To investigate the haemostatic state in children with ALL using global and standard hemostasis assays.

Methods: Seventy patients aged 1 to 17 years with ALL before the first consolidation phase in ALL-MB-2015 protocol were enrolled in this study. Standard coagulation tests (APPT, TT, PT, fibrinogen, ATIII, D-dimer concentrations), Thromboelastography(TEG), Thrombodynamics(TD) and Thrombomodulin concentration were used to assess coagulation status in patients during the treatment.

Results: TEG parameters and standard tests were in normal(89%) or in hypocoagulation(11%) area during the treatment. The concentrations of fibrinogen and ATIII lowered during the treatment in 87% of patients. Thrombosis occurred in 36 patients (51%). TD revealed hypercoagulation in 89%. We‘ve divided patients in two groups, 1st with low ATIII concentration and 2nd with normal ATIII concentration. There was 45% of thrombosis in 1st group in patients with hypercoagulation

by TD and normal D-dimer levels and only 3% with high D-dimer levels. Even there was 42% of thrombosis in 2nd group in patients with hypercoagulation by TD and low D-dimer levels and 13% with high D-dimer levels despite normal ATIII concentration. There was no thrombosis in both groups with TD parameters in normal area. This is confirmed by threefold increase in free thrombomodulin concentration in patients with thrombosis($p < 0.001$). Similar hypercoagulation by TD parameters but lower ATIII and D-dimer levels revealed reducing lysis potential in patients with ALL.

Conclusions: Thrombodynamics revealed significant hypercoagulability in patients with ALL. The reduced lysis potential confirmed by low ATIII, normal D-dimer levels and hypercoagulation confirmed by TD is the hypothetic group of parameters to predict thrombotic complication in children with ALL.

PB 1429 | Association between FTO rs9939609 Polymorphism and Risk of Recurrent Venous Thromboembolism

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Background: Multiple genetic variations have been identified in *FTO* (fat mass and obesity-associated) gene, among them, *FTO* rs9939609 polymorphism has been shown to be associated with the risk of primary venous thromboembolism (VTE). However, its role in recurrent VTE is not known.

Aims: The aim of our study was to investigate the association between *FTO* rs9939609 polymorphism and the risk of VTE recurrence in a prospective follow up study.

Methods: *FTO* rs9939609 polymorphism was analyzed in Malmö thrombophilia study (MATS, followed for ~10 years) by using TaqMan PCR.

Results: MATS patients (n=1,050) were followed from the discontinuation of anticoagulant treatment until diagnosis of VTE recurrence or the end of the study. One hundred twenty-six patients (12 %) had VTE recurrence during follow up. Cox regression analyses showed no evidence of an association between *FTO* rs9939609 polymorphism and the risk of VTE recurrence in the study population as a whole. However, by including an interaction term in the analysis we found that gender modified the effect of *FTO* rs9939609 polymorphism: male patients with *FTO* polymorphism had significantly higher risk of VTE recurrence (Hazard ratio [HR] = 2.05, 95% confidence interval [CI] = 1.2-3.5, P= 0.009 and HR=2.03, CI= 1.2-3.6, P=0.013) as compared to females on uni- and multi-variate Cox regression analysis (after adjusting for BMI, family history, acquired risk factors for VTE and risk of thrombophilia) respectively.

Conclusions: Our results show that *FTO* rs9939609 polymorphism is associated with higher risk of VTE recurrence in males but not in females independent of other well-known risk factors for VTE.

PB 1430 | The Protein Z System Dynamic in Patients Undergoing Off-pump Coronary Artery Bypass Surgery

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Background: A large number of studies has evaluated multiple markers of coagulation in patients undergoing cardiac surgery. Nonetheless, evidence regarding the protein Z (PZ) system in patients scheduled for off-pump coronary artery bypass surgery (OPCAB) so far is lacking.

Aims: In this pilot study, we evaluated the effect of OPCAB surgery on the protein Z system by measuring the levels of PZ, protein Z-dependent protease inhibitor (ZPI) and factor X (FX) before surgery and one week after surgery.

Methods: The sample consisted of 30 men aged between 45 and 77 years who underwent elective first-time isolated OPCAB surgery. Ethical approval for the study was obtained from the Ethics Committee of Collegium Medicum. Written informed consent was received from all of the study subjects. Fasting blood samples were obtained from the participants before surgery and one week after surgery. PZ, ZPI and FX in plasma were measured by ELISA kits.

Results: The Wilcoxon test was used to compare the levels of PZ, ZPI and FX at the two measurement time points. Of note, both plasma PZ and FX levels were significantly higher one week after surgery than before surgery (median, 3.95 μg/mL versus 3.38 μg/mL, p=0.02; 122.27% versus 111.71%, p=0.002, respectively). In contrast, the levels of ZPI were significantly lower one week after surgery; that is, the median value was 5.96 μg/mL compared with 7.11 μg/mL (p=0.004).

Conclusions: We demonstrated the impact of OPCAB surgery on the plasma levels of PZ, ZPI and FX. However, further studies are necessary to elucidate the exact role of the protein Z system in patients undergoing this surgical procedure.

PB 1431 | Relevance of Co Morbidities, D-Dimer, Red Cell Distribution Width and Factor VIII Levels in Thrombosis

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Background: Arterial or venous thrombosis is a leading cause of mortality worldwide accounting for 1-2 deaths/ 1000 population per year. The interest in recent years have been in identifying comorbidities as risk factors and the usefulness of D- Dimer and red cell distribution width (RDW)in of thrombosis.

Aims: To compare the relevance of co morbidities, factor VIII, D-Dimer levels and RDW in patients with thrombosis.

Methods: 68 patients who attended thrombosis screening programme in a tertiary care hospital in India were included in this study after informed consent and ethical clearance. The clinical history, comorbidities (hypertension, diabetes), D-Dimer(Normal < 250 ng/ml),RDW(Normal range11.5- 15.5%) and Factor VIII assay (Normal range 50 -150%)levels were noted in patients with history of thrombosis. The data was analyzed.

Results: The 68 patients included 9 (13%) subjects with stroke, 26 (38%) subjects with deep vein thrombosis, 10 (15%) with heart diseases and 10 (15%) subjects with other conditions like recurrent abortions and chronic kidney disease. 13 patients (19%) had comorbid conditions without an event while 81% had thrombosis.51% of patients with thrombosis had elevated factor VIII levels of which 69% were seen in venous thrombosis .D Dimer levels were elevated in 33% and RDW in 15% cases respectively. In venous thrombosis all 3 parameters were elevated in 4 patients and either of 2 parameters in 10 patients. However there was no statistically significant difference for D-dimer (F=1.167, p=0.331), RDW (F=0.419, p=0.740) and Factor VIII (F=1.305, p=0.283) values for the various clinical conditions of thrombosis.

Conclusions: Comorbidities may or may not be associated with an event. Factor VIII levels followed by D Dimer and RDW were elevated . Elevation of two or more markers were seen in complications like pulmonary embolism , inferior vena cava / portal vein thrombosis or polycythemia. These simple test combinations can help to predict outcome and risks in thrombosis especially venous thrombosis.

PB 1432 | Longitudinal Assessment of Coagulation and Fibrinolysis in Pregnant Women with Risk Factors for Preeclampsia

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Background: Preeclampsia-PE is a multifactorial disease whose pathophysiology was not fully elucidated. No laboratory marker to present favorable cost-effectiveness ratio was proposed for PE diagnosis, which is primarily done based on blood pressure and proteinuria determination and clinical symptoms.

Aims: To assess coagulation and fibrinolysis markers in pregnant women with risk factors for PE.

Methods: Women were selected and followed throughout pregnancy. D-Di and PAI-1 plasma levels were determined by ELISA in 23 samples from 11 pregnant women who developed PE and 55 from 17 women who did not develop the disease in the following gestational periods 12-19,20-29,30-34 and 35-40 weeks.

Results: There was no significant difference in D-Di and PAI-1 levels in pregnant women who developed PE compared to those who did not develop the disease, in each of the 4 gestational periods evaluated. However, a significant increase in D-Di levels in pregnant that developed PE was observed when comparing the 12-19x30-34 gestational periods(P=0.045), 12-19x35-40(P< 0.001), 20-29x30-34(P=0.048), 29x35-40 weeks(P=0.030). Similar results were found in the ones that did not develop the disease: 12-19x30-34 periods(P=0.001), 12-19x35-40(P=0.000), 20-29x30-34(P=0.003), 29x35-40 weeks(P=0.001). A significant increase in PAI-1 levels was also obtained in pregnant women who developed PE, comparing the periods 35-40x12-19(P=0.001), 35-40x20-29(P< 0.001) and 35-40x30-34 weeks(P< 0.001) and in women who did not develop the disease in 12-19x20-29(P=0.004), 12-19x30-34(P< 0.001), 12-19x35-40(P=0.001), 20-29x35-40(P=0.009) and 30-34x35-40 weeks(P=0.002).

Conclusions: There was a trend of increase in D-Di and PAI-1 levels throughout gestation in pregnant women who developed and who did not develop PE. This increase was more evident in those who developed the disease, which allow inferring that D-Di and PAI-1 are promising candidates to integrate a diagnostic algorithm for PE, to be applied after a clinical score and associated with imaging tests

PB 1433 | Role of Oxidized High-density Lipoprotein (oxHDL) on Coagulation and Platelet Adhesion Modulation

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Background: High density lipoprotein (HDL) exerts some antithrombotic properties by inhibiting coagulation and modulating platelet reactivity. Nevertheless, when oxidative modification of HDL takes place due to pathological condition, subsequent generation of oxHDL occur and unknown detrimental properties emerge.

Aims: To determine the effects of oxHDL on coagulation function and platelet adhesion.

Methods: Primary rat mesenteric endothelial cells (RMEC) were isolated from SD rats (180 g approximately). In addition, an endothelial cell line was obtained form ATCC. Endothelial cells were stimulated with HDL or oxHDL (50 µg/mL) and alteration of coagulation and thrombosis-related protein expression was determined. Furthermore, blood serum from vehicle- and oxHDL-treated rats was subjected to coagulation and thrombosis-related protein expression analysis and coagulation activity measurements. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Results: TF and P-selectin protein expression in RMEC increased significantly in response to oxHDL treatment, compared to control condition. Also, we observed a significant increase in platelet adhesion in response to oxHDL treatment, when compared to control. (Preliminary)

Conclusions: Our results provides evidence which suggests that oxHDL modulates coagulation and promotes platelet adhesion.

PB 1434 | Inter-individual Variability and Normal Ranges of Whole Blood and Plasma Thrombin Generation and Correlation with Clotting Factor Levels in a Large Healthy Control Population

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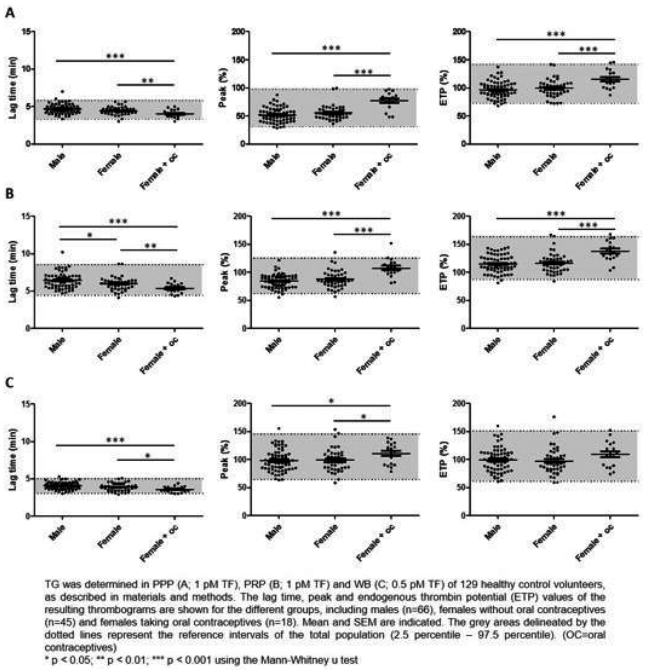
Background: Global assays measuring thrombin generation (TG) in plasma increasingly gained attention in the field of thrombosis and hemostasis. Adaptation of the method enabled the measurement of TG in whole blood (WB). Despite their potential, TG assays did not yet reach the stage of universal clinical application, partly due to the absence of normal ranges.

Aims: To accurately determine normal ranges/inter-individual variability of TG and correlate results with coagulation factor levels, sex and oral contraceptive (OC) usage.

Methods: The study protocol was evaluated by the local medical ethical board. 129 healthy volunteers gave full informed consent. Clotting factors were determined with STA-R technology; normal ranges of TG in platelet-poor/platelet-rich plasma (PPP/PRP) and WB according to CLSI guidelines.

Results: Our study is the first to measure normal ranges of TG in PPP, PRP and WB in a large healthy cohort. Significant correlations were found between TG in plasma and WB. Inter-individual variability of TG in WB was comparable to that of plasma (10 to 27% for all parameters). OC use increased TG in PPP/PRP/WB (figure).



TG was determined in PPP (A; 1 pM TF), PRP (B; 1 pM TF) and WB (C; 0.5 pM TF) of 129 healthy control volunteers, as described in materials and methods. The lag time, peak and endogenous thrombin potential (ETP) values of the resulting thrombograms are shown for the different groups, including males (n=66), females without oral contraceptives (n=45) and females taking oral contraceptives (n=18). Mean and SEM are indicated. The grey areas delineated by the dotted lines represent the reference intervals of the total population (2.5 percentile – 97.5 percentile). (OC=oral contraceptives)
* p < 0.05; ** p < 0.01; *** p < 0.001 using the Mann-Whitney u test

FIGURE 1 Effect of sex and oral contraceptive use on TG parameters determined in PPP, PRP and WB

The inhibitory effect of thrombomodulin on TG was significantly lower in females compared to males and this effect was more pronounced upon OC use. Main clotting factor determinants for TG parameters depended on the TF concentration but were similar in WB/PRP/PPP.

Conclusions: Establishing normal ranges for TG brings us one step closer to clinical use. Good correlations between plasma and WB (also regarding clotting factor determinants for TG) suggest that also WB TG can be reliably used in clinic. The relatively high inter-individual variability in TG is important to consider. Previously, inhibition of TG by a fixed concentration of anticoagulant -also direct FXa/thrombin inhibitors- proved to be highly variable from one individual to another. These data may argue against the idea that one standard dose of new oral anticoagulants fits for all.

PB 1435 | Venous Thromboembolism Events over Five Years Presenting with a Normal D-Dimer

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Background: Failure of the d-dimer level in its negative predictive capability to rule out VTE, can be a real concern. To address this problem in a large hospital, data has been collated on d-dimer levels between 2012 and 2016, in PE and DVT diagnoses. Community acquired thrombosis (CAT) is by far the main source of this data, with an aim to determine patterns in VTE diagnosed in the context of a negative d-dimer.

Aims: To identify all VTE events presenting with normal d-dimer levels over five years. Determine whether there are specific risk factors, medical history or presentation in those VTE events, compared to patients presenting with elevated d-dimer levels.

Methods: Observational cohort study reviewing CAT events, associated with a d-dimer test, Liatest D-I plus, from January 1st 2012 to December 31st 2016. VTE diagnosis is determined from radiology records, with demographic information and VTE risk factors obtained from patient notes. The mean age and standard deviation was calculated for all VTE events. The student's t-test was used to detect significance between positive and negative d-dimer groups.

Results: 3079 VTE events (1366 DVT & 1713 PE) associated with a d-dimer test were identified. 72 events (38 DVT 34 PE), having a normal d-dimer (< 0.50 ug FEU/ml). Normal d-dimer patients had a mean age of 47.34 (SD ± 16.30) significantly lower than the mean age of all VTE patients being 62.70 (SD± 18.230) (p< 0.0001). Patients with a previous VTE and normal d-dimer comprised 25/72 (35%) compared with 680/3079 and raised d-dimer (22%) (p=0.011).

Conclusions: Over 5 years, patients presenting with a VTE event and a normal d-dimer were significantly younger and more likely to have had a previous thrombosis than those with a raised d-dimer. The younger age at presentation may be explained by the age-related association with d-dimer levels.

PB 1436 | Biomarkers for the Diagnosis of Venous Thromboembolism: D-dimers, Thrombin Generation, and Phospholipid-dependent Clotting Time

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Background: The diagnostic algorithms for venous thromboembolism (VTE) currently involve a composite of clinical pre-test probability, laboratory D-dimer (DD) and specific imaging techniques. However, different DD assays show differences in sensitivity and specificity, and other research tests might assist in the identification of VTE patients.

Aims: To assess the accuracy of different laboratory tests (DD, thrombin generation and phospholipid-dependent clotting time) as biomarkers of acute VTE.

Methods: Samples arriving at the Coagulation Laboratory at Mater Dei Hospital (Msida, Malta) from the Accident and Emergency Department with a request for DD measurement were collected. The following tests were performed: Innovance DD (Siemens Healthcare Diagnostics), HemosIL DD HS (Instrumentation Laboratory), Thrombin Generation (using the CAT), STA Procoag PPL (Diagnostica Stago). According to Innovance DD, samples were divided in 3 groups: negative DD (group 1), positive DD without VTE (group 2), positive DD with VTE confirmed by lower limb compression ultrasonography or computed tomography pulmonary angiography (group 3).

Results: These are preliminary data of 38 out of 75 samples planned for this study (13 in group 1, 13 in group 2, 12 in group 3). The agreement between Innovance and HemosIL DD in the categorization of patients was 95%, since 2 patients with positive Innovance DD but no evidence of VTE, were classified as negative by HemosIL DD. The phospholipid-dependent clotting time was slightly shorter in VTE patients vs non-VTE patients (median 32.2 sec in Group 3 vs 35.5 in Groups 1 and 2 joined, p=0.34).

On the thrombin generation curve, lag time was prolonged (5.25 vs 4.33 min, p=0.18), peak thrombin concentration increased (303 vs 279 nM, p=0.23) and velocity index increased (118.4 vs 96.9 nM/min, p=0.07).

Conclusions: The results of this study suggest that some laboratory research tests might have the potential to support the diagnosis of VTE. However, this data should be confirmed on a larger sample size.

PB 1437 | Risk Factors and Clinical Characteristics Favoring Pulmonary Embolism Vs DVT in Hormonal Therapy (HT) Associated Thromboembolism. Retrospective Analysis of 73 Cases from a Single Hemostasis Unit

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Background: In women who use combined oral contraceptives (COC) the risk of Thromboembolism (TE) is increased 3-6 fold compared to non- users. Inherited thrombophilias synergize to an excess risk of thrombosis among users of OC. Age and family history constitute risk factors for VTE among women on hormonal treatment. However, limited data exist regarding risk factors and clinical characteristics leading either to HRT induced PE or DVT.

Aims: Our objectives were to analyze the baseline characteristics of women who suffered VTE during the use of COC and to investigate possible risk factors favoring the development of PE or DVT.

Methods: We studied 73 COC users, who suffered TE, with a median age of 28 years, which received COC for a median period of 2 months. Among them, 4 (5.5%) suffered ischemic strokes while 69 VTE. Lower limbs were the predominant site of VTE n=42(60.9%), followed by Pulmonary Embolism (PE) n=17(27.5%), Upper limbs n=7(10.1%), CNS n=7(10,1%) and Abdomen n=4(5.8%). 17/73 (23,3%) presented TE recurrence, while in total 19/73 (26,0%) suffered PE either as 1st episode or during recurrence. We analyzed, patients age, duration of hormonal treatment intake, smoking, family history, thrombophilia profile and assess their impact on the development of PE or DVT. For that purpose we used SPSS 20.0 package with lower limit of sensitivity set at 0,05.

Results: Median age of women that developed PE was 33,6 years contrasting the median age of the non-PE population which was 28,7 years p= 0,034. 9/21(42.9%) of women that reported family history of thrombosis presented PE, while 10/52 (19.2%) with negative family history manifested PE p= 0,037.PE was not associated with duration of hormonal treatment, smoking, and thrombophilia profile. Elevated plasma homocysteine was encountered more often, albeit not significantly (p= 0.07).

Conclusions: In HRT-VTE increased age and positive family history seem to favor PE phenotype. Although small and retrospective, our study is the first to demonstrate the above.

PB 1438 | The Influence of Ethnicity on Thrombin Generation

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Background: Ethnicity is touted as an independent risk factor for venous thromboembolism (VTE) although the basis for this is varied and contentious. Comparison of plasma thrombin generation using calibrated automated thrombogram (CAT) across ethnic groups offers a modality that objectively measures global haemostatic function to evaluate this influence. Direct comparative data across ethnic groups is currently not available.

Aims: To establish the influence of ethnicity on plasma thrombin generation.

Methods: 60 normal subjects, matched for age and gender, equally representing 4 ethnic groups - Caucasian, Chinese, Indian and Malay,

were recruited. TG parameters {lag time, time to peak, peak and endogenous thrombin potential (ETP)} in platelet-poor plasma were measured using CAT.

Results: Mean ETP±SD for the different ethnic groups were as follows: Caucasians - 1338.18±194.19nM.min, Chinese - 1318.91±108.90nM.min, Indians - 1389.81±182.61nM.min and Malays - 1436.21±184.24. Caucasian subjects have the longest mean lag time - 2.59±0.37s; Indian subjects had the highest mean peak - 284.22±30.74nM and Malay subjects had the longest mean time to peak - 5.47±0.59s apart from the highest mean ETP. Statistical analysis based on ethnicity however did not demonstrate any significant difference for all thrombin generation parameters.

Conclusions: In a population of healthy subjects, thrombin generation mediated by plasma factors is not influenced by ethnicity and does not explain the reported racial differences in VTE incidence. In view of the absence of significant difference, the use of separate normal ranges according to ethnic groups for plasma thrombin generation is not essential.

PB 1439 | Is Platelets - Bound C4d a Marker for Active Disease and Correlates with Hypercoagulability in Patients with Systemic Eritematosus Lupus?

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Background: Platelets - bound C4d (P-C4d) is a potential biomarker of active disease and hypercoagulability in patients with systemic lupus erythematosus (SLE). Patients positive for P-C4d have shown disease activity related to a higher frequency of cardiovascular events associated with thrombosis than patients negative for P-C4d. In fact, considering the biological role of platelets in hemostasis and coagulation, it is believed that high levels of P-C4d may be related to the thrombotic tendency in patients with SLE. By other side, Thrombomodulin (TM) and D dimer (DDi) are recognized biomarkers for endothelial lesion and hypercoagulability status, respectively.

Aims: This study has evaluated P-C4d, TM and DDi levels and their relationship to disease activity in SLE women under treatment, compared to controls.

Methods: Disease activity was defined by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI- 2K). This study included 90 women with similar age distributed into 3 groups: 1) Healthy women (control, n=30); 2) SLE women with low disease activity (SLEDAI 2K ≤ 4, n=30), and 3) SLE patients with /moderate high disease activity (SLEDAI 2K > 4, n=30). Analysis of P-C4d was performed by flow cytometry using anti-CD42a antibody to identify platelet population and antibody specific for C4d. Endothelial lesion and hypercoagulable status were assessed by thrombomodulin and D-dimer measurements in citrated plasma samples, respectively. Data analysis was performed by Mann Withney using GraphPad Prism software 6™.

Results: The results are shown in Table 1.

Conclusions: Increased levels of P-C4d, TM and DDi, compared to controls, are directly linked to disease activity defined by SLEDAI-2K index and may indicate a thrombotic potential associated with the SLE inflammatory process. Support: CNPq,CAPES and FAPEMIG

PB 1440 | Profiling of Thrombotic and Inflammatory Mediators in Synovial Fluid from Patients Undergoing Primary Total Joint Arthroplasty

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Background: Synovial fluid is an intra-articular fluid whose main function is to lubricate joints and decrease the coefficient of friction. The role of inflammatory and thrombotic mediators in degenerative joint disease (DJD) has been well established. Previous reports have suggested that increased plasma levels of biomarkers such as IL-1b, CRP, and (MP-TF) play a role in the DJD. It is hypothesized that the levels of these biomarkers in synovial fluid may provide additional information on the pathogenesis of DJD.

Aims: This study is designed to profile synovial fluid samples for various inflammatory biomarkers and to demonstrate their relevance to the progression of DJD.

Methods: This study consisted of 25 patients undergoing primary total joint arthroplasty (TJA) of the hip or knee for DJD. Patients were consented prior to surgery in accordance with LUMC IRB. At the time of arthrotomy, a sample of synovial fluid was obtained using a syringe and

TABLE 1 *P-c4d, Thrombomodulin and D dimer levels expressed as medians in women with Systemic Lupus Erythematosus under treatment*

	Controls (n=30)	Patients with SLE (n=60)	SLE Patients with Low Activity (n=30)	SLE Patients with Moderate/ High Activity (n=30)	P value*
P-C4d(P-C4d/μL)	a,b,c5.080	a 42.47	b,d 12.70	c,d 69.45	a0,0000 b0,0003 c0,0000 d0,0000
TM(ng/mL)	a,b,c 0.21	a 0.29	b,d 0.25	c,d0.34	a0,0341 b0,0000 c0,0007 d0,0002
DDi(ng/mL)	a,b,c 374	a 1155	b,d 930	c,d 1563	a0,0133 b0,0000 c0,0198 d0,0004

placed into a tube containing calcium citrate for preservation and analysis. Biomarkers including IL-1b, MP-TF and CRP were measured using commercially available ELISA methods from R&D laboratories (Minneapolis, MN). Total protein levels were measured by nantrope technology.

Results: Total protein levels ranged from 5-65 mg/ml (mean 18 mg/ml +/- standard deviation 13 mg/ml). IL-1b levels ranged from 0.04-0.96 pg/ml (0.19pg/ml +/-0.18pg/ml). MP-TF levels ranged from 0-55 pg/ml (14pg/ml+/-19pg/ml). CRP levels ranged from 0-7 ug/ml (1.5 ug/ml +/- 1.9ug/ml).

Conclusions: The presence of IL-1b and CRP in synovial fluid is highly suggestive of an ongoing inflammatory process, whereas MP-TF represent is a thrombotic biomarker which is a complex of microparticles with tissue factor. The wide variation in the levels of these markers may suggest differing degrees of degenerative and inflammatory processes in DJD. These levels can be quantified to provide more insight into the role of these mediators in the pathogenesis of DJD.

PB 1441 | The Proteins with Prothrombine Origine in Blood Plasma of Ischemic Stroke Patients as a Markers of Hipercoagulation

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Background: Shifts of the balance between coagulation and fibrinolysis play a crucial role in pathogenesis and treatment of cerebral ischemia.

Aims: In this study, we characterized the main markers of hipercoagulations as proteins with prothrombin origin in patients in acute as well as post acute phase of ischemic stroke.

Methods: Blood plasma samples were taken from 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardioembolic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. Vitamin K-dependent plasma proteins fraction was obtained by sorption on barium sulfate. Systemic generation of the proteins with prothrombin origin at the vitamin K-dependent fractions was done by ELISA. Fraction composition was described throuhg size-exclusion chromatography on Healthcare „HiLoad 16/60 Superdex 200 pg" column.

Results: Increased concentration of hipercoagulation marker in blood plasma of acute ischemic stroke patients was showed. Concentrations become more close to physiological one year past. Results of chromatographic separation of fraction obtained from the patients with ischemic stroke in acute and post acute phase suggests the presence of proteins with Mr from 70 to higher 200 kDa. Mostly all peaks were presented in each tested fractions. Just the high of peaks was different for each tested fraction. The fact is that just one vitamin K-dependent protein - prothrombin with Mr 70 kDa is in bloodstream of healthy donors in the highest amount and its level become lover during the acute stroke. In this frame we could assume important role of prothrombin in the pathological process of the ischemic stroke development.

Conclusions: The first characterization of the coagulation marker in acute and post acute period was done. The difference in quantity and quality composition of the fractions could be used as a potential target for the prevention of the stroke repetition.

PB 2085 | Molecular Genetic Investigations in Hereditary Hemorrhagic Telangiectasia in Hungary; Identification of a Founder Mutation

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Background: Hereditary hemorrhagic telangiectasia (HHT; Osler-Rendu-Weber syndrome) is an autosomal dominant vascular disease characterized by the presence of epistaxis, mucocutaneous telangiectases and visceral arteriovenous malformations (AVMs). Mutations in the genes for endoglin (*ENG*), the activin receptor-like kinase 1 (*ACVRL1*) and *SMAD4*, which encode proteins of the transforming growth factor-beta superfamily are responsible for the disease. Approximately 85 % of HHT cases have heterozygous family-specific mutations either in the *ENG* or *ACVRL1* genes, causing HHT type 1 and 2, respectively. Clinical diagnosis of the disease is based on the four Curacao criteria.

Aims: Our aims were to identify causative mutations of HHT in Hungary and to examine if common mutations exist.

Methods: Genetic analysis was performed in HHT index patients and their relatives (total n=85) between 2012 and January 2017. All exons and flanking intronic regions of *ENG*, *ACVRL1* and *SMAD4* were determined by direct fluorescent sequencing. Genotyping of markers D12S1677, D12S85, D12S2196, D12S1712, D12S270, rs2071219, rs706815 and rs706816 around *ACVRL1* gene was performed in 14 carriers of the *ACVRL1* c.625+1 G>C and 50 healthy controls to ascertain the possibility of a founder effect.

Results: The mutation detection rate was 75% among the 41 HHT index patients, 46% were detected with ENG (11 known and 6 novel), 29% with *ACVRL1* (3 known and 4 novel) mutations and no *SMAD4* mutations were found. A novel *ACVRL1* c.625+1 G>C mutation was detected in 6 apparently unrelated HHT families. Haplotype analysis of the above-mentioned genetic markers suggested a founder effect. The genealogical analysis revealed that the possible common ancestors were married in 1779.

Conclusions: The identification of a founder mutation in HHT is helpful, since the clinical presentation of the disease becomes more predictable and it might simplify the molecular genetic diagnosis algorithm.

PB 2086 | How Well can Laboratories Make a Diagnosis of Type 3 VWD? Results from a UK NEQAS (Blood Coagulation) Diagnostic Challenge Exercise

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Background: Proficiency testing (PT) in the Haemostasis laboratory usually involves assay of a specified factor assay or screening test on a single sample, as directed by the PT provider. However, this does not reflect "real life" in laboratories where a panel of tests on the same sample is often required to make a diagnosis for a patient with a bleeding history.

Aims: We describe here an exercise where laboratories were provided with plasma and brief clinical details and asked to perform whichever tests they deemed necessary to make a diagnosis.

Methods: 73 centres took part in this exercise. The history given was a 10 year old male with a history of bleeding into joints, with a prolonged APTT. No details were available on the parents. The sample was from a patient previously diagnosed with type 3 VWD.

Results: 41/73 centres correctly identified type 3 VWD in this exercise, with 5 reporting VWD requiring subtyping, and 1 reporting haemophilia or VWD, with further investigation required. 17/73 centres reported haemophilia A, all these centres failed to perform a VWF assay (11 of these centres are registered for VWF assays in the UK NEQAS BC programme, so probably have this assay available, 5 of these centres do not perform VWF assays but did not suggest referral for this investigation). One centre diagnosed a lupus anticoagulant in this sample, and did not perform either FVIII or VWF assays.

The number and pattern of tests employed in this exercise varied markedly - 40/73 centres did not confirm the reported APTT, and 7 performed only a FVIII assay. Mixing studies were carried out in 46 centres, with 14 performing incubated mixes to exclude an inhibitor.

Conclusions: The failure of 18/73 centres to perform sufficient assays to correctly diagnose severe VWD highlights the need for expertise within departments to fully investigate bleeding disorders, and also highlights the value of proficiency testing exercises such as this.

PB 2087 | Rivaroxaban but Not Apixaban Causes False-positive Results in Lupus Anticoagulant Testing that Can Be Overcome with Andexanet Alfa

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Background: Direct Factor Xa (FXa) inhibitors are capable of inducing false-positive lupus anticoagulant (LA) dRVVT results, however information on inter-patient variability and dose response is limited. Andexanet alfa is a recombinant FXa decoy protein, and its incorporation *in-vitro* during LA testing may overcome FXa inhibitor-induced false-positive LA results.

Aims: To determine inter-individual variability and dose-dependent effects of rivaroxaban and apixaban on LA testing and whether andexanet alfa can overcome this interference.

Methods: After informed consent, plasma from 8 healthy volunteers and 12 patients on therapeutic doses of rivaroxaban (n=6) or apixaban (n=6) with known LA status (3/6 LA positive in each group) were collected. Normal pooled plasma, known concentrations of rivaroxaban, apixaban, andexanet alfa and affinity purified LA+ and control IgG were used in experiments. dRVVT and anti-FXa assays were performed on the Sysmex CS5100 analyser.

Results: Plasma from healthy volunteers yielded false-positive dRVVTs when spiked with 200ng/mL rivaroxaban, but not apixaban (100% and 0% of samples, respectively). In pooled normal plasma, rivaroxaban caused more dose-dependent prolongation of the dRVVT Screen relative to the Confirm, leading to a false-positive LA ratio at concentrations ≥100ng/mL. In contrast apixaban proportionately prolonged the dRVVT Screen and Confirm, and even at high concentrations (>1,200ng/mL) the LA ratio remained negative. The addition of andexanet alfa abolished the rivaroxaban-induced false-positive LA results in LA-negative patient plasma without compromising the interpretation of true LA-positive patient results; this was replicated in LA-positive IgG spiking experiments.

Conclusions: Rivaroxaban but not apixaban causes an exaggerated dose-dependent prolongation of the dRVVT Screen, yielding a false-positive LA test interpretation. Andexanet alfa corrects the false-positive LA ratio in patients on rivaroxaban but may not be required for patients on apixaban.

PB 2088 | The Feasibility of Heparin Neutralization Assay to Replace Serotonin Release Assay in the Diagnosis of Heparin-induced Thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) remains diagnostically challenging. Nearly all patients with HIT produce antibodies that recognize the PF4-heparin complex that can be detected by immunoassays. However, these assays are also positive in many heparin-treated patients who do not have HIT. In these cases, functional assays such as the serotonin release assay (SRA) are useful for identifying pathogenic HIT antibodies. However, the SRA is labor intensive and not widely available.

Aims: We assessed the clinical utility of heparin neutralization assay (HNA) and evaluated whether it improves the diagnostic specificity for HIT.

Methods: We implemented a heparin neutralization assay (HNA) in conjunction with PF4/heparin enzyme-linked immunosorbent assay (ELISA) over a one year period. 1194 patient samples were submitted to the laboratory for HIT testing from December 2015 to November 2016.

Results: Using SRA as the "gold standard", the positive predictive value of a positive ELISA (Opitical Density, or OD≥0.4) is only 25%. In fact, ELISA OD < 0.9 or HNA %inhibition < 70% essentially exclude HIT. HNA significant improves the diagnostic specificity in conjunction with ELISA. With ELISA OD≥1.4 and HNA %inhibition ≥70%, the sensitivity of the test combination is 94%, and the specificity 93%. With a more stringent diagnostic criteria for HIT using 4T score and ELISA OD, ELISA/HNA has similar diagnostic accuracy to SRA.

Conclusions: HNA is a much easier procedure than SRA, and it has the potential to facilitate more timely and accurate HIT diagnosis. Based on our data, we propose a new diagnostic algorithm for HIT, which mostly eliminates the need for SRA.

PB 2089 | One Stage and Chromogenic FVIII Assays in Spiked and Post Infusion Samples Containing rFVIIIFc or Recombinant FVIII: Data from a UK NEQAS for Blood Coagulation Survey

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Background: Variability in FVIII:C measurement is a recognised problem although there are few data for samples containing recombinant Factor VIII Fc fusion protein (rFVIIIFc) . Many studies use samples for which factor concentrate has been spiked into FVIII deficient plasma in vitro. This approach should be validated by comparison to samples obtained from patients after infusion of product.

Aims: To assess one stage and chromogenic FVIII assay results in spiked and post infusion samples.

Methods: Four samples were distributed in a UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS) survey. One was constructed by adding Advate, a recombinant FVIII

TABLE 1 One stage FVIII assay results obtained with different APTT reagents

		Post Advate Infusion	Post Advate Infusion	Sample Spiked with Advate	Sample Spiked with Advate	Post rFVIIIC Infusion	Post rFVIIIC Infusion	Sample Spiked with rFVIIIFc	Sample Spiked with rFVIIIFc
APTT reagent used in FVIII assay	n	Median (IU/dL)	Range (IU/dL)	Median (IU/dL)	Range (IU/dL)	Median (IU/dL)	Range (IU/dL)	Median (IU/dL)	Range (IU/dL)
Actin FS	17	56	48 - 68	51	42 - 61	47	42 - 58	46	41 - 58
Actin FSL	2	62	61 - 64	60	59 - 61	56	51 - 62	54	54 - 55
Cephascreen	3	66	57 - 71	58	55 - 66	56	54 - 59	54	53 - 60
PTT Auto	2	59	55 - 63	55	52 - 58	55	49 - 60	46	38 - 54
SynthaSIL	24	57	48 - 72	57	45 - 69	46	39 - 60	42	32 - 53
All one stage	57	57	48 - 72	53	40 - 69	47.4	39 - 62	45.2	32 - 60

TABLE 2 Factor VIIII results obtained with different Chromogenic assay kits

		Post Advate infusion	Post Advate infusion	Spiked Advate	Spiked Advate	Post rFVIIIFc infusion	Post rFVIIIFc infusion	Spiked rFVIIIFc	Spiked rFVIIIFc
Kit used in Chromogenic FVIII assay	n	Median (IU/dL)	Range (IU/dL)	Median (IU/dL)	Range (IU/dL)	Median (IU/dL)	Range (IU/dL)	Median (IU/dL)	Range (IU/dL)
Biophen	12	61	54 - 67	59	55 - 68	61	51 - 66	59	47 - 67
Coamatic	1	51	-	51	-	49	-	51	-
Coatest	3	54	53 - 74	52	51 - 69	53	51 - 78	55	53 - 76
Electrachrome	4	55	51 - 61	53	48 - 63	55	47 - 57	49	44 - 60
Siemens	5	69	66 - 74	69	66 - 74	63	57 - 65	64	59 - 68
Technoclone	2	75	73 - 77	72	69 - 74	83	82 -84	84	83 -84
All Chromogenic	27	61	51 - 77	61	48 - 73	61	47 -84	59	44 - 84

(rFVIII) concentrate to FVIII deficient plasma, one was from a severe Haemophilia A patient after infusion of Advate, one was prepared by addition of rFVIIIFc (trade name Elocta or Eloctate) to FVIII deficient plasma and the 4th was collected from a severe Haemophilia A patient following rFVIIIFc infusion. Fifty-three haemophilia centres in the UK and Scandinavia performed FVIII assays using one stage methods and 27 performed chromogenic FVIII assays. Centres calibrated assays with their local plasma standard.

Results: One stage assay results were lower than chromogenic by 7% and 15% for the two Advate samples, and by 29% and 31% for the 2 rFVIIIFc samples. The inter-laboratory variation was similar for all samples with CVs of 12-16% for chromogenic assays and 10-13% for one stage assay results. Results according to different reagents are shown in table 1 and 2. For both materials there was a highly significant correlation between results obtained on spiked and post infusion samples for both one stage and chromogenic assays.

Conclusions: In this study inter lab variability was similar for rFVIII and rFVIIIFC, and the data indicate that either can be safely monitored by one stage or chromogenic assay. Spiked samples behaved in a similar way to post infusion samples for both products and are therefore suitable for use in proficiency testing exercises.

PB 2090 | Epitope Mapping of Anti-FVIII Antibodies in Hemophilia A Patients Using LumiTope, a FVIII Domain-specific Multiplex Microsphere Based Immunoassay

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Background: FVIII neutralizing antibodies develop in response to replacement treatment in 30% of patients with severe hemophilia A. Such inhibitory antibodies represent a serious complication in the treatment of these patients.

Aims: The aim of the present study was investigation of domain specificity of anti-FVIII antibodies in hemophilia A patients in a time-frame of two years using a multiplex microsphere-based immunoassay on the Luminex™ system.

Methods: FVIII protein was expressed and purified in several single and multi-domain fragments using a baculovirus expression system in insect cells. The domains were purified (>80%) and individually coupled to colored-coded magnetic microspheres. Commercially available full length (FL-FVIII) and B-domain deleted (BDD-FVIII) FVIII proteins were immobilized accordingly. The coupled target proteins were used to establish the Luminex™ based assay, termed *LumiTope*.

Results: In total 765 samples from 266 hemophilia A patients (20 mild, 43 moderate and 203 severe) were analyzed. Among these 200 patients were without and 66 patients were with inhibitor. For all samples domain and isotype specificity of the anti-FVIII antibodies was determined. Our results show that *LumiTope* is a sensitive test for detection of anti-FVIII antibodies. The test revealed positive results against FL-FVIII beads for all patients with antibody titers> 0.6 BU/ml and positive results on FVIIII-ELISA antibody test. Moreover, we were able to identify several monoclonal and polyclonal antibodies against A2a2, a3A3, LC, C1, C2 and C1C2 domains. Detected antibodies were predominantly directed against the A2a2 and C2 domains of FVIII. The IgG1 and IgG4 subclasses contributed to the majority of anti-FVIII IgG response.

Conclusions: Our new immunoassay, *LumiTope*, provides a sensitive and fast method for characterization of inhibitory anti-FVIII-antibodies in hemophilia A patients. The characterization of the binding regions of these antibodies may provide the basis for understanding of inhibitory mechanisms.

PB 2091 | Investigation of an Incidental Finding of Factor 8 Gene Duplication Detected by Chromosomal Microarray Analysis

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Background: A 32 year old female patient (YL) with recurrent pregnancy loss (X4) was referred to the National Hemophilia Center for investigation of an incidental finding of a duplication involving a part of the Factor 8 (F8) gene, which was observed in chromosomal microarray (CMA) analysis performed in her 4th miscarried male fetus.

Aims: To confirm or exclude the involvement of F8 gene in the above duplication with the aim of establishing hemophilia A carrier status for YL.

Methods:

1. Multiplex ligation-dependent probe amplification (MLPA) was used to detect duplication of F8 gene exons.
2. Genotyping based on STR markers was used to establish the grandparental origin of the fetal F8 gene copy involved in the duplication.

Results:

1. The duplicated region indicated by CMA, Xq28 (154,225,507-154,285,587; GRCh37/hg19 genome build), includes F8 exons 1-3. MLPA analysis demonstrated duplication of F8 exons 1-3 in both the miscarried fetus and in YL.
2. Based on STR analysis, YL´s mother was found to be negative for the above duplication, and the fetus was found to carry the haplotype of YL´s father, who was not available for DNA or FVIII level analysis.

Conclusions: Using MLPA, we confirmed the involvement of F8 gene in duplication of a chromosome X segment containing a part of the F8 gene, which was incidentally detected in a miscarried fetus. Furthermore, we detected the same duplication in the mother, suggesting that she may be a hemophilia A carrier. In her future pregnancy, we will be able to offer prenatal diagnosis based on MLPA. In view of the uncertainty regarding the association of the duplication of F8 exons 1-3

with hemophilia A, we will offer fetal blood sampling to measure fetal FVIII level if such duplication is detected in a male fetus in the future.

PB 2092 | Thromboxane Formation Assay to Identify High on-treatment Platelet Reactivity to Aspirin

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Background: Platelet inhibition by aspirin is indispensable in secondary prevention of cardiovascular events. Nevertheless impaired aspirin antiplatelet effects (high on-treatment platelet reactivity [HTPR]) are frequent. This is associated with an enhanced risk of cardiovascular events. The current gold standard to evaluate aspirin induced platelet inhibition is the light-transmittance aggregometry (LTA). However, the pharmacologically most specific test is measurement of arachidonic acid (AA) induced thromboxane (TX) formation. TX is the specific product of the transformation of AA by cyclooxygenase-1 (COX-1). By now, the optimal cut-off to define HTPR to aspirin by inhibition of TX formation is not known.

Aims: We aimed to compare the results of LTA with the TX ELISA assay and calculate a cut-off level for detecting HTPR by TX ELISA assay.

Methods: We measured platelet function in 2507 samples. Arachidonic acid (AA) induced TX formation by ELISA assay and AA induced LTA was used to measure aspirin antiplatelet effects and to detect aspirin specific HTPR.

Results: TX formation correlated nonlinear with the maximum of aggregation in the AA induced LTA (Spearman's rho R=0.7396; confidence interval [CI] 0.7208 - 0.7573, p< 0.0001). Receiver operating characteristic (ROC) analysis revealed 209.8 ng/ml as the optimal cut-off value to detect HTPR to aspirin with the TX ELISA assay (area under the curve: 0.92, P< 0.00001, sensitivity of 82.7 %, specificity of 90.3 %).

Conclusions: TX formation ELISA is reliable in detecting HTPR to aspirin. The calculated cut-off level needs to be tested in trials with clinical end points.

PB 2093 | Prospective Multicenter Validation of Standard and Age-adjusted D-Dimer Testing in Patients with Suspected Pulmonary Embolism (PE) and Deep Venous Thrombosis (DVT)

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Background: D-dimer testing, with or without age-adjustment, is the standard of care for ruling out pulmonary embolism (PE) or deep vein thrombosis (DVT).

Aims: To determine the test characteristics of an automated D-dimer assay for the exclusion of PE and DVT in emergency department (ED) patients using standard and age-adjusted cutoff formulas.

Methods: Prospective observational study of consecutive ED patients with suspected DVT or PE in 24 centers (18 USA, 6 Europe). All patients had low or intermediate Wells scores for PE or DVT. We evaluated the INNOVANCE D-dimer assay on the CS-5100 platform. For the standard cutoff, we considered a D-dimer result < 0.50 mg/L negative. For the age adjusted cutoff, we adjusted cutoff in patients > 50 years old to consider a result negative if D-dimer (in mg/L) was < Age * 10 (years). The diagnostic standard was imaging demonstrating PE (excluding subsegmental PE) or DVT (excluding calf vein DVT, including recurrent and chronic DVT) within 3 months. Patients without 3-month follow up data were excluded. We calculated test characteristics using standard methods. We also explored modifications of the age adjustment formula.

Results: Among 3,586 patients, those evaluated for PE (n=1834) had mean age of 48 ± 16 years, 676 (37%) were male, 1081 (59%) were white and PE prevalence was 5.5% (101/1834). Among those evaluated for DVT (n=1752) the mean age was 53 ± 16 years, 710 (41%) were male, 1172 (67%) were white, and DVT prevalence was 6.4%

TABLE 1 Test Characteristics INNOVANCE D-dimer

	PE (%)				DVT (%)			
Method	Sens.	Spec.	NPV	PPV	Sens.	Spec.	NPV	PPV
Standard cutoff (0.5)	98.02	54.54	99.73	13.75	92.04	45.77	98.61	12.08
Age-adjusted cutoff (Age (if >50)*10)	97.03	59.00	99.63	14.89	92.04	52.51	98.79	13.56

(113/1752). Test characteristics using standard and age-adjusted cut-offs are reported in Table 1. Altering the age adjustment formula did not improve specificity but did increase false negatives.

Conclusions: The INNOVANCE D-dimer assay is highly sensitive and has reasonably high specificity. Age-adjustment allows slightly more patients to be safely excluded, without increasing false negatives. Further modification of this formula does not appear to be beneficial.

PB 2095 | The Detection of Cross-reactive Antibodies to Recombinant Porcine FVIII

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Background: Recombinant porcine FVIII (rpFVIII) is licensed for the treatment of bleeding in adults with acquired haemophilia A (AHA). Its safety and efficacy have not been established in patients with >20 Bethesda units (BU) of cross-reactive antibodies to rpFVIII.

Aims: The aim was to determine the stability, sensitivity of FVIII:C assay and baseline inhibitor titre to rpFVIII in patients with AHA.

Methods: rpFVIII was reconstituted in water then diluted to 1IU/ml of labelled potency in 0% FVIII plasma containing VWF (George King). One-stage FVIII:C (FVIII:C1) using Actin FS (AFS) and 0% FVIII (Siemens) was measured at times 0, 1 and 2 hours post reconstitution against human plasma standard (SHP, Siemens). rpFVIII was serially diluted, 1.25 to 0.06 IU/ml, in 0% FVIII and FVIII:C1, using a single batch of AFS and Synthasil (Werfen), or Chromogenic FVIII (FVIII:CR, Siemens) was assayed against SHP or rpFVIII standard. The Nijmegen modified Bethesda assays (BA) of 9 patients with AHA were performed with rpFVIII used as the source of FVIII. Residual FVIII was measured by FVIII:C1 with AFS. All assays were performed with Sysmex CS5100i instrumentation.

Results: There was no difference in FVIII:C1 between t0 and t2 hours post reconstitution of rpFVIII (t0 was 1.63 IU/ml and t2 was 1.62 IU/ml). rpFVIII measured against SHP was underestimated by FVIII:CR (67% of target) and overestimated by FVIII:C1 (45% with AFS, 20% with Synthasil). The recovery of FVIII:C1 was improved by the use of rpFVIII as the standard however further investigation is required.

Cross-reactive antibodies to rpFVIII were detected in 6 patients with AHA. Porcine titres ranged from 0.83-52.0 BU. 3 of these patients demonstrated non-linear assays common in AHA. 3 patients with antibodies to human FVIII did not have antibodies to rpFVIII (table1).

TABLE 1 Human and porcine inhibitor titre in patients with AHA

Patient ID	FVIII:C1 (IU/ml)	rpFVIII BA (BU)	Linearity	Human FVIII BA (BU)
P1	<0.01	52	linear	>360
P2	0.02	0		8.2
P3	0.03	0		2.5
P4	0.02	0.83	linear	>74
P5	0.03	0		>9
P6	0.04	>5.3	non-linear	>200
P7	0.11	>42	non-linear	>530
P8	0.02	2.0	linear	>380
P9	<0.01	>0.98	non-linear	>102

Conclusions: Not all individuals with AHA have antibodies to rpFVIII. The antibody kinetics varied between patients. The assessment of antibodies to rpFVIII may be useful prior to treatment of patients with AHA.

PB 2096 | Mixing Test to Discriminate between Lupus Anticoagulant and Direct Oral Anticoagulant in Diluted Russell's Viper Venom Time Test

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Background: Lupus anticoagulants (LA) are associated with an increased risk of thrombosis and laboratory detection is major importance. One of the recently developed anticoagulants is rivaroxaban, a direct Xa inhibitor. The false positive results of LA tests were reported in the samples with rivaroxaban.

Aims: The aim of this study is to discriminate between LA and rivaroxaban in LA tests, and distinguish the true positive.

Methods: Thirteen samples positive for LA in APTT and dRVVT reagents were assayed. Ten LA negative samples with rivaroxaban were also used in this study. dRVVT screen and confirm tests were performed in undiluted and 1:1 mix with normal pooled plasma. Screen / confirm normalized ratio was calculated in all samples including the mixing plasmas. Index of circulating anticoagulant (ICA) was used to show the mixing test results in not only screen but also confirm

reagent. ICA = (b - c) / a X 100 where a, b and c are the clotting times of the patient's plasma, the 1:1 mixture, and normal plasma respectively.
Results: The means of screen / confirm ratios in LA and rivaroxaban samples were 1.6, 1.6 for undiluted and 1.3, 1.3 for 1:1 mixing, respectively. The means of ICA values in screen reagent were 18.9 and 24.2 in LA and rivaroxaban samples, respectively. There were no significant differences in these indexes. The means of ICA values in confirm reagent were 2.2 and 19.4, respectively and showed significant difference.
Conclusions: There was a large difference between LA and rivaroxaban group in ICA of confirm. The clotting times were not prolonged in LA samples due to the high phospholipid concentration, but rivaroxaban samples prolonged because of a direct Xa inhibitor in both undiluted and 1:1 mix plasmas. When screen / confirm ratio shows more than cut-off value in rivaroxaban suspicious samples, ICA of confirm could be useful to interpret the ratio results. The mixing test of confirm reagent can discriminate between LA and rivaroxaban samples.

PB 2097 | Assessment of Three Contact Activation Reagents for the Diagnosis of Factor XI Deficiency

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Background: FXI deficiency is usually diagnosed by prolonged aPTT (ratio>1.3) and confirmed by coagulation assays (FXI:C< 70%). The low bleeding risk of carriers and the limitations of current diagnostic methods suggest that FXI deficiency might be underestimated. Sensitive and reliable methods are required.
Aims: To assess 3 contact activation commercial reagents in subjects with congenital FXI deficiency.
Methods: Plasma of 140 cases with FXI deficiency (9 homozygous or compound heterozygous and 131 heterozygous for 12 mutations) were collected. Under identical conditions, samples were evaluated in ACL TOP 500 coagulometer with the following reagents:
1) SynthASil (SS; silica-based),
2) SynthAFax (SF; ellagic acid-based), both from Instrumentation Laboratory, and
3) EA-DG-APTT (EA; ellagic acid-based) from Grifols. *F12* rs1801020 was genotyped usingTaqman probes.
Results: Severe FXI deficiency prolonged aPTT with all reagents. However, a high proportion of mild deficiencies would not be detected using aPTT, with false negatives of 77% for EA, 22% for SS and 12% for SF. 42-46% of false negatives corresponded to cases with qualitative deficiency (CRM+). The common *F12* SNP significantly prolonged aPTT when activated by silica. FXI:C values obtained with ellagic acid were higher than those with silica (SF: 47.7±12.7%, EA: 46.3±10.9% *vs.* SS: 40.4±14.9%). Thus, silica gave 2-3-fold lower rate of false negatives (2.1%) than ellagic acid; most of them were CRM+. Noteworthy, FXI:C levels were not determined by current adjustments with ellagic acid in cases with severe deficiency.
Conclusions: Mild FXI deficiency, particularly CRM+, might be underestimated due to the high rate of false-negative results that render aPTT assays. Other elements of the contact phase, as *F12* SNP, and the strength of the activation may explain the high rate of false negatives. Our results suggest that the best method to detect and quantify FXI deficiency is FXI:C using silica.
Funding: SEHH. PI15/00079; CB15/00055 (ISCIII, FEDER); 19873/GERM/15.

PB 2098 | Platelet Reactivity in a Large Population with the Bruneck Cohort

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Background: The Bruneck Study began in 1990 as a population-based study in 1000 people on the epidemiology and pathogenesis of cardiovascular disease with a follow-up every 5 years. Extensive platelet reactivity using multiple concentrations of agonists has rarely been studied in large cohorts due to constraints of blood volume, time and expertise. We have developed a high throughput plate-based (Optimul) assay requiring a relatively small amount of blood and minimal technical skill.
Aims: To provide in-depth platelet reactivity phenotyping in a large population.
Methods: Fasting blood was taken from 338 people (age 55-98, 51% male) in citrate (0.105M) vacutainers and platelet rich (PRP) and poor (PPP) plasma was obtained by centrifugation. Light transmission aggregometry (LTA; AA 1mM; ADP 5 & 20μM; collagen 0.4, 4 & 10μg/ml; TRAP-6 amide 25μM; U46619 10μM) and Optimul aggregometry (AA 0.3-1.5mM; ADP 1-30μM; collagen 0.4-30μg/ml; epinephrine 0.6-10μM; ristocetin 0.1-1.5mg/ml; TRAP-6 0.1-25μM; U46619 0.01-10μM) were performed within 2 hours of blood draw. % aggregation was calculated and data were analyzed using R with the nlpr package. Data is reported as mean±s.d.
Results: Mean % aggregation to AA (30±33%), ADP 5μM (58±14%) and 20μM (62±12%), collagen 0.4μg/ml (35±27%), 4μg/ml (60±14 %), 10μg/ml (60±15%), TRAP-6 (64±12%) and U46619 (65±11%) was generated by LTA. Additionally, maximum % aggregation to agonists were calculated from the Optimul assay for AA (78±17%), ADP (85±6%), collagen (87±8%), epinephrine (76±19%), ristocetin (89±6%), TRAP-6 (88±5%) and U46619 (87.9±3.7%).
Conclusions: This is the first complete study of platelet reactivity using LTA and the Optimul assay in a large population. We are

currently interrogating the platelet reactivity data according to clinical characteristics such as sex, age and smoking status. The potential of our assay in gathering data easily in large cohorts is unprecedented and can only advance the field of platelet reactivity testing.

PB 2099 | Evaluation of IgG4 Anti-FVIII as Strategy to Improve the Diagnosis of Factor VIII Inhibitor in Association with Modified-Bethesda Assay

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Background: Alternative methods for better evaluate the presence of factor(F) VIII inhibitor have been assessed during the last years.

Aims: The aim of this study was evaluate the use of FVIII-IgG4 in order to improve the inhibitor testing, currently based on modified-Bethesda assay (MBA).

Methods: Samples from 47 hemophilia A patients, and 4 acquired FVIII inhibitor patients were assessed. Each plasma sample was tested to MBA and FVIII-IgG4 by ELISA.

Results: In total 269 plasma samples, divided into 3 groups, were analyzed. The correlation of all samples for MBA and FVIII-IgG4 were 0.70 (p< 0.0001). Group A: 44 samples with MBA negative (< 0.6BU), with median MBA of 0.11BU (0-0.56), had median FVIII-IgG4 not detectable (ND) (ND-1:640). Group B: 94 samples with MBA 0.6-5BU, and median of 1.32BU, with median FVIII-IgG4 titer of 1:80 (ND-1:2560), being ND in 19% of samples. Group C: 55 samples with MBA >5BU and median of 32.58BU (5.2-1254BU) with median FVIII-IgG4 titers of 1:1280 (1:20-1:81920). Interestingly, one patient who developed inhibitor, showed first 3 consecutives MBA negative results with high titers of FVIII-IgG4 (1:320-1:640). When MBA became positive the levels of FVIII-IgG4 were maintained. Patients with similar MBA results showed different FVIII-IgG4 titers, suggesting a higher sensitivity of FVIII-IgG4 compared with MBA. For 76 samples, from 11 patients under ITI, the MBA was performed using both coagulometric and chromogenic methods. Samples from 6 patients had concordance in all 3 tests. However, for 3 patients with both chromogenic MBA and FVIII-IgG4 negative, the coagulometric MBA was 0.6-6.4BU.

Conclusions: The classification of inhibitor in high and low titer is an important clinical information. This study suggests that FVIII-IgG4 was able to improve the specificity of inhibitor detection in comparison to MBA assay.

PB 2100 | Comparison of Age-adjusted D-dimer and Clinical Probability-adjusted D-dimer for Diagnosing Pulmonary Embolism

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Background: Diagnosing pulmonary embolism (PE) in the emergency department can be challenging and often results in the over-utilization of CT pulmonary angiography (CT-PA). Age-adjusted D-dimer has been shown to decrease CT utilization rates. Recently, clinical probability-adjusted D-dimer has been promoted as an alternative strategy to reduce CT scanning.

Aims: To compare the safety and efficacy of the age-adjusted D-dimer and clinical probability-adjusted D-dimer rules in Canadian ED patients tested for PE.

Methods: This was a retrospective chart review of ED patients investigated for PE at two hospitals from April 2013 to March 2015. Inclusion criteria were ED physician ordered CT-PA, Ventilation-Perfusion (VQ) scan or D-dimer for investigation of PE. Patients under the age of 18 were excluded. PE was defined as CT/VQ diagnosis of acute PE or acute PE/DVT in 30-day follow-up. Trained researchers extracted anonymized data. The age-adjusted D-dimer and clinical probability-adjusted D-dimer rules were applied retrospectively. The rate of CT/VQ imaging and false negative rates were calculated.

Results: In total, 1,189 patients were tested for PE. 1,129 patients had a D-dimer and a Wells score less than 4.0, or a Wells score greater than 4.0 with or without a D-dimer. 364/1,129 patients (32.3%, 95%CI 29.6-35.0%) would have imaging for PE if the age-adjusted D-dimer rule was used. 1,120 patients had a D-dimer and a Wells score less than 6.0, or a Wells score greater than 6.0 with or without a D-dimer. 217/1,120 patients (19.4%, 95%CI 17.2-21.2%) would have imaging for PE if the clinical probability-adjusted D-dimer rule was used. The false-negative rate was 0.3% (95%CI 0.1-0.9%) for the age-adjusted D-dimer and 1.0% (95%CI 0.5-1.9%) for the clinical probability-adjusted D-dimer.

Conclusions: The false-negative rates for the age-adjusted D-dimer and clinical probability-adjusted D-dimer are low. The clinical probability-adjusted D-dimer results in a 13% absolute reduction in CT scanning compared to age-adjusted D-dimer.

PB 2101 | The Screening aPTT Mixing Test Is Not Sufficient to Rule Out the Presence of Inhibitor

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Background: Many laboratories routinely perform screening aPTT mixing test to evaluate the presence of inhibitors, which if positive triggers the investigation with the modified-Bethesda assay (MBA).

Aims: The aim of this study was evaluate the sensitivity/specificity of screening aPTT mixing test to identify factor (F) VIII inhibitors.

Methods: This study had 2 phases. Phase 1: Evaluation of 15 laboratories for their capacity to assess FVIII inhibitors presence using aPTT mixing test. Each laboratory received 3 lyophilized plasma samples from patients with hemophilia A (HA), acquired HA and health individuals (controls), with 3.45, 5.67 and 0.4BU, respectively. Phase 2: According to the results the central lab evaluated each step of the procedure, including FVIII source and different mathematical approaches.

Results: 4/15 (27%) laboratories showed inadequate results for aPTT mixing test in at least one sample, including false positive or negative results. In the phase 2, the central lab analyzed 59 samples from 44 HA and 6 acquired HA patients. These samples were tested in parallel to MBA and aPTT mixing test, with previous incubation at 37°C for 2h. In MBA assay, 38 samples were negative for inhibitor (< 0.6BU) and 21 positive. No correlation between aPTT mixing test and MBA results was observed. aPTT mixing test was false positive in 12/59 (25%) samples using not buffered in house pool, and 4/59 (7%) using commercial plasma. For the mathematical approach, classical aPTT reference range correction and Rosner index had 5% of false negative results, and using WFH previous recommendation 14%. The sensitivity/specificity was calculated for 10 procedures combinations. The range of sensibility to inhibitor detection was from 70 to 100% and the specificity 64 to 93%.

Conclusions: Although, aPTT mixing test is simple with low cost, it is not enough to rule out the presence of FVIII inhibitor. This suggests that aPTT mixing test should not be recommended as screening test, due to the occurrence of false negative results.

PB 2102 | Quantitation of Dabigatran by Global and Specific Coagulation Assays

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Background: The direct oral thrombin inhibitor dabigatran offers the advantage of fixed dosing without the need for laboratory monitoring, in contrast to warfarin. Therapeutic ranges for dabigatran have not been established and no assay for its activity is approved by the US FDA; the EMEA has approved a thrombin time test with dabigatran calibrators (HYPHEN), available in the EU.

Aims: We sought to assess the ability of common tests such as the thrombin time (TT), global hemostasis assays such as thromboelastography (TEG), and a specific ecarin clotting time (ECT) activity assay to quantify dabigatran.

Methods: 110 plasma samples from 11 healthy volunteers who underwent testing of dabigatran pharmacokinetics in IRB-approved research (Circulation 2016 134:1909-1911) were tested with the thrombin time (TT), an ECT dabigatran assay (Stago Diagnostica), and TEG (Haemonetics) assays. Dabigatran levels for each sample were determined by liquid chromatography tandem mass spectroscopy (LCMS).

Results: Samples with 0- 221 ng/ml dabigatran (by LCMS) were tested. Table 1 lists the correlation between each test parameter and LCMS dabigatran level. The TT was highly correlated with dabigatran LCMS values. A normal TT (< 20.5 seconds) excluded dabigatran values of > 3.4 ng/ml. An ECT dabigatran assay likewise was highly correlated with dabigatran MS values. TEG R times obtained without kaolin activator ("native" TEG) demonstrated variable correlation with LCMS dabigatran levels. R times obtained by TEG with kaolin activator ("kaolin" TEG) similarly had poor correlations with, and sensitivity to dabigatran.

TABLE 1 Correlation of Various Assay Parameters with LCMS Dabigatran Level.

	Thrombin Time	ECT	R (native) TEG	R (kaolin) TEG
Correlation Range for 11 Individual Subjects (r)	0.93-1.0	0.86-0.99	0.55-0.94	0.51-0.90
Mean of 11 Subject Correlations (r)	0.98	0.95	0.74	0.73
Correlation for All Samples Combined (r)	0.86	0.91	0.56	0.43

Conclusions: The TT was extremely sensitive to, and highly correlated with dabigatran levels from 3.4 ng/ml to 131 ng/ml. The ECT assay was likewise highly correlated with dabigatran LCMS between 0 and 221 ng/ml. TEG R times (native or kaolin) showed poor sensitivity to, and inferior correlations with dabigatran LCMS levels, and were limited by high inter-subject variability.

PB 2104 | Influence of the Statistical Approach on Cut-off Values of IgG/IgM Anticardiolipin and Anti-β2 Glycoprotein I Antibodies in Classification of Antiphospholipid Syndrome

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Background: IgG/IgM anticardiolipin (aCL) and anti-β2 glycoprotein I (aβ2GPI) antibodies are laboratory criteria for classification of antiphospholipid syndrome (APS). Recommendations published in 2014 by the SSC-ISTH state that threshold for positivity should be determined by the 99th percentile (p) of at least 120 healthy subjects.

TABLE 1 The performance characteristics of aβ2GPI at different cut-off levels (*: 90% CI statistically not possible on the data)

			N=120			N=200		
		Insert cut-off	99th NPP	99th NPP (Tukey)	99th NPP (Reed)	99th NPP	99th NPP (Tukey)	99th NPP (Reed)
aβ2GPI IgM	Cut-off (70%* CI)	20,0	31,1 (---) (N=120)	3,5 (---) (N=99)	19,4 (---) (N=119)	20,0 (12,2-34,0) (N=200)	2,3 (---) (N=154)	16,9 (11,8-20,0) (N=199)
	Sens (95% CI)	7,14 (4,29-11,05)	6,35 (3,67-10,11)	26,59 (21,24-32,50)	7,14 (4,29-11,05)	7,14 (4,29-11,05)	34,13 (28,29-40,34)	8,73 (5,55-12,92)
	Spec (95% CI)	95,95 (93,17-97,83)	97,20 (94,74-98,71)	82,87 (78,29-86,82)	95,64 (92,79-97,60)	95,95 (93,17-97,83)	76,32 (71,29-80,87)	95,02 (92,03-97,12)
	OR (95% CI)	1,82 (0,88-3,79)	2,35 (1,02-5,41)	1,75 (1,17-2,62)	1,69 (0,82-3,46)	1,82 (0,88-3,79)	1,67 (1,16-2,41)	1,82 (0,94-3,55)
aβ2GPI IgG	Cut-off (70%* CI)	20,0	136,2 (---) (N=120)	11,4 (---) (N=115)	45,5 (---) (N=119)	48,8 (11,4-159,4) (N=200)	11,4 (11,4-11,4) (N=195)	31,3 (11,4-49,0) (N=199)
	Sens (95% CI)	26,19 (20,87-32,08)	17,86 (13,33-23,15)	32,14 (26,42-38,29)	21,03 (16,17-26,59)	20,63 (15,81-26,16)	32,14 (26,42-38,29)	22,22 (17,24-27,87)
	Spec (95% CI)	95,95 (93,17-97,83)	98,75 (96,84-99,66)	89,41 (85,51-92,55)	98,13 (95,98-99,31)	98,13 (95,98-99,31)	89,41 (85,51-92,55)	96,88 (94,35-98,50)
	OR (95% CI)	8,41 (4,51-15,66)	17,23 (6,10-48,62)	4,00 (2,57-6,23)	13,98 (5,90-33,13)	13,65 (5,76-32,37)	4,00 (2,57-6,23)	8,89 (4,43-17,83)

TABLE 2 The performance characteristics of aCL at different cut-off levels (*: 90% CI statistically not possible on the data)

			N=120			N=200		
		Insert cut-off	99th NPP	99th NPP (Tukey)	99th NPP (Reed)	99th NPP	99th NPP (Tukey)	99th NPP (Reed)
aCL IgM	Cut-off (70%* CI)	20,0	209,2 (---) (N=120)	13,8 (---) (N=106)	64,1 (---) (N=119)	64,2 (33,5-247,8) (N=200)	12,6 (---) (N=179)	63,5 (32,5-64,2) (N=199)
	Sens (95% CI)	12,70 (8,85-17,45)	0,79 (0,10-2,84)	16,27 (11,94-21,42)	6,35 (3,67-10,11)	6,35 (3,67-10,11)	17,46 (12,98-22,72)	6,35 (3,67-10,11)
	Spec (95% CI)	92,52 (89,08-95,15)	99,07 (97,29-99,81)	87,85 (83,77-91,22)	97,51 (95,15-98,92)	97,51 (95,15-98,92)	86,92 (82,73-90,40)	97,51 (95,15-98,92)
	OR (95% CI)	1,80 (1,03-3,14)	0,85 (0,14-5,11)	1,41 (0,88-2,26)	2,65 (1,12-6,30)	2,65 (1,12-6,30)	1,41 (0,89-2,22)	2,65 (1,12-6,30)
aCL IgG	Cut-off (70%* CI)	20,0	42,5 (---) (N=120)	3,2 (---) (N=103)	23,8 (---) (N=119)	24,5 (7,9-47,3) (N=200)	3,2 (---) (N=174)	21,1 (7,9-24,5) (N=199)
	Sens (95% CI)	21,03 (16,17-26,59)	18,65 (14,04-24,02)	39,68 (33,60-46,01)	19,84 (15,10-25,31)	19,84 (15,10-25,31)	39,68 (33,60-46,01)	19,84 (15,10-25,31)
	Spec (95% CI)	98,44 (96,40-99,49	99,07 (97,29-99,81)	77,57 (72,61-82,02)	98,44 (96,40-99,49)	98,44 (96,40-99,49)	77,57 (72,61-82,02)	98,44 (96,40-99,49)
	OR (95% CI)	16,83 (6,62-42,83)	24,30 (7,47-79,11)	2,28 (1,58-3,27)	15,64 (6,13-39,89)	15,64 (6,13-39,89)	2,28 (1,58-3,27)	15,64 (6,13-39,89)

However, no specific recommendations regarding statistical methods are indicated.

Aims: To determine the impact of statistical methods on the calculated 99thp for aCL and aβ2GPI.

Methods: 99thp was calculated on 120 and 200 healthy subjects using a non-parametric percentile method (NPP) with different tests identifying outliers (Tukey/Reed). Specificity (spec), sensitivity (sens) and odds ratio (OR) were calculated in a patient cohort applying different

cut-offs. We included 114 patients with thrombotic APS, 138 with non-APS thrombosis, 138 with auto-immune disease and 183 healthy controls (no clinical criteria for APS). IgG/IgM aCL and aβ2GPI were determined by a chemiluminescent immunoassay (HemosIL®AcuStar).

Results: Results of the normal population were not Gaussian distributed; Box-Cox/logarithmic transformations did not normalize the data. The manufacturer's cut-off was valid according to the transference principle (NCCLS-C28-A3). The calculated 99thp and their confidence intervals (CI) are shown in table 1 and 2.

Tukey classified more subjects as outliers lowering the 99thp by up to 93%. Large CI were observed.

Conclusions: The choice of the statistical method influences the calculated cut-off. We recommend the use of conservative methods to detect outliers. Nonetheless, excluding outliers by Reed hardly influences the performance characteristics. CI illustrate that larger sample sizes (n>200) are needed for a reliable calculation of the 99thp. Although guidelines recommend a local cut-off calculation, a multicenter approach to collect a large number of normals may be a good alternative, as well as transference of the manufacturer's cut-off if populations are comparable.

PB 2105 | Reference Interval Mean Clotting Times Should Not Be Used to Calculate Lupus Anticoagulant Mixing Test Ratios Unless They Match the Normal Pooled Plasma Clotting Time

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Background: Lupus anticoagulant (LA) testing can be enhanced if clotting times (CT) are converted to ratios via normal pooled plasma (NPP) or reference interval mean (RIM) CT as denominator. The advantage of NPP is that it reflects immediate assay system performance but NPPs with CTs distant from RIMs can systematically bias towards false-positive or -negative results. There are no data on how applying RIM CTs to mixing tests that mix test plasma with NPP of RIM-distant CTs affects interpretation.

Aims: To assess mixing test interpretations in one assay with similar NPP and RIM CTs and another where they are discordant.

Methods: Diagnostic data on 311 samples from non-anticoagulated patients positive for LA by dilute Russell's viper venom time (dRVVT) and/or dilute activated partial thromboplastin time (dAPTT) using RIM CTs for screen, confirm and 1:1 mix ratios were further evaluated using NPP CTs as mix test ratio denominators. 104 of the patients had antiphospholipid syndrome (APS), 34 had systemic lupus erythematosus (SLE) and persistent LA, 27 had SLE and first LA positive testing, and 146 were clinically appropriate patients being investigated for APS.

Results: All were LA-positive in undiluted plasma, 92 in dRVVT only, 156 in dAPTT only and 63 in both. RIM CTs (s) for dRVVT and dAPTT screen were 43.8/41.4 respectively, mean NPP CTs (n=38) 44.0/36.0 respectively. Locally established mix ratio cut-offs derived from RIM CT and NPP CT were >1.07/>1.13 respectively for dRVVT, and >1.10/>1.15 respectively for dAPTT. Mixing test interpretations are in Table 1.

TABLE 1 dRVVT and dAPTT mixing test ratio interpretations derived from reference interval mean or normal pool plasma clotting time denominators

Mixing test interpretations from ratios derived from RIM CT or NPP CT as denominator	RIM + NPP +	RIM - NPP -	RIM + NPP -	RIM - NPP +
dRVVT (n=155)	96 (61.9%)	42 (27.1%)	10 (6.5%)	7 (4.5%)
dAPTT (n=219)	55 (25.1%)	111 (50.7%)	0 (0%)	53 (24.2%)

Conclusions: Most dRVVT mix interpretations (89%) agreed, doubly negative samples arising from the dilution effect. 17 discordant results arose from slightly different cut-offs. dAPTT mix was less sensitive as 50.7% were doubly negative. The lower NPP than RIM CT elevated a further 24.2% of dAPTT mix ratios above the cut-off as they reflected LA's effect on plasma in which they were mixed. If NPP and RIM CTs are discordant, NPP CT should be used for mix ratios.

PB 2106 | Lupus Anticoagulant-hypoprothrombinemia Syndrome (LAHPS): Report of 4 Cases

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Background: LAHPS, the association of acquired FII deficiency and lupus anticoagulant (LA), is a rare disorder caused by aFII antibodies. It is drastically different from antiphospholipid syndrome (APS); it may cause predisposition to severe bleeding, in addition or not to thrombosis.

Aims: We report and discuss 4 cases of LAHPS.

Methods: LA was detected using a sensitive APTT and DRVVT, according to the ISTH guidelines. Hypoprothrombinemia was defined as FII under reference value (< 70IU/dL); abnormal PT and FII, were corrected by normal plasma (NP). Coagulation factors were determined by one-stage methods; those tests based on APTT were interfered by LA.

Results: Table 1 summarises laboratory results and clinical features. All cases showed abnormal PT, corrected by NP, and prolonged APTT, not corrected by NP. Cofactor effect on APTT was observed when FII was ≤25IU/dL. No time-temperature dependent inhibition was detected. Cases showed APTT and DRVVT tests compatible with LA, as well as low FII (8-52IU/dL) corrected by NP. In 3/4 cases, low FVIII (1.2-50IU/dL) and FIX (2-40IU/dL) and increased apparent activity on progressive dilutions of sample were observed. Acetic acid

TABLE 1 Laboratory results and clinical features

CASE (sex/ age)	Platelet count (plat/µL)	PT(%)/ Mixing test	FII (IU/ dL)	APTT(sec)/ Mixing test/ Confirmatory test (RV: 37-48)	DRVVT/ Mixing test/ Confirmatory test	TT (ratio) (RV:0.75-1.25)	Other coagulation factors (IU/dL)	Clinical features
1 (M/27)	70000	50/C	25	138/NC/+	Prolonged/NC/+	0.95	FV:70; FVII:96; FVIII:4; FIX:2; FX:100; abnormal CST	Intracerebral haematoma
2 (F/34)	216000	47/C	8	94/NC/+	Prolonged/NC/+	1	FV:80; FVII:80; FVIII:1.2; FIX:8; X:82	Severe bleeding, requiring blood transfusion, after caesarean section
3 (M/57)	154000	58/C	38	57/NC/+	Prolonged/NC/+	1.12	FV:80; FVII:90; FVIII:50; FIX:40; X:88	Asymptomatic
4 (M/59)	172000	65/C	52	62/NC/+	Prolonged/NC/+	1.05	FV:70; FVII:110; FVIII:60; FIX:60; X:100	Severe bleeding, requiring blood transfusion, after tooth extractions and plastic surgery

M: male; F: female; C: corrected by normal plasma; NC: not corrected by normal plasma; (+): positive confirmatory test; CST: acetic acid clot solubility test

clot solubility test was abnormal, suggesting low FXIII in 1 patient; he also showed thrombocytopenia. Other tests, like those to study von Willebrand disease and platelet function, were normal. One case was asymptomatic, the rest had suffered from bleeding. No other associated condition was found.

Conclusions: LAHPS has a heterogeneous spectrum of appearance. While it has been reported associated with other clinical conditions (primary APS, infections, drugs, systemic lupus erythematosus), in any of cases presented here, an associated condition was found; all except one, referred bleeding symptoms. LAHPS diagnosis is challenging; it should be suspected when abnormal PT+positive LA results were observed, mainly in the context of bleeding complications. A careful analysis should be done in order to achieve accurate diagnosis.

PB 2107 | Fibrinogen Prothrombin Time Derived Method Is Not Useful in Anticoagulated Patients by LMWH or Rivaroxaban

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Background: Fibrinogen prothrombin time derived method (FibPT-d) is an easily available and economic method in laboratories with automated cogulometers. There are few reports on the behavior of this test in anticoagulated patients with DOACs or LMWH.

Aims: Compare Fibrinogen results obtained by Clauss method (FIBC) and FibPT-d with two thromboplastins in anticoagulated patients.

Methods: Population: 285 consecutive patients with a normal PT and APTT before initiating anticoagulation:102 with vitamin -K antagonist (VKA), 50 with unfractioned heparin (UFH), 35 with LMWH, 50 with rivaroxaban (RIVA) and 48 with dabigatran (DABI) and 100 healthy controls (NC).

Methods: Fib C by HemosiL Fibrinogen C (FIBC) or reagent with 100 NHI thrombin U/mL for DAB samples (HemosiL Q:F:A or TriniCLOT Fibrinogen); Fib PT-d with rabbit brain (HemosiL PT Fibrinogen HS plus, Fib PT HS) and with human recombinant (HemosiL Recombiplastin 2 G, FibPTRP) thromboplastins; heparin level by HemosiL Liq anti Xa; RIVA level by Liq Anti Xa with specific calibrators (HemosiL Rivaroxaban calibrators) and DABI level by HemosiL Direct Thrombin Inhibitor Assay. All FIB assays were calibrated with Calibration Plasma and performed on ACL TOP platform.

Data Analysis: BIAS % between FibC and FibPT-d with each thromboplastin were calculated. Comparisons between methods was performed by Bland Altman plots.

Results: The table shows the BIAS % between FIBC and Fib PT-d with each trhomboplastins. Bland Altman analysis shows that there is a positive bias ($p < 0.001$) for FibPT-d in either NC or anticoagulated samples, but BIAS was significantly higher compared to NC for RIV and VKA samples with FIB PT HS in a concentration dependent manner. LMWH samples present a higher bias compared with NC with both thromboplastins used.

TABKE 1 % BIAS FIB C vs FIbPT-d with both thromboplastin. (p vs NC)

Group	BIAS (%) Fib-PTHS	BIAS (%) Fib-PTRP
Normal controls n=100	13,19 ±10.93	16,38±9.45
VKA anticoagulated pts n=102 (INR:1,10 - 11,18)	31.82±16.36 p<0.0001	20.72±10.56 p<0.0001
UFH anticoagulated pts n=50 (0,16-1,08 anti Xa/mL)	19.54±15.18 p=0.068	22.86±9.80 p=0.017
LMWH anticoagulated pts n=35 (0.10-1.27 anti Xa /mL)	24.62±16.54 p<0.0001	26.86±13.63 p<0.0001
Rivaroxaban anticoagulated pts n=50 (13.94 - 845 ng/mL)	34.52±17.41 p<0.0001	16.38±12.05 p=0.47
Dabigatran amticoagulated pts n=48 (25-995 ng/mL)	18.04±13.14 p=0.0368	17.83±9.60 p=0.49

Conclusions: Although the FibPT-d is an economic and rapid method, it should not be used in all situations because of the FIB PT- d mathematical algorithm is only validated in normal subjects without anticoagulation.

PB 2108 | Development of Novel Assays for Factor VIII Potency Determination

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Background: The determination of factor VIII (FVIII) plasma levels is widely used for diagnosis of patients and monitoring of their treatment with clotting factor concentrates. Furthermore, the potency determination of plasma-derived and recombinant FVIII concentrates is used during manufacturing and batch release. Several assays have been established for the determination of FVIII but the one-stage clotting assay and the chromogenic assay are predominantly used. For potency measurements of FVIII concentrates, the European Medicines Agency (EMA) requires the chromogenic method in accordance with the European Pharmacopoeia, while the US FDA requires the one-stage clotting assay. However, modified recombinant FVIII products such as B-domain deleted as well as fusion proteins yield different potencies with these two assays and also with different reagent kits.

Aims: We want to improve fluorogenic and clotting-based FVIII assays in order to reduce discrepancies in potency determination of different types of FVIII products.

Methods: Based on previous studies we have further developed our fluorogenic assay using low amounts of FIXa as trigger and by addition of corn trypsin inhibitor (CTI) to prevent spontaneous activation of the intrinsic pathway. The conditions of this assay were transferred to a clotting assay with optical readout.

Results: By addition of CTI to commercially available FVIII-deficient plasma we achieved a significant improvement and simplification compared to previous assays. With both readouts the assays displayed a large measurement range and high reproducibility. The optical clotting assay could be adapted to an automated analyser.

Conclusions: The adaptations of our FVIII assays may help to resolve assay discrepancies between FVIII products while offering a simple and automated format for the processing of clinical samples as well as concentrates.

PB 2109 | Evaluations of Automated Chromogenic Assays for the Diagnosis and Monitoring of Haemophilic Patients

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Background: One-stage clotting assay is still the most widely used method for measuring both FVIII and FIX activities in haemophilic patients. Chromogenic assay is increasingly diffusing, in particular for monitoring the recovery of the novel longer acting concentrates.

Aims: To evaluate the analytical performance of newly developed applications of BIOPHEN FVIII:C and BIOPHEN Factor IX (HYPHEN BioMed, France) on Sysmex CS 2400 (Sysmex, Kobe, Japan) analyzer and their diagnostic accuracy evaluating possible discrepancies between one-stage and chromogenic assays.

Methods: Sixty patients with haemophilia A and 40 with haemophilia B (severe, moderate and mild), were enrolled together with 40 healthy Italian subjects (aged 20-62 years). The clotting tests were performed on citrate platelet poor plasmas stored at -80°C. BIOPHEN FVIII:C was compared with another chromogenic automated assay (COAMATIC Factor VIII, CROMOGENIX on ACL TOP analyzer; Instrumentation Laboratory, Italy).

Analytical performance and method comparison analysis were performed according to the Clinical & Laboratory Standards Institute (CLSI) guidelines.

Results: Imprecision analysis results are reported in Tables 1 and 2. Linearity was good up to 1/128 dilution for both assays (r=0.99); mean recovery was 91.74 % for BIOPHEN FVIII:C and 97.28% for BIOPHEN Factor IX.

Generally, a good correlation was found between one-stage and chromogenic assays for both FVIII:C and FIX:C (Spearmen's Rank correlation: 0.983 and 0.994, respectively) and between the two FVIII chromogenic assays (Spearmen's Rank correlation: 0.991).

TABLE 1 BIOPHEN FVIII:C imprecision

		Mean ± SD (IU/dL)	CV%
Intra-assay (repeatability)	Normal Control 87 (70-104) IU/dL	91.26±1.45	1.59
	Pathologic Control 28 (21-35) IU/dL	30.27±0.51	1.68
	Pool A	121.61±6.28	5.16
Inter-assay (reproducibility)	Normal Control 87 (70-104) IU/dL	90.96±1.48	1.63
	Pathologic Control 8 (21-35) IU/dL	31.09±0.41	1.32
	Pool B	140.04±3.81	2.72

TABLE 2 BIOPHEN Factor IX imprecision

		Mean ± SD (IU/dL)	CV%
Intra-assay (repeatability)	Normal Control 109 (87-131) IU/dL	97.50±2.05	2.11
	Pathologic Control 34 (25-43) IU/dL	30.70±0.89	2.89
	Pool A	87.50±1.45	1.66
Inter-assay (reproducibility)	Normal Control 109 (87-131) IU/dL	104.33±7.72	7.40
	Pathologic Control 34 (25-43) IU/dL	35.56±2.42	6.81
	Pool B	98.69±7.22	7.32

Conclusions: Our results showed a good analytical performance of both the assays. The introduction of chromogenic FVIII and FIX tests as part of routine analytical panel could help to determine the most suitable functional haemostatic assay for the diagnosis and the monitoring of haemophilic patients treatment, particularly using new longer acting products.

PB 2110 | Evaluation of Utility of ISTH Scoring System and its Modifications in Diagnosis of Disseminated Intravascular Coagulation in a Tertiary Care Centre in Southern India

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Background: DIC is an acute emergency whose diagnosis is based on clinico-pathologic picture. In our setup routine diagnosis is based on clinical profile along with microangiopathic haemolysis and FDP positivity. However in contrast to a scoring system, this lacks objectivity.

Aims: To evaluate the

1. ISTH scoring system and its modifications in patients suspected with DIC
2. agreement among routine method, ISTH scoring system and its modifications.

Methods: Blood samples were collected in EDTA and Citrate for cell count with peripheral smear and coagulation assays respectively. Scoring was done and analysis of agreement between the scores considering ISTH score as reference standard was done by kappa analysis. Various scoring algorithms are given in Table 1.

TABLE 1 Components of ISTH score, Fibrinogen exclusion score (Modified ISTH score) and Modified ISTH score with schistocytes

Parameter involved	ISTH score	Fibrinogen exclusion score	Modified ISTH Score with Schistocytes
Platelet count	>1,00,000 =0 51,000-1,00,000 =1 <50,000 =2	>1,00,000 =0 51,000-1,00,000 =1 <50,000 =2	>1,00,000 =0 51,000-1,00,000 =1 <50,000 =2
D - Dimer / FDP	No increase=0 Moderate increase=2 Strong increase=3	No increase=0 Moderate increase=2 Strong increase=3	No increase=0 Moderate increase=2 Strong increase=3
Prolongation of PT	<3 sec=0 >3 sec - <6 sec=1 >6 sec=2	<3 sec=0 >3 sec - <6 sec=1 >6 sec=2	<3 sec=0 >3 sec - <6 sec=1 >6 sec=2
Fibrinogen (g/l)	≥1 =0 <1 =1	Not included	Not included
Schistocytes	Not included	Not included	Absent=0 Present=1
Diagnostic of DIC	5/8	4/7	5/8
Considered diagnostic score (Since FDP quantification not done)	5/7	4/6	5/7

FDP quantification could not be performed in our lab, hence FDP positive was taken as moderate increase for scoring. Approval from Institute Ethics Committee and informed consent from participants were obtained.

Results: Among the total of 56 cases, 36 (64.28%), 9 (out of 52 - 17.3%), 22 (39.3%) and 17 (30.4%) were diagnosed positive by routine diagnosis, ISTH score, Fibrinogen exclusion score (Modified ISTH score) and Modified ISTH score with schistocytes respectively. Fibrinogen assay was not performed in 4 cases. Frequency of derangement in various parameters among cases diagnosed positive by scoring systems are mentioned in Table 2.

The agreement between routine diagnosis, Fibrinogen exclusion score (Modified ISTH score) and Modified ISTH Score with schistocytes with ISTH DIC score was fair (κ = 0.215, p = 0.012); moderate (κ = 0.502, p = 0.000) and substantial (κ = 0.681, p = 0.000) respectively.

TABLE 2 Frequency of deranged parameters in positive cases according to diagnostic modalities under consideration

Parameter	All cases (n=56)	Routine diagnosis (n=36)	Fibrinogen Exclusion score (n=22)	Modified ISTH score with Schistocytes (n=17)	ISTH score (n=9) (out of 52 cases)
Thrombocytopenia (≤10000/μL)	47 (83.92%)	33 (91.66%)	20 (90.90%)	16 (94.11%)	9 (100%)
PT prolongation (>3 sec)	26 (46.42%)	17 (47.22%)	19 (86.36%)	14 (82.35%)	9 (100%)
Fibrinogen (< 100 mg/dl)	2 (3.85%) (not done in 4 cases)	2 (6.06%) (not done in 3 cases)	2 (10%) (not done in 2 cases)	2 (13.33%) (not done in 2 cases)	2 (22.22%)
FDP / D-Dimer (present)	26 (46.42%)	23 (63.88%)	16 (72.72%)	14 (82.35%)	7 (77.77%)
Schistocytes (present)	39 (69.64%)	33 (91.66%)	15 (68.18%)	15 (88.23%)	7 (77.77%)

Conclusions: The best agreement was observed between Modified ISTH score with schistocytes and ISTH score. A considerable number of cases were under diagnosed by ISTH score and Fibrinogen assay in every case is difficult in a resource constraint setup. Hence in cases where ISTH score is difficult to assess Modified ISTH score with schistocytes can be considered.

PB 2111 | Method Comparison of the Atellica COAG 360 System* with Sysmex CS-2000i System and BCS XP System

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Background: The Atellica COAG 360 System* is a novel, fully automated random-access blood coagulation analyzer. The system includes coagulometric, chromogenic, immunological, aggregation, and high-sensitivity LOCI®-based immunoassay technology. Cap-piercing and advanced preanalytical sample integrity (PSI™) checks provide fill-volume checks for primary tubes and assay-specific warning levels for HIL interferences.

Aims: The aim was to compare suitability and efficiency in coagulation testing of the new Atellica™ COAG 360 System* (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) with other coagulation analyzers in a multicenter validation study.

Methods: Method validation and comparisons were performed for selected parameters including PT, APTT, fibrinogen, Ddimer, antithrombin, and vWF activity using >60,000 routine samples from various sources, including general physicians and intensive-care centers.

Results: Atellica COAG 360 System* based analysis is highly consistent with BCS® XP System and Sysmex® CS-2000*i* System results (>100 samples/parameter), leading to high correlation coefficients: r > 0.975 for all assays tested. The Atellica COAG 360 System* provided robust and reliable results. Atellica COAG 360 System* software and hardware features, including speed of diagnostics, result flagging, continuous reloading of reagents and consumables, high onboard reagent stability, graphical display of reaction curves, and ability to import assigned values for controls via file transfer, were appreciated by the users.

Conclusions: Coagulation analyses with the novel Atellica COAG 360 System* are comparable and non-inferior to those performed with the established BCS XP and Sysmex CS-2000*i* systems. Considering the various novel functionalities provided by the Atellica COAG 360 System*, the system is expected to be well-suited for routine and specialty testing requirements in coagulation laboratories.

*Not available for sale in the U.S. Product availability varies by country.

PB 2112 | Evaluation of an Automated Chemiluminescent Assay and two ELISA's for Detection of Heparin Induced IgG Antibodies in the Diagnosis of Heparin Induced Thrombocytopenia (HIT)

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Background: Heparin Induced Thrombocytopenia (HIT) is an adverse drug reaction to heparin in which the patient forms platelet factor 4 (PF4) antibodies (PF4Ab). The antibodies form PF4-Ab/heparin complexes binding FcγIIa receptors on platelets activating them, with formation of microparticles and clots. Correct and rapid diagnosis is crucial because of high risk of thrombosis and death. Diagnosis based solely on clinical symptoms is not straightforward. Confirmation with a rapid and reliable assay is therefore of the utmost importance.

Aims: Performance characteristics of an automated chemiluminescent assay (HemosIL® AcuStar HIT IgG, Instrumentation Laboratory, Bedford, MA, USA) and two enzyme linked immunosorbent assays (ELISA) (Asserachrom® HPIA HIT-IgG, Diagnostica Stago, S.A.S. France and Zymutest HIA IgG, Hyphen Biomed, Neuville-sur-Oise, France) for detection of heparin induced IgG antibodies in the diagnosis of HIT were evaluated.

Methods: Citrated plasmas from 153 patients with clinical suspicion of HIT were evaluated with all assays and confirmed with a functional, flowcytometric assay. Manufacturers's cut-off and optimized cut-off calculated by receiver operating curve (ROC) were evaluated.

TABLE 1

Pre- and post-test probability for Zymutest HIA IgG, HemosIL® AcuStar HIT IgG and Asserachrom® HPIA HIT –IgG with cut-off calculated per run as recommended by the manufacturer, per 4T score class.

			Post-test probability (%)					
			Zymutest HIA IgG		HemosIL® AcuStar HIT IgG		Asserachrom® HPIA HIT–IgG	
		Pre-test probability (%)	Negative	Positive	Negative	Positive	Negative	Positive
4T score	Low R (2/54)	3.57 [0.98-12.12]	0.00 [0.0-7.87]	18.18 [2.28-51.78]	3.7 [0.45-12.75]	0.00 [0.00-84.19]	0.00 [0.00-7.71]	20.00 [2.52-55.61]
	Intermediate R (9/58)	13.43 [7.23-23.60]	0.00 [0.00-8.41]	36.00 [17.97-57.48]	0.00 [0.00-6.38]	81.82 [48.22-97.72]	0.00 [0.00-7.71]	42.86 [21.82-65.98]
	High R (9/21)	30.00 [16.66-47.88]	0.00 [0.00-23.16]	56.25 [29.88-80.25]	0.00 [0.00-20.59]	64.29 [35.14-87.24]	0.00 [0.00-21.8]	71.43 [47.82-88.72]

* 95% CI between []; number of HIT positive/HIT negative samples between ()

Cut-off for AcuStar: 1.00 U/mL, ELISA: mean for all batches (Zymutest: 0.34 OD, Asserachrom: 0.24 OD)

TABLE 2

Pre- and post-test probability for Zymutest HIA IgG, HemosIL® AcuStar HIT IgG and Asserachrom® HPIA HIT –IgG with ideal cut-off calculated with ROC, per 4T score class.

			Post-test probability (%)					
			Zymutest HIA IgG		HemosIL® AcuStar HIT IgG		Asserachrom® HPIA HIT–IgG	
		Pre-test probability	Negative	Positive	Negative	Positive	Negative	Positive
4T score	Low R (2/54)	3.57 [0.98-12.12]	0.00 [0.00-6.85]	50.00 [6.67-93.24]	0.00 [0.00-7.71]	20.00 [2.52-55.61]	0.00 [0.00-6.98]	40.00 [5.27-85.34]
	Intermediate R (9/58)	13.43 [7.23-23.60]	0.00 [0.006.60]	69.23 [38.57-90.91]	0.00 [0.00-7.71]	20.00 [2.52-55.61]	0.00 [0.00-6.72]	64.29 [35.14-87.24]
	High R (9/21)	30.00 [16.66-47.88]	0.00 [0.00-21.80]	60.00 [32.29-83.66]	0.00 [0.00-7.55]	45.00 [23.06-68.47]	0.00 [0.00-19.51]	69.23 [38.57-90.91]

* 95% CI between []; number of HIT positive/HIT negative samples between ()

Cut-off for AcuStar: 0.22 U/mL, Zymutest: 0.71 OD, Asserachrom: 0.59 OD

Results: 20 out of 153 patients were confirmed positive. Sensitivity for Asserachrom® and Zymutest was 100.00% with specificity of 81.20% and 75.94%, respectively. Altering the cut-off, improved specificity to 91.00% for Asserachrom® HPIA HIT-IgG and 90.90% for Zymutest HIA IgG, without loss of sensitivity. With the insert cut-off, sensitivity for AcuStar was 90.00 % with a specificity of 93.23 %. Given that already two cases were missed, no higher cut-off was considered.Pre- and post-test probability was calculated for different cut-offs (table 1 and 2).

Conclusions: This study showed excellent performance of Asserachrom® HPIA IgG and Zymutest HIA IgG, but specificity varies with the selected cut-off. Although less sensitive,HemosIL® AcuStar HIT IgG had best specificity.

PB 2113 | The Influence of a High Fat Meal, Smoking, Coffee, Exercise and Sample Storage on Light Transmission and Impedance Aggregometry in Healthy Volunteers

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Background: Platelet function testing (PFT) is an important part of the workup of patients with a bleeding tendency. Logistic and pre-analytical aspects in PFT are crucial since platelets are viable cells and samples cannot be frozen and analyzed on a later moment in time. Guidelines give recommendations on pre-analysis in PFT but not all advices are evidence based. Some of the recommendations are troubling patient's logistics or are patient unfriendly.

Aims: The aim of this study was to investigate sample storage temperature (4°C, room temperature (RT) and 37°C) and storage time (3, 6, 24 hours), high fat meal, smoking, exercise and coffee drinking on the most used methods for platelet function testing: light transmission aggregometry (LTA) and multiple electrode impedance aggregometry (MEIA).

Methods: Per test condition 5 to 10 healthy volunteers were included. Informed consent was obtained. Citrated/hirudin whole blood and platelet rich citrated (PRP) plasma was used for testing. Platelet aggregation was measured using Chronolog LTA and Multiplate® analyzer. LTA was executed using 1mM arachidonic acid, 5µM ADP, 2µg/ml collagen and 5µM epinephrine as agonists. The Multiplate® was executed with 0.5mM arachidonic acid, 6.4µM ADP, 3.2µg/ml collagen and 32µM thrombin receptor activating peptide as agonists. Statistical analysis were performed using Wilcoxon signed rank test.

Results: No significant differences in platelet aggregation results (LTA and MEIA) could be detected in healthy subjects before and after smoking, high fat meal, exercise and coffee consumption. Citrated whole blood for LTA could be stored for 6 hours on RT. Storage of citrated PRP was agonist dependent possible for 3 or 6 hours. Multiplate results were stable after storage of hirudin whole blood for 6 hours at RT for all agonists.

Conclusions: These results are a first step for improvement of the patient friendliness and logistics in platelet function diagnostics. Prolongation of storage time beyond 6 hours needs new sample stabilizators.

PB 2114 | Automated Data Management for Thrombin Generation

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Background: The thrombin generation (TG) test is widely used nowadays in both fundamental and clinical research. The test itself can easily be adapted to meet specific needs of the investigator. However the use of currently available software for the calculation of TG parameters restricts its flexibility for the development of new applications. Moreover the use of “black box” software might restrict the interpretation of results that are obtained under experimental conditions that are not foreseen by such software.

Aims: The aim of this work was to develop a fully automated and „transparent“ algorithm for the calculation of thrombin generation data and to compare it to the existing black box software.

Methods: Two fully automated algorithms for the calculation of TG parameters were developed. One, written in C, performed a five-parameter fit to obtain ETP, peak, lag time and time to peak and can be used on batches of hundreds of fluorescence curves. The other was designed in Wolfram Mathematica and in addition compensated for temperature irregularities during the course of the experiments.

The performance of both programs was tested in comparison with the standard software on a data set of approximately 5000 raw TG curves.

Results: The data calculated by the two new algorithms were consistent with the data calculated by the standard software for the TG curves obtained in normal pool plasma and plasma of healthy donors (correlation coefficient > 0.97), except for the measurement of the lag time which consistently was longer with the standard program. Discrepancies were observed in the sets of TG data of patients on anticoagulants (both VKA´s & NOACs) and for hemophiliacs. The differences appeared to be mainly due to the algorithms used for the subtraction of α2macroglobulin-thrombin activity.

Conclusions: Both developed algorithms showed itself robust and reliable. The open code in the easily accessible Mathematica programming language makes it a flexible tool for research purposes.

PB 2115 | The Laboratory Control of Anticoagulant Thromboprophylaxis during Early Postpartum Period after the Caesarean Delivery

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Background: The incidence of VTE after caesarean section reaches 0.6% and the widespread use of it draws attention to this group. The dosage and duration of low molecular weight heparin (LMWH) prophylaxis is estimated by anamnestic risk-scales, which predictive potency can be low in case of individual patient's risk estimation. The laboratory hemostasis assays are supposed to solve this problem.

Aims: The aim of this study was to estimate a potency of tests to reflect the coagulation state of patients, receiving prophylactic doses of LMWH in early postpartum.

Methods: We conducted an observational study in 97 women undergoing caesarean section (CS). All gave their written informed consent. The protocol was approved by ethics committee. Blood samples were collected 3-5 hours after the CS (P1) and 2 days after the CS (P2). Standard tests (Fg, APTT, prothrombin, D-dimer), anti-Xa assay, ROTEM and thrombodynamics/thrombodynamics-4D were performed. ROC-analysis, Wilcoxon signed rank test and Mann-Whitney U-test were used for statistical analysis of the data.

Results: At P1 fibrinogen (Median[5-95%]: 5.7[3.3-7.3]; reference range: 2.0-4.7 g/l) and D-dimer concentrations (5118[1532-9999]; 0-550 μg/l), CFT (156[93-292]; 164-430 sec) and α (61[46-73]; 32-60°) in ROTEM, Vi (63[54-70]; 38-56 μm/min), V (39[32-57]; 20-29 μm/min), Vst (38[32-46]; 20-29 μm/min) in thrombodynamics and Ast (206[97-344]; 40-100 AU/l) in thrombodynamics-4D were shifted to hypercoagulation and had the tendency to normalization at P2. Coagulation parameters were compared in groups formed in presence or absence of LMWH for estimation of laboratory assays sensitivity to LMWH influence. Vs had maximal AUC in ROC-analysis (AUC=0.76), Ast had AUC=0.75. For all other tests AUC varied from 0.45 to 0.55.

Conclusions: Coagulation assays reveal hypercoagulation after the delivery and tendency to normalization of coagulation during early postpartum. Parameters of thrombodynamics/thrombodynamics-4D had the highest sensitivity to the presence of LMWH-prophylaxis.

PB 2116 | Forty Years of Riddle Solving to Decipher Puzzling Test Results in the Coagulation Laboratory

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Background: The coagulation laboratory is responsible for performing all the routine and specialized tests in the coagulation field. Occasionally the laboratory activity is "spiced up" by puzzling results requiring out of the box thinking, and solving those issues contributes to enhancement of the professional level of the laboratory, expands clinicians´ expertise and provides great benefit to the patients.

Aims: This abstract illustrates my personal experience accumulated over 4 decades of work, starting as a young technician and stepping up to become the laboratory manager.

Methods: Various coagulation and molecular biology methods have been used to evaluate puzzling test results.

Results: The following cases will be presented:

- Ten possible reasons for very prolonged INR of 5-10.
- Pitfalls in measurements of fibrinogen levels.
- Distinguishing between high D-Dimer levels occurring in vitro or present in vivo.
- Severe bleeding due to rare acquired inhibitor to factor X.
- Thrombosis due to acquired non-neutralizing inhibitor of Protein S.
- How to establish Hemophilia A carriership in women randomly found to have low factor VIII levels.
- Severe thrombosis due to "smart" inhibitor that distinguishes between native Protein C and activated protein C (APC).
- Requirements for antibodies development in factor XI deficiency.
- Infusion of disguised factor XI can cause inhibitor development in factor XI deficiency.
- Bleeding due to exposure to animal's clotting factors.

Conclusions: Each of those ten cases will be presented including a short description, path of laboratory investigation and conclusions. References to published data will be provided. It should be noted that my experience represents collaboration and team work with laboratory personnel and clinicians, to whom I thank from the bottom of my heart.

PB 2117 | "Function over Form"? Low Dose V/Q SPECT is Superior to Planar V/Q and Has a Diagnostic Yield Approaching that of CTPA in the Diagnosis of Acute Pulmonary Thromboembolic Disease

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Background: Since revision of the guidelines for Ventilation/Perfusion (V/Q) scintigraphy by the European Association of Nuclear Medicine (EANM) in 2009, it was suggested that V/Q Single Photon emission computed tomography (SPECT) should be more readily available due to its high sensitivity and specificity for the diagnosis of pulmonary embolism (PE) and low radiation burden.

Aims: To assess diagnostic yield and rate of indeterminate scans when performing low dose V/Q SPECT with holistic interpretation in the diagnosis of acute pulmonary thromboembolic disease and to compare this with published rates.

Methods: All patients that underwent V/Q SPECT (using 100 MBq Tc99m MAA and 20 MBq Technegas) over one year were included in the study. Pregnant patients were excluded. Scans positive and negative for PE and indeterminate scans were recorded. Rates were compared with those published in the literature.

Results: 343 patients were included in the analysis. 14.9% of scans were positive and 76.4% excluded PE. Rate of indeterminate scans was 8.7%, higher than published rates of 3% (Leblanc et al). However, only 19 of 30 patients with indeterminate scans went on to have Computed Tomography of the Pulmonary Arteries (CTPA), (5.5%). This suggests SPECT is superior to planar V/Q, as it has been shown to yield a rate of indeterminate scans requiring CTPA of 20% in the literature. A dichotomous report confirming or excluding PE was provided in 91.3% of cases. This gave a non-diagnostic scan rate of 8.7%, comparable to CTPA similar to Bajc et al where CTPA was non-diagnostic in 6%.

Conclusions: Low dose V/Q SPECT with holistic interpretation is feasible, superior to Planar V/Q and has a diagnostic yield approaching that of CTPA. Given the low radiation burden, and the constant increasing demand on CT services, we believe V/Q SPECT to be an underutilized investigation. It is suggested that this functional test could become a more prominent tool in the assessment of patients with suspected acute pulmonary thromboembolic disease.

PB 2118 | Point-of-care INR with CoaguChek XS Pro is comparable to laboratory INR in a hospital setting

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Background: Point-of-care-testing (POCT) of International Normalized Ratio (INR) to monitor vitamin K antagonist (VKA) anticoagulant therapy is fast, easy and minimal invasive. POCT INR with CoaguChek XS Pro is widely used in patients on stable long-term VKA therapy in outpatient and home settings. However, it is unclear whether POCT INR methods are reliable in clinical practice with patients who undergo changes in anticoagulant treatment.

Aims: To investigate whether POCT INR using CoaguChek XS Pro is comparable to laboratory INR in a clinical patient population.

Methods: POCT INR measurements were performed in patients admitted to the cardiology ward and compared to the reference INR method (manual tilt-tube using HepatoQuick thromboplastin) and the routine INR method (automated method using Innovin reagent). In addition, five other laboratory INR methods were compared. Analytical and clinical agreement was estimated using error grids.

Results: Analytical comparisons of CoaguChek XS Pro and laboratory INR methods with the reference INR method showed a substantial number of deviating INR results in several INR methods. Comparison of CoaguChek XS Pro with the routine INR method revealed an analytical agreement of 79% and a clinical agreement of 83%. The median analytical agreement of all laboratory INR methods compared to the routine INR method was 73% (range 41%-94%) and the median clinical agreement 80% (range 70%-87%).

Conclusions: The analytical and clinical agreement of CoaguChek XS Pro with the routine laboratory method for VKA monitoring in a clinical patient population is acceptable in the context of the observed differences between INR methods.

PB 2119 | Evaluation of Assay Performance Monitoring Edoxaban Plasma Concentration with Technoview® Edoxaban and Technochrom® Anti-Xa

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Background: The increasing use of the new oral anticoagulant edoxaban creates the need of it's measurement in clinical routine in some special clinical settings such as urgent invasive procedures or in cases of bleeding.

Aims: The aim of this study was to evaluate the performance of measurement of edoxaban with a system composed of an anti-Xa assay using lyophilized calibrators and controls, as well as correlation of patients sample results with those measured with Mass Spectrometry Liquid Chromatography (LC-MS).

Methods: The one stage chromogenic assay for the determination of Xa inhibitor activity in human citrated plasma, Technochrom® anti-Xa, is used for edoxaban measurement on Ceveron® alpha. Lyophilzed calibrators with assigned edoxaban values are used for assay calibration. Controls with assigned edoxaban values as well as in vitro spiked

plasma are measured to calculate assay performance parameters. Anti-Xa assay results of patient samples are compared to those obtained with LC-MS reference method to evaluate assay correlation.

Results: Calibration curves in low range 0-150ng/mL and in high range 0-500ng/mL are made using two adjusted analyzer settings. All calibration curves has a linearity of R^2=1.0±0.1. LLoQ is determined at 25ng/mL. The recovery of control is within 100% ± 10% of target value for all concentrations and precision is very good with intra-assay and inter-assay variations of < 15%. For method comparison the correlation of spiked samples as well as patient samples shows a Passing and Bablock regression with a Slope of 1.0 +/- 0.1, an Intercept < 15ng/mL and r >0.95.

Conclusions: Our data demonstrate that using the lyophilized calibrator set Technoview® Edoxaban for calibration of Technochrom® anti-Xa assay in optimized settings on Ceveron alpha the determination of edoxaban plasma concentrations in patient samples can be performed with very good performance, patient sample results correlating with the LC-MS results.

PB 2120 | Evaluation of Platelet Response by Whole Blood Multiple Electrode Aggregometry Method in Patients Using Antiplatelet Therapy

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Background: Platelets play a key role in both hemostasis and thrombosis. Platelet function tests are used for monitoring the efficacy of antiplatelet drugs, with the aim of predicting the adverse events such as bleeding or thrombosis in arterial thrombotic diseases.

Aims: To evaluate results of platelet response by multiple electrode aggregometry in patients using antiplatelet drugs (Aspirin and/or Clopidogrel).

Methods: Platelet aggregation by multiple electrode aggregometry was performed in 650 patients using antiplatelet therapy (Aspirin n=400; Clopidogrel n=47; Aspirin and Clopidogrel n=203). Additionally the test was performed in normal controls (n=59). The blood was collected in hirudin tubes and the platelet aggregation studies were performed in whole blood using Multiplate® Analyser (Roche) equipament. The agonists used were: Adenosine Diphosphate (ADP) final concentration: 6.5 µM and Arachidonic Acid (AA) final concentration: 0.5 mM. The normal range used to compare the response to antiplatelet therapy was 57-113 U for ADP and 71-115 U for AA.

Results: 92%of patients using Aspirin were poor responsive for AA (mean=20U) and 8% were normal responsive for AA (mean= 90U).

In patients using only Clopidogrel, 76.6% were poor responsive with ADP (mean=26U) and 23.4% patients were normal responsive for ADP (mean= 75U).

Regarding patients using combined therapy (Aspirin and Clopidogrel), 91.1% were poor responsive with AA (mean=15U) and 8.9% were normal responsive with AA (mean=92U); 84.2% of patients were poor responsive with ADP (mean=27.7U) and 15.8% were normal responsive with ADP (mean=75U).

Normal controls were 100% responsive to AA (mean 100U) and ADP (mean= 90U).

Conclusions: The use of whole blood multiple electrode aggregometry method is an important tool to evaluate platelet response in patient's using antiplatelet drugs and permits to distinguish patients with normal platelet activity despite a positive history of antiplatelet medication intake.

PB 2121 | Evaluation and Comparison of the Productivity of the Atellica COAG 360 System* and the BCS XP System in Daily Routine Setting

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Background: The Atellica™ COAG 360 System* is a fully automated, high-volume coagulation analyzer. The system includes novel elements to extend system availability time and reduce technician hands-on time.

Aims: The purpose of this study was to evaluate and compare the performance of the Atellica COAG 360 System* and the BCS® XP System, specifically evaluating instrument availability time and technician hands-on time in daily laboratory setting with equal conditions.

Methods: One Atellica COAG 360 System* and three BCS XP Systems (routine devices) were included in this study. Plasma samples (n = 1775) were analyzed routinely over 5 days on one of the BCS XP Systems and on the Atellica COAG 360 System* at Labor Lademannbogen.

Results: The results show extended system availability for the Atellica COAG 360 System*, with an average increase of 54% compared to the BCS XP System. Hands-on technician time was examined at two levels. First, the overall time savings for device preparation using the Atellica COAG 360 System* compared to one BCS XP System was 5 hours 21 minutes over the course of the study week (51% reduction in technician hands-on time). Second, there was an 83% reduction (1 hour 40 minutes vs. 17 minutes per 1000 samples) in technician hands-on time required for sample management and a 41% reduction (3 hours 1 minute vs. 1 hour 46 minutes) for reagent management.

Conclusions: In this study, the Atellica COAG 360 System* performed with increased system availability and reduced technician hands-on time, resulting in the same productivity on one Atellica COAG 360 System* as three of the BCS XP Systems. This advantage over the standard equipment has the potential to affect the overall efficacy and productivity of a high-volume coagulation laboratory.

*Not available for sale in the U.S. Product availability varies by country.

PB 2122 | Rotational Thromboelastometry Parameters Have Variable Correlation with Plasma-based Assays of Coagulation across Trimesters of Pregnancy: A Cross-sectional Study

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Background: In pregnancy and postpartum, women exhibit hypercoagulability and physiologic changes in of coagulation factors. We hypothesized that normative ranges of rotational thromboelastometry (ROTEM) across trimesters of pregnancy correlate with changes in plama-based assays of coagulation

Aims: To compare ROTEM with plasma-based assays in pregnancy.

Methods: After IRB approval, citrated blood was collected from 72 healthy pregnant women and grouped by trimester: 1st (11±3 weeks gestation, n= 20), 2nd (21±5, n=23), and 3rd (33±3, n= 29). EXTEM, INTEM, and FIBTEM were performed on ROTEM (IL, USA) to establish reference ranges. Routine, plasma-based coagulation tests, i.e., activated partial thromboplastin time (PTT), prothrombin time (PT), and Fibrinogen were performed on a STA-R Evolution analyzer (Stago, USA). Data analysis was performed Pearson correlation (SPSS v23, 2016, IBM, USA, significance = P< 0.05).

Results: Fibrinogen showed highest correlations with MCF FIBTEM ($r = 0.92$, $p < 0.001$) and A10 FIBTEM ($r = 0.90$, $p < 0.001$), while CT INTEM correlated modestly with PTT ($r = 0.51$, $p < 0.001$.) Interestingly, CT EXTEM did not correlate with PT ($r = 0.11$, $p = 0.35$,) but rather did correlate with PTT ($r = 0.48$, $p < 0.001$). Fibrinogen level showed a significant contribution to CFT, α-angle, and MCF for both INTEM and EXTEM (Table).

TABLE Correlation of Fibrinogen with INTEM and EXTEM values

Fibrinogen correlation with:	CFT	α-angle	MCF
INTEM	r = -0.67, p < 0.001	r = 0.70, p < 0.001	r = 0.81, p < 0.001
EXTEM	r = -0.71, p < 0.001	r = 0.73, p < 0.001	r = 0.87, p < 0.001

Conclusions: The high correlation of MCF FIBTEM with fibrinogen suggests that FIBTEM can be reliably used to guide clinical management. A10 values can be used similar precision and more rapid results (10-14 min) over MCF or plasma-based fibrinogen testing. As CT INTEM correlates only modestly with PTT values, clinical correlation studies in pregnancy are needed. Elevated fibrinogen levels during pregnancy substantially contribute to shortening of CFT and increasing α-angle and MCF on INTEM and EXTEM tracings that should be interpreted as physiological changes rather than pathologic hypercoagulability.

PB 2123 | Thrombin Generation Assay CANNOT Identify Antithrombin Resistance During Anticoagulant Therapy

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Background: Variant thrombins derived from missense mutant prothrombins at Arg596 codon are resistant to inactivation by antithrombin (AT), causing susceptibility to venous thromboembolism (VTE). Prothrombin mutations associated with AT resistance (ATR) have been identified in VTE patients worldwide (e.g., Japan, Serbia, India, Italy). There is no simple laboratory test that can detect ATR, although we have reported a clinical test detecting ATR, which is not widespread.

Aims: We examined whether thrombin generation assay (TGA), a widely used blood coagulation function test, can identify ATR.

Methods: We tested TGA for plasma samples from ATR patients with or without anticoagulant therapy (warfarin or heparin) and non-ATR VTE patients taking warfarin. TGA was performed with tissue factor trigger at a final concentration of 5 pM using Calibrated automated thrombography.

Results: In the ATR patients without anticoagulant therapy (N=10), the average of total thrombin generation (ETP) and the duration of thrombin generation (StartTail) were 3,733 nM and 65 sec, whereas those in the non-ATR VTE patients with warfarin therapy (N=3) were 531 nM and 37 sec, respectively. In the healthy subjects (N=3), those were 1,600 nM and 24 sec, respectively. ETP of the ATR patients with anticoagulant therapy (N=6) ranged from 702 to 2,055 nM, similar to those of healthy subjects. On the other hand, StartTail of the ATR patients with anticoagulant therapy apparently prolonged, ranging from 45 to 66 sec. Thus, it seemed that a prolongation of StartTail was one of the characters of ATR, although StartTail of the non-ATR VTE patients with warfarin therapy and the AT deficiency patients without anticoagulant therapy were prolonged.

Conclusions: TGA may be useful as an ATR screening test only in the patients without anticoagulant therapy. To efficiently identify the ATR of VTE patients with or without anticoagulant therapy, we hope that the ATR detection method reported by us will be widely spread.

PB 2124 | Clot Wave Form Parameters of Prolonged APTT in Different Situations: Is There Any Difference between Them?

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Background: ACL TOP coagulometers give APTT clot reaction parameters: Clotting time (CT), Maximum first derivative of Absorbance= Velocity (Max 1), Maximum and minimum second derivative= Acceleration (Max 2) and deacceleration (Min 2). There is not much information of them in other causes of APTT prolongation than hemophiliacs.

Aims: To evaluate APTT parameters, APTT SP in ACL TOP, in Unfractioned Heparin (UFH), Dabigatran (DABI), Lupus anticoagulant (LA), anti-Vitamin K antagonists (AVK) and single factor deficiencies (SFD) of factor XII, XI, IX, V, and VIII (HA and vWD).

Methods: 222 curves from 61 normal controls (NC), 31 LA, 41 UFH, 20 DABI, 34 AVK and 33 SFD were analyzed. Parameters: CT, Max 1, Max 2 and Min 2. Second derivative curve morphology (SDM): single peak (SP), double peak (DP), peak with shoulder (PSH) and wide peak (WP). Parameters differences between groups and SDM were analyzed by ANOVA test with post HOC Bonferroni. Statistics was performed by SPSS 23 software.

Results: Max 1 was lower in all groups compared to NC ($p< 0.0001$), but UFH and Dabi tended to be higher than others. Max 2 was lower than NC in all groups, without differences between them, except Dabi higher than LA ($p= 0.011$). Min 2 expressed as absolute value was lower in all groups compared to NC, but in LA, AVK and SFD was lower than UFH and DABI ($p< 0.001$),

Samples with DP had significantly lower Max 1 and Max 2 than SP ($p< 0.001$ and $p=0.005$), but similar to PSH and WP. However, Min 2 of DP was significantly lower than SP, PSH and WP, table 2.

TABLE 2 APTT reaction curve parameters according different second derivative morphology (SDM) in patietns with prolonged APTT

SDM	CT Mean (SD)	Max 1 Mean (SD)	Max 2 Mean (SD)	Min 2 Mean (SD)
SP (N:74)	75,6 (47.2)	219,4 (109.6)	318,4 (217.4)	-160,6 (99.3)
DP (N: 50)	69,3 (30.3)	153,0 (63.7)	207,9 (135.2)	-39,5 (62.0)
PSH (N: 30)	62,8 (29.0)	179,0 (50.9)	236,7 (138.7)	-67,5 (82.7)
WP (N: 7)	80,2 (42.5)	156,6 (70.3)	159,7 (132.3)	-36,7 (30.7)

Conclusions: SDM of UFH and DABI is like NC (SP), but different to LA, AVK and SFD which present higher prevalence of DP and PSH. Max 1 and Max 2 are lower in all patients´ groups compared to NC, but similar between them. Min 2 was lower in LA, AVK or SFD compared to UFH and DABI. Looking at SDM and Min 2 could be useful to discriminate prolongations of APTT by UFH or DABI to those secondary to factor deficiencies or LA in low complexity laboratories.

PB 2125 | Hereditary Angioedema: Role of Genetic Test in Diagnosis

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Background:

- Laboratory tests for the diagnosis of HAE in Argentina are underdeveloped. Most patients are diagnosed with C1-INH tests, but for patients with high clinical suspicion of HAE and normal test for C1-INH, testing for mutations in the Factor XII (FXII) gene may allow diagnosis in some patients.

Aims:

- To show the outcomes of patients diagnosed in Argentina with hereditary angioedema (HAE) and the utility of genetic tests results.

Methods:

- Patients and samples: A total of 587 patients with clinical suspicion of HAE were studied.
- Quantification of complement factors: serum samples are collected to measure C3, C4 and C1-INH levels using a turbidimetric method.

TABLE 1 APTT clot reaction curve parameters in different group of patients

GROUP	CT Mean (SD)	Max 1 Mean (SD)	Max 2 Mean (SD)	Min 2 Mean (SD)	SP (% cases)	DP (% cases)	PSH (% cases)	WP (% cases)
NC	28.5 (3.2)	292.9 (51.2)	818.4 (187.3)	-332.5 (74.9)	100	0	0	0
LA	73.7 (36.5)	164.9 (89.7)	203.3 (195.1)	-79.4 (91.0)	6.5	35.5	54.8	3.2
UFH	88.8 (53.9)	219.0 (126.6)	248.6 (174.7)	-149.7 (95.4)	100	0	0	0
DABI	63.6 (19.1)	232.7 (46.7)	383.0 (189.8)	-195.6 (75.0)	100	0	0	0
AVK	61.4 (26.7)	157.4 (52.6)	266.5 (173.1)	-51.5 (41.6)	19.4	55.5	8.3	16.7
SFD	63.4 (28.5)	180.1 (73.2	255.4 (174.7)	-53.9 (111.1)	12.1	57.6	30.3	0

Spectrophotometric assays are used to measure the functional activity of C1-INH.

- SERPING1 genetic test: involves isolation of patients' DNA and sequencing of PCR-amplified exons 1-8 and the underlying intronic regions.
- FXII genetic test: the most common mutation c.983C>A in exon 9.

Results: A total of 587 samples were received and 144 (24.5%) patients were diagnosed,125 (21.3%) patients with HAE type 1 (HAE 1), 15 (2.5%) type 2 (HAE II) and 4 (0.7%) with normal C1 inhibitor (HAE-nC1). SERPING1 genetic test: A genetic test was performed in 23 families and confirmed HAE I/II. Forty-two percent mutations were consistent with the common mutation c.1031-20a>g. No mutation was found in the coding region in 2 cases.

FXII genetic test: This test was only performed in patients with strongly clinically suspected HAE, but functional C1-INH was normal. Four out of the 14 tested patients had the FXII mutation (28%), thereby confirming HAE diagnosis based on mutations in FXII gene.

Conclusions: A total of 587 samples were received and 144 patients were diagnosed. Mutations of the SERPING1 gene were found in 91% of patients. HAE-nC1 was diagnosed based on the FXII mutation gene in 28% of patients with normal C1-INH function, being important in the diagnostic algorithm.

PB 2126 | Alterations in the Parameters of Classic, Global and Innovative Assays of Hemostasis Caused by Sample Transport via a Pneumatic Tube System

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Background: Pneumatic tube system(PTS) is presently an integral part of blood sample transport in large medical facilities. However, pre-analytical effects of PTS use on different hemostasis assays are only partially characterized.

Aims: To evaluate the effect of PTS on haemostasis using a large array of laboratory assays.

Methods: Blood was collected from 9 healthy donors. All gave their written informed consent, and the study protocol was approved by the institutional ethics committee. TEG, Thrombodynamics(TD), Thrombodynamics-4D(TD-4D), TGA, LTA with 4 inductors, and routine tests(APTT, PT, TT, Fib) were determined. Platelet function(levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and mepacrine release) was determined using flow cytometry with/without stimulation by collagen-related peptide plus SFLLRN.

Results: PTS significantly increased collagen-indused LTA(84 vs 72%,p< 0.05) and decreased epinephrine-induced one(64 vs 72%, p< 0.05). Flow cytometry revealed significant platelet pre-activation in PTS samples: procoagulant platelets were increased from 0.5 to 1.8%(p< 0.05), while mepacrine accumulation was impaired by 10%(p< 0.05). Activation markers were also higher in the stimulated platelets. Significant changes were registered in all global hemostasis tests: TD, TEG, TGT, TD-4D. TD showed increased clot growth velocity: Vi and Vst were 68 vs 58 µm/min and 42 vs 32 µm/min(p< 0.05), respectively. In all tube-delivered samples there was spontaneous clotting. TEG parameters R and K were significantly decreased(13.8 vs 20.8 min and 4 vs 7.6 min(p< 0.05)), angle was increased (42.3 vs 26.8,p< 0.05). TGA increase parameters ETP and Amax were changed: 1372 vs 1056nM*min and 103 vs 89nM(p< 0.05), respectively. TD-4D showed increased parameter Ast: 72.8 vs 53.9nM(p< 0.05). In routine tests no significant difference was revealed.

Conclusions: The results indicate that the PTS does no affect routine assays, moderately affects platelet activation, and induces significant procoagulant deviation in global haemostasis tests.

PB 2127 | Productivity Evaluation of a Novel Blood Coagulation Analyzer in the 24/7 Clinical Coagulation Laboratory Environment

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Background: The Atellica™ COAG 360 System* is a fully automated random-access blood coagulation analyzer with cap-piercing technology. Its novel elements include a refrigerated chamber and anti-evaporation caps, which prolong onboard reagent stability, as well as continuous management of reagents and consumables.

Aims: The study aimed to compare the productivity of the Atellica COAG 360 System* with a routine laboratory setup.

Methods: Hands-on/walkaway time managing samples, reagents, and consumables were assessed over 5 days in the coagulation laboratory at Labor Berlin. A total of 1732 plasma samples (492 manually loaded) were collected from the routine laboratory setup (two blood coagulation analyzers, one connected to the 24/7 laboratory automation track, the other used stand-alone for specialty hemostasis assays) prior to archiving and additionally analyzed on the Atellica COAG 360 System* (off-track).

Results: In comparison with the routine laboratory setup, we observed a reduction in hands-on time with the use of one Atellica COAG 360. For reagent and consumables (cuvettes/cleaning solution) management the total hands-on time per day was reduced by 90% and 70% respectively (Mean 90.1%, SD ±4.2% and Mean 69.9%, SD±11.9%). In addition, the prolonged reagent stability feature was noted to save up to 31min 15s in average for un/freezing of specialty assays daily. The sample analysis was interrupted during the week in total 33min on the Atellica COAG 360 compared to 6h 42min on both Stago STA R Max analyzers. Total hands-on time per day on

manual plasma samples loading was reduced by 89% (Mean 88.5%, SD ±4.3%).

Conclusions: In terms of overall time savings, especially hands-on time and, importantly, reduction in time needed for active sample and reagent management, the Atellica COAG 360 System* is well-suited for routine and specialty testing in high-volume coagulation laboratories. *Not available for sale in the U.S. Product availability varies by Country.

PB 2128 | Mutation Screening for the C677T Variant in the MTHFR Gene by Melting Point Analysis with the Real Time PCR: Detection of a Rare Variant Val225Ile

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Background: The genetic determinant affecting plasma homocysteine levels is a polymorphism *C677T* (rs1801133) in the 5,10-methlylene-tetrahydrofolate reductase (*MTHFR*) gene substitutes Ala222Val. The "T "variant produces a thermolabile and less efficient enzyme. As a result, T/T homozygote produces a small increase in plasma homocysteine levels. This is a risk factor for venous thromboembolism. We used a melting peak analysis with fluorescence hybridization probes Real Time PCR.

Aims: We describe the results that showed atypical melting curves in the analysis of *C677T*.

Methods: The patient was a 49-year-old man who presented with an idiopathic thrombosis in the right renal vein. The thrombophilia study included the analysis of phospholipids antibodies, antithrombin, Protein S, Protein C and Factor V Leiden, *F2G20210A*, *F12C46T*, *C677T* in the *MTHFR* gene mutations. The *C677T* was analyzed by Real Time PCR with fluorescence probes for the specific allele. The sequencing was performed with Sanger method.

Results: Genetic analysis by Real Time PCR of the patient's results showed atypical melting curves in the analysis of *C677T*. Subsequent sequencing of the corresponding PCR fragment reveals a very rare polymorphism that was heterozygous, rs200100285 A/G that substitutes Val225Ile amino acid. This variant is listed in the genetic public databases by the 1000 Genomes Project. Its prevalence is very low in Caucasian populations. This variant was not detected in 500 analysis of *C677T* with Real Time PCR over a period of 10 years in our centre.

Conclusions: The Real Time PCR with specific fluorescence probes is an efficient, accurate and robust technique to study variations that may have clinical application.

Spanish grant RD12/0042/0032.

PB 2129 | Evaluation of Potential Sources of Variation in 1-stage Factor IX Assays

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Background: Monitoring of effective replacement therapy in Haemophilia B patients is routinely measured using a 1-stage clotting factor assay. Recent pharmaceutical advances have delivered new products for the management of bleeding in Haemophilia B and movement towards pharmacokinetic based personalised dosing requires laboratory assays to accurately reflect in vivo activity.

Aims:

1. To evaluate on a single platform using the Tcoag Destiny Max analyser variation in resulting factor IX levels applying a 1-stage factor IX assay (1-st FIX assay) using either optical or mechanical end-point detection mechanisms.
2. To evaluate resulting factor IX levels associated with various combinations of manufactures reference material and reagents.

Methods: 1-st FIX optical and mechanical assays using all reagent combinations were performed on the Destiny Max analyser using aPTT HS (Tcoag), APTT-SP (Werfen), Actin FS (Dade-Behring), factor IX deficient plasma (Tcoag, Technoclone, Werfen), reference plasma (Tcoag and Technoclone) and quality control TriniCHECK 1 and abnormal control (Tcoag).

Results: Overall reagents combinations and reference sources generated 36 reference curves. Based on normal and abnormal quality control results there was no statistically significant difference between optical (range p = 0.41 - 0.93) and mechanical FIX levels (range p = 0.65 - 0.85) across all reagent and reference combinations. Reference curves were run at nine points from 1/5 to 1/100 dilutions, with Actin FS giving statistically significant shorter clotting times (p = 0.002). Linearity r values ranged from 0.999 - 0.987 with Werfen FIX deficient plasma curves linear over dilutions 1/5 to 1/25.

Conclusions: No major variation was detected in levels of FIX quality control results across detection systems or reagent and reference plasma combinations investigated. However, linearity findings could have an impact on calculation of results particularly at low levels. Further work is in progress to include chromogenic assay and clinical samples.

PB 2130 | The Calibrated Automated Thrombography in Normal Pregnancy

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Background: Normal pregnancy is associated with hypercoagulable state. It manifests by high level of coagulation factors VII, VIII, IX and

TABLE. 1 Parameters of CAT in normal pregnancy (Me, 90% (CI) (* - p<0.05 compare to controls)

Parameters of CAT	I trimester (n=18)	II trimester (n=36)	III trimester (n=18)	Controls (n=20)
LT, min (TM-)	2.30 (1.70-2.60)	2.60 (1.90-3.60)	2.55 (1.70-3.20)	2.33 (1.68-2.67)
LT, min (TM+)	2.30* (2.00-2.80)	2.60* (2.30-3.60)	2.60* (1.70-3.00)	2.33 (1.69-2.67)
ETP, nM*min (TM-)	2354.60* (1883.60-2850.10)	2110.45* (1656.50-3118.50)	2257.10* (1697.50-2805.50)	1577.75 (1344.53-1871.10)
ETP, nM*min (TM+)	1838.05* (1377.70-2253.10)	1660.45* (1244.00-2682.00)	1676.35* (1498.00-2346.00)	714.58 (480.95-947.80)
Peak, nM (TM-)	368.00* (284.20-447.30)	383.80* (257.60-579.70)	350.35* (251.70-536.60)	285.61 (241.98-336.68)
Peak, nM (TM+)	327.20* (252.10-402.50)	329.35* (222.20-546.30)	329.10* (247.40-498.00)	159.16 (107.25-199.60)
TTP, min (TM-)	5.23 (4.50-6.00)	5.00 (4.30-7.00)	5.20 (4.30-7.60)	5.03 (4.01-5.55)
TTP, min (TM+)	5.00* (4.30-5.50)	5.00* (4.30-6.50)	5.00* (4.30-6.30)	4.33 (3.97-4.86)

Von Willebrand. At the same time anticoagulation system factors change too. The Calibrated Automated Thrombography (CAT) can be useful in the functional testing of coagulation system of pregnant women. When thrombomodulin (TM) is used thrombin generation (TG) becomes sensitive to the disorders of the protein C system.

Aims: The aim of this study was to estimate normal parameters of CAT for all trimesters of physiological pregnancy.

Methods: The study involved 74 healthy pregnant women (19 - 40 years, I, II and III trimester - 18, 36 and 18 respectively) and 20 non-pregnant controls. TG was measured according to Hemker et al. at 5pM TF and 4 μM phospholipids platelet poor plasma (PPP) with PPP plasma +/- TM reagent (Thrombinoscope BV, Maastricht, The Netherlands). Such parameters were assessed: lag time (LT, min), endogenous thrombin potential (ETP, nM*min), peak thrombin (Peak, nM) and time to peak (TTP, min). STATISTICA 6.0 was used. Results are given as median (Me) with 90% confidence intervals (CI) for each gestational period. P< 0.05 was considered statistically significant.

Results: Data are shown in the table. All parameters of CAT were markedly reduced in the presence of TM. ETP and Peak in the absence and presence of TM were significant increased during pregnancy. LT and TTP were significantly higher with TM vs. without it. This fact can indicate the hypercoagulation in healthy pregnant women.

Conclusions: Calibrated Automated Thrombography with and without TM allows the effectively detecting of hypercoagulability that is very important for clinical practice. Extremely high levels of CAT parameters can indicate some complications of normal pregnancy and delivery.

PB 2131 | The Impact of Pre-analytical Variables and Reagents on the Results of Thrombin Generation - Lessons Learned from the Generation of Normal Reference Ranges

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Background: Haemostasis is a complex interplay between coagulation factors, platelets and vascular endothelium. Clinicians often rely on routine clotting assays that assess a small part of the haemostatic process. Global haemostatic assays such as thrombin generation provide an important means of eliciting dynamic information about coagulopathic or thrombotic states.

Aims: The objective was to generate data for normal reference ranges using an in house normal plasma pool (NPP) as a quality and normalisation control. The data were analysed to detect any significant difference between use of reagents and differences in sample processing.

Methods: Ethical approval and informed consent for samples from healthy volunteers was obtained via the KD Coagulation Research Plasma Bank. Individuals with a known coagulation defect or on antiplatelet or anticoagulant medication were excluded. Table 1 gives the details of the different sample processing and tissue factor (TF) reagents used. Thrombin generation (TGA) was performed as per Hemker et al.

TABLE 1 TGA assay conditions for normal reference ranges; Platelet count adjusted to 150 x 10^9/L.

PRP (with CTI)	Unadjusted platelet count	Adjusted platelet count
	PRP reagent	PRP reagent
	Innovin 0.5 pM	Innovin 0.5 pM
	Innovin 1 pM	Innovin 1 pM
PPP (with CTI)	Double spun	Triple spun
	PPP low	PPP low
	Innovin 1 pM	Innovin 1 pM & 2 pM
PPP (no CTI)	Double spun	
	PPP reagent	
	Innovin 5 pM	

Results: 21 male and 20 female volunteers were recruited to the study. Data were collected for the in house NPP and the coefficients of variation (CVs) were found to differ between in-house and ready-made TF reagents, with the latter being closer to 10% (acceptable for a diagnostic assay).

Results from PRP samples with unadjusted and adjusted platelet counts were compared and found to be significantly different (see Table 2). Results of PPP samples processed on the same day or after thawing of frozen aliquots were also compared. Significant differences were seen in the results,

(see Table 2).

TABLE 2 Results of paired t tests for adjusted vs unadjusted platelet count and fresh vs frozen PPP samples; p values (< 0.05 significant)

PRP Adjusted vs unadjusted platelet count	PRP reagent p value	Innovin 0.5 pM p value	Innovin 1 pM p value		
LT	0.032	0.219	0.752		
ETP	0.132	0.014	0.259		
Peak	0.005	0.004	0.008		
ttPeak	<0.0005	<0.0005	<0.0005		
Fresh vs frozen PPP samples	PPP Low (CTI) p value	Innovin 1 pM (CTI) p value	Innovin 2 pM (CTI) p value	PPP reagent p value	Innovin 5 pM p value
LT	0.146	0.113	0.045	0.171	0.015
ETP	0.883	0.255	0.053	0.006	0.613
Peak	0.976	0.122	0.001	0.290	0.003
ttPeak	0.267	0.257	0.064	0.145	0.007

Conclusions: The findings of this study underline the potential impact of different methods of sample processing and TF reagents on results from thrombin generation. This is a highly useful test, but in order to maximise use of the output and to minimise variability, each part of the protocol should be carefully considered.

PB 2132 | High Level of Concordance for Routine Assays between Results Obtained with Two Automated Coagulation Analyzers

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Background: The Sekisui CP3000 coagulation system was reviewed.
Aims: The analytical performance characteristics of the Sekisui CP3000 Coagulation System were assessed in comparison with STAGO STA-R Evolution for several haemostasis assays.
Methods: Testing was performed using remnant samples for PT and APTT (n=215), TT (n=30), fibrinogen (n=74), D-dimer (DD)(n=40), antithrombin (AT)(n=47) and Protein C (PC)activity (n=33). Anti-Xa (heparin) activity was determined in 34 and 10 samples from patients on LMWH and UFH, respectively. Results were compared between the two analysers using regression statistics, Pearson correlation and qualitative agreement methods. Time to first result was studied and user experience was assessed with a weighted scoring survey.
Results: Within-run and within-laboratory %CVs were < 5%, except for abnormal level PT and AT and normal level fibrinogen, but all were clinically acceptable. Significantly better imprecision results (1.1% and 4.2%, respectively) were obtained for DD at normal level than the manufacturer's claim. Correlation coefficients (r) ranged from 0.86 (APTT) to 1.0 (INR) in comparison with STA-R Evolution. Qualitative agreement results were 79.0% and 97% for APTT and TT when the STAGO-recommended reference ranges were used. Total agreement for PT results was 90%. Reference ranges were successfully verified for APTT, PT/INR, TT and AT, results indicated need for adjustment for fibrinogen, DD and PC. The average time to result was 133 seconds for PT, and 327 seconds for the panel of PT, APTT and fibrinogen. CP3000 scored significantly higher in the user experience survey than the STAR-Evolution for size, QC monitoring, weekly maintenance and troubleshooting, but lower for calibration, QC processing, start-up, and noise.
Conclusions: CP3000 showed high level of repeatability, reproducibility, and strong correlation with the STA-R Evolution results. Time to first result was rapid, and the analyzer was rated favorably for its size, QC monitoring, maintenance and troubleshooting.

PB 2133 | Current Practice Related to D-dimer Testing in Croatia

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Background: D-dimer assay provides an important contribution to the venous thromboembolism diagnostic workup. However, the lack of standardization among different assays and laboratories is the main aspect that still complicates its diagnostic use.
Aims: The aim of this study was to present current practice related to D-dimer testing in Croatia.
Methods: The results are based on a review of data obtained in a national survey related to overall haemostasis testing in the form of a questionnaire,conducted between June and September 2015.
Results: Among all respondents to the questionnaire (104/170; 61.2%), 42/104 (40.4%) of laboratories declared to perform the D-dimer assay. More than half of them reported to use Siemens Innovance D-dimer assay (25/42), while other commercial D-dimer reagents were represented as followed: bioMerieuxVidas D-dimer (5/42), Roche Cardiac D-dimer (3/42), Roche immunoturbidimetric assay (3/42), Olympus

D-dimer reagent (2/42), Nycocard D-dimer (2/42), IL-Dimer HS500 (1/42) and Abbott D-dimer (1/42). Among them, 24/42 indicated to use fibrinogen equivalent unit (FEU) and 18/42 declared to use D-dimer unit (DDU) reporting units for expressing D-dimer results. Survey results showed a number of different measurement units currently in use: mg/L (19/42), mg/L FEU (12/42), µg/L (5/42), µg/L FEU (4/42), µg/mL (1/42), ng/mL (1/42) and ng/mL FEU (1/42). All 42 laboratories declared to use fixed cut-off value and no one laboratory declared to use age-adjusted cut-off values. The most commonly used cut-off values were 0.5 mg/L DDU (15/42) and 0.5 mg/L FEU (10/42).
Conclusions: Results of our survey showed that different combinations of measurement and reported units are currently used for reporting D-dimer results. These results confirm and underline the need for further standardization in D-dimer results reporting at national level. Obviously, an effort should be directed towards harmonization of available commercial D-dimer methods.

PB 2134 | Usefulness of APTT Reagents for LA Detection and Frequency of LA Positive Patients in APTT Prolonged Patients

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Background: Lupus anti-coagulant (LA) is an antibody that inhibits phospholipid-dependent coagulation reactions and of the several tests to detect LA, APTT is among the most accessible. However, APTT test sensitivity to LA varies depending on the reagent, with poor sensitivity in some cases resulting in false negatives.
Aims: The aim of this study was to verify the usefulness of the two types of APTT reagents used in our hospital for LA detection.
Methods: 3.2% sodium citrate blood samples from 26 patients were analysed using the APTT test on both the CP 2000 with the Coagpia APTT-N reagent (APTT-N) and the CS 2100i with the Actin FS reagent (ACTIN). The samples were also used for mixing tests, dRVVT and factor assays.
Results: In the APTT-N prolonged/ACTIN normal group (N=8),there was a significant difference between the mean values for APTT-N and ACTIN of 72.9 vs 28.7 sec respectively (P< 0.01). In this group,various bleeding or thrombogenic factors were identified in all specimens. In the LA positive (LA+) group (N=9), the mean value for APTT-N of 87.7 sec was significantly longer than that for ACTIN, 36.0 sec (P< 0.01). Among the APTT prolonged specimens, the LA+ rate was 50% (9/18). Furthermore, in the LA+ group, the abnormality rate for APTT results was 100% with APTT-N (9/9) and 55.5% with ACTIN (5/9).
Conclusions: APTT-N was able to detect all instances of APTT prolongation in LA+ patients, with mean values for LA+ specimens also showing significantly higher prolongation times compared to ACTIN. As such, it was considered that the sensitivity of APTT-N to LA and its utility are high. Even within the results of this study, which tested at random a number of APTT-prolonged specimens, the number of LA+ patients reached 50%. Based on this, predisposition to thrombophilia maybe more common among patients than is conventionally thought, and as such use of an APTT reagent with high LA sensitivity should be given due consideration for its benefit to patients.

PB 2135 | An Evaluation of the Activated Partial Thromboplastin Time Waveform in Various Diseases

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Background: The activated partial thromboplastin time (APTT) waveform includes several parameters relating to various underlying diseases.Another benefit of optical endpoint coagulation analyzers is the ability to visualize the clot reaction curve as the prothrombin time (PT) and APTT. Previous reports have highlighted the usefulness of the visual inspection of the APTT clot reaction curves, and abnormal biphasic clot reaction curves have been reported to be associated with the early detection of disseminated intravascular coagulation (DIC).
Aims: In this study, we measured and analyzed the APTT wave form in patients with various diseases, and examined the relationship between the disease and the parameters of the APTT waveform.
Methods: The APTT waveform was examined in various diseases.
Results: Regarding the pattern of APTT waveform, a biphasic pattern of acceleration or velocity curve is observed in patients with hemophilia and those positive for antiphospholipid antibody (aPL) or inhibitor for coagulation factor VIII (FVIII). The time of acceleration, velocity and fibrin formation at 1/2 height are prolonged in patients with hemophilia, those with an inhibitor, those positive for aPL, those treated with an anti-Xa agent and those with DIC, and those all tend to become shorten in pregnant women. The height of the acceleration peak 1 is significantly lower in patients with hemophilia, those with an inhibitor, those positive for aPL, those treated with an anti-Xa agent and those with DIC, and that it tend to be significantly higher in pregnant women. The height of velocity is significantly lower in patients positive for FVIII inhibitor and it is also significantly higher in patients treated with an anti-Xa agent and in pregnant women. The width of acceleration peak 1 and peak 2 and the velocity were significantly longer in almost all diseases.
Conclusions: An analysis of the APTT waveforms was thus found to provide us with useful information.

PB 2136 | Identification of Rare Inherited Diseases by Next Generation Sequencing

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Background: Genomic studies on rare inherited diseases in recent years have revolutionized molecular diagnosis for inherited disease.

Recent whole genome and targeted gene studies have found novel somatic mutations with pathological importance in inherited diseases. We performed mutational screening to reach accurate diagnosis of diseases and want to apply a targeted gene panel for the detection of possible mutation in a diagnostic molecular pathology laboratory.

Aims: To implement an NGS panel for genetic diagnosis of more than 100 inherited diseases. The gene panels targets 552 regions known to haven pathogenic mutations in genes with potential involvement in severe recessive, pediatric onset diseases.

Methods: We performed next generation sequencing (NGS) using Trusight Inherited disease panel which included 552 genes covered 110 inherited rare diseases. Research protocol was approved by the Institutional Review Board (ERC/IRB) and conformed to the tenets of the Declaration of Helsinki. We extracted genomic DNA of apparently undiagnosed eight cases from peripheral blood by QIAamp® Blood mini kit, Qiagen. DNA quality/quantity was checked by Qubit® 2.0 flurometer (life technology®) and then used TruSight™ DNA Amplicon Sequencing chemistry for library preparation and normalization. Sample was run on Next generation sequencer MiSeq Illumina™.

Results: In total 10509 variants were detected, after excluding intronic and synonymous variants, 1851 missense variants were found in eight undiagnosed patients. We identified p.Q96* mutation in *FERMT3* gene in homozygous state which is responsible for rare inherited bleeding disorder Leukocyte adhesion molecule III Deficiency. A most frequent mutation p.A177T found in *RNASEH2B* gene that cause Aicardi-Goutières syndrome type 2 (AGS-2).

Conclusions: Targeted gene panel testing by NGS was applicable for accurate detection of inherited diseases. We are able to provide correct diagnosis with respect to individualize genetic pattern in the clinical setting.

PB 2137 | Type and Frequency of Hemoglobinopathies, Diagnosed in the Area of Karachi, in Pakistan

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Background: Hb comprises four subunits, each having one polypeptide chain and one heme group.The polypeptide chains of adult Hb themselves are of two kinds, known as alpha and beta chains, similar in length but differing in amino acid sequence. The alpha chain of all human Hb, embryonic and adult, is the same. The non-alpha chains include the beta chain of normal adult Hb ($\alpha 2\beta 2$), the gamma chain of fetal Hb ($\alpha 2\beta 2$), and the delta chain of HbA2.

Aims: Aim was to know the frequency distribution of hemoglobinopathies in the Karachi,which will help in formulating various strategies for the effective control and prevention of these disorders.

Methods: The blood samples were collected from the patients of low Hb,for testing of parameters like, CBC, peripheral smear, Hb analysis by electrophoresis (at alkaline pH),high-performance liquid chromatographyand sickling test. CBCs were done by Sysmex XN 1000 analyzer, peripheral blood smears were stained with Leishman's stain, grading of hypochromia, anisocytosis, microcytosis, macrocytosis, and polychromasia was done according to the standard criteria. Hb electrophoresis at alkaline pH was performed on Genio analyzer.

Results: Out of total 2731, 935 (34.2%) patients had hemoglobinopathies. Out of these 935 patients who had hemoglobinopathies, beta thalassemia minor 51.8%, beta thalassemia major 24.1%, HbD trait 6.7, sickle/beta thalassemia 4.5%, sickle cell disease 3.9%, HbE trait 1.9%, and sickle cell trait 1.7% were most common hemoglobinopathies. Less prevalent were delta/beta thalassemia, HbE homozygous, HbD homozygous, and HbH disease.

Conclusions: Our study is an attempt to determine the type and frequency of various hemoglobinopathies in Karachi region that can be useful in prevention and management of various hemoglobinopathies, which may play a vital role in the hospital blood bank as well as in the formulation of transfusion policies.

FIBRINOLYSIS & PROTEOLYSIS

PB 161 | Affimers for Modulation of Fibrinolysis: An Alternative Approach for Bleeding Disorders

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Background: Restance of a fibrin clot to lysis can determine clot stability and has implications for bleeding disorders. We hypothesised that fibrinogen-targeting affinity proteins, termed Affimers, represent a tool to manipulate fibrin network characteristics and the potential to identify novel therapeutic targets for conditions characterised by excessive bleeding.

Aims: Modulate fibrin clot properties and enhance resistance to fibrinolysis using fibrinogen-specific Affimers.

Methods: A phage display system was used to screen a large library ($n=10^{10}$) of random Affimers for fibrinogen binding. Effects of Affimers on fibrin clot properties were assessed by turbidimetric assays, confocal microscopy and ROTEM. Mass spectrometry (MS) was used to elucidate interaction sites.

Results: 112 fibrinogen-binding Affimers were isolated from two independent screens, of which 14 demonstrated distinct sequences. Two of these, F5 and B9, prolonged plasma clot lysis time from 42.2±12.3 mins (mean±SD) to 234.4±7.4 and 102.3±8.0 mins respectively at 20:1 Affimer:fibrinogen molar ratio ($p< 0.01$ for both). A dose response curve showed the lowest molar ratio to have an effect was 5:1 and 10:1 for F5 and B9, respectively. In whole blood ROTEM, B9 reached maximum clot lysis after 75 minutes, compared to control at 54 minutes, F5 only reached 22.3±6.1% ($p< 0.01$) lysis by the 2 hour endpoint. F5 caused a decrease in maximum clot firmness

(MCF) from 45.7±1.5mm to 35.3±2.1mm (p< 0.01), while B9 did not influence MCF. F5 and B9 had no significant effect on clot structure when assessed by confocal microscopy. MS analysis of trypsin digest products of Affimer-fibrinogen complexes suggested that F5 binds 2 regions on the fibrinogen α chain, while B9 binds to the fibrinopeptide B region.

Conclusions: Our data show that Affimers can modulate fibrinolysis while maintaining clot structure, in plasma and whole blood. This novel approach may help to develop new antifibrinolytic therapeutic agents for use in bleeding disorders.

PB 162 | A Novel Tick Salivary Protein Modulates Fibrinolysis by Interacting with Plasminogen and Tissue Plasminogen Activator, and Prevents Arterial Thrombosis in vivo

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Background: Tick saliva is a rich source of anti-hemostatics, including inhibitors of platelet aggregation and blood coagulation. Here, we have characterized a novel family of salivary proteins named Tick Salivary Fibrinolysis Modulator (TSFM) from the vector of Lyme Disease, *Ixodes scapularis*. TSFM is among the most abundant transcripts and proteins expressed in the salivary gland.Molecularly, TSFM is a basic 10 Kda non-enzymatic protein which contains 6 disulfide bonds and a C-terminal domain rich in lysine residues.

Aims: Identify the function of TSFM which has been elusive for more than a decade.

Methods: TSFM was generated by chemical synthesis. *In vitro* assays for fibrinolysis and arterial thrombosis model in mice have been employed to study the mechanism of action of TSFM.

Results: Assays with chromogenic substrate (S-2251) demonstrate that synthetic TSFM accelerates plasminogen activation by tissue-type Plasminogen Activator (TPA), with a *Km* of 20 nM. This activity is inhibited by lysine analogue, e-aminocaproic acid. Additionally, Surface Plasmon Resonance (BIAcore) results show that TSFM exhibits high-affinity binding to TPA, and to plasminogen (K_D 5-10 nM), but not to Urokinase. Conceivably, lysine residues mediate interaction of TSFM with components of the fibrinolytic cascade, providing a scaffold for formation of a productive TSFM/plasminogen/TPA ternary complex. TEG experiments with whole blood show that TSFM decreases the α angle, suggesting interference in the rate of fibrin formation. *In vivo*, TSFM inhibits $FeCl_3$-induced thrombosis in mice.

Conclusions: TSFM emerges as a novel modulator of vascular biology from tick saliva.

PB 163 | Comparison and Characterization of Two Assays (TAFIa/ai ELISA vs. Activity Based Selective CPU Assay) for the Monitoring of Carboxypeptidase U (CPU, TAFIa, CPB2) Levels in Patients

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Background: The influence of the procarboxypeptidase U (proCPU, TAFI, proCPB2) system in thrombotic disorders has been investigated elaborately. As a consequence, interest in the potential of CPU inhibitors is rising. Nevertheless, the measurement of CPU (TAFIa, CPB2) is challenging, with both critical pre-analytical and analytical factors. Well characterized assays to monitor plasma CPU levels in (pre)clinical studies are indispensable.

Aims: This research focuses on the comparison of two assays that can be used for the monitoring of CPU plasma levels in patients.

Methods: An *in-house* activity based CPU assay using the selective and specific substrate Bz-*o*-cyano-Phe-Arg was compared with a commercially available TAFIa/ai ELISA, both for monitoring of CPU plasma levels in patients and with regard to the influence of *in vitro* added CPU inhibitors. The performance of the assays was compared both using spiked CPU standards and control plasma samples (n=10). Additionally, CPU levels were measured in plasma samples of a selected number of thrombolyzed patients.

Results: Analysis of spiked CPU standards before and after thermal inactivation shows a lower reactivity of the ELISA antibody against thermally inactivated CPU compared to active CPU. Addition of a CPU inhibitor influences the reactivity as well. In thrombolyzed stroke patients, a clear increase in CPU plasma levels was demonstrated with both assays, but marked differences in kinetics were observed.

Conclusions: Both assays measure different aspects of CPU generation in patients. The differences in kinetic profiles provide additional information. Although the ELISA is able to detect the overall activation of the CPU system, it cannot accurately show the time-dependent inactivation of CPU. On the contrary, our activity based assay detects peak CPU levels more accurately if enough time points during thrombolysis are available and also detects both the increase and decrease of CPU plasma levels. It seems advantageous to combine both assays in (pre)clinical trials.

PB 164 | Optimising the Degradation of NETs to Destabilise Fibrin Clot Structure and Promote Fibrinolysis

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Background: Neutrophils release DNA and histones as Neutrophil Extracellular Traps (NETs), to kill invading pathogens. NETs also

promote thrombosis by activating coagulation and inhibiting fibrinolysis, causing thrombotic disease.

Aims: To understand regulation of fibrinolysis by NETs and identify strategies to enhance thrombolysis by NET destabilisation.

Methods: Precise methods are available to monitor effects of DNA and histones on clotting and lysis, to determine binding constants for histones and heparinoids, and the effects of histone post-translational modifications, proteases and DNase.

Results: Although histones stimulated plasminogen activation by tPA or streptokinase in solution they delayed fibrinolysis by altering clot structure, producing thicker fibres with a higher mass/length ratio. Histone subtype separation by heparin chromatography identified histone core (H2, H3, H4) as more significant than H1 linker. Histones were digested by neutrophil elastase>plasmin>>activated protein C, but were protected by DNA. Fragmentation of histones reversed their effect on clot structure, but still impeded fibrinolysis. Only brief treatment with DNase is required to promote clot lysis, indicating large DNA fragments in NETs are needed to influence clotting and lysis rates.

Therapeutic concentrations of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) bind tightly to histones: the IC50 for 0.2 IU/ml UFH is 1.1 and 2.7 µg/ml for lysine-rich (H1) and arginine-rich fractions (H3), respectively. Citrullination of histones by PAD4 weakened the IC50 with UFH around 3-fold.

Conclusions: Arginine-rich core histones regulate fibrinolysis more than lysine-rich H1 and citrullination reduces effects on clot structure. Though heparin neutralisation remains effective there is a need to develop non-anticoagulant heparinoids. Susceptibility of NETs to proteolysis is variable and modulated by DNA. DNases offer most immediate promise to improving thrombolysis by degrading NETs.

PB 165 | Extracellular Histones Enhance Fibrin Degradation by Prourokinase in a Factor Seven Activating Protease (FSAP)-dependent Manner

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Background: Histones have been described to inhibit fibrinolysis by modifying fibrin structure and rheological properties. This observation, however, was restricted to tissue plasminogen activator (tPA)-mediated fibrinolysis.

Aims: To evaluate the effect of histones on fibrinolysis by other plasminogen activators.

Methods: Plasma or purified fibrinogen, supplemented with calf thymus histones or vehicle, was clotted with tissue factor in the presence of either tPA (40 ng/ml), urokinase (uPA, 100 U/ml) or prouPA (10 µg/ml), and fibrinolysis was assessed by a turbidimetric method as clot lysis time.

Results: In plasma, histones (≥ 50 µg/mL) inhibited tPA or uPA-mediated fibrinolysis. On the contrary, histones dose-dependently potentiated prouPA-driven fibrinolysis, with a significant effect at 2.5 µg/ml and maximal stimulation at 40 µg/mL (23±4.3 min vs 111±24 min in controls). Recombinant histone subtypes, particularly H4, H3 and H2A, recapitulated the effect of the mixture. Binding of histones to negatively charged molecules (DNA, unfractionated heparin or de-N-sulfated heparin), as well as cleavage of histones by activated protein C did not abolish but rather potentiated the effect on prouPA-induced lysis. Stimulation of fibrinolysis by histones was independent of thrombin (similar results with reptilase-clotted plasma) and progressively declined by incubation of histones in plasma. In purified system, histones dose-dependently impaired prouPA (40 ng/ml) fibrinolytic activity, indicating that a plasma factor was required for stimulation. In plasma, indeed, histones generated a proteolytic activity towards p-Glu-Pro-Arg-MNA that was quenched by a neutralizing antibody against FSAP. In the clot lysis assay, inhibition of FSAP abolished the effect of histones on prouPA-mediated fibrinolysis.

Conclusions: While inhibition of tPA-mediated fibrinolysis by histones can be relevant to thrombogenesis, enhancement of prouPA activity may contribute to extravascular proteolysis and tissue damage.

PB 166 | Localization of the C-terminal Cleavage Site of Human Alpha-2-antiplasmin

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Background: Native alpha-2-antiplasmin (a2AP), the main natural inhibitor of plasmin, consists of 464 amino acids. The C-terminus of a2AP is crucial for the initial interaction with plasmin(ogen) and the rapid inhibitory mechanism. Circulating a2AP exists in different molecular forms: ~65% is C-terminally intact and can bind to plasmin(ogen) (plasminogen-binding a2AP/PB-a2AP), whereas due to proteolytic cleavage, ~35% has lost its C-terminus (non-plasminogen binding a2AP/NPB-a2AP) and thereby its rapid inhibitory capacity. To date, the exact C-terminal cleavage site of PB-a2AP is unknown. It is known that a commercially available monoclonal antibody (mAb) against a2AP (TC 3AP, Technoclone) detects PB-a2AP but not NPB-a2AP, indicating that the epitope of this mAb should be located C-terminally from the C-terminal cleavage site.

Aims: To determine the epitope of TC 3AP and to localize the C-terminal cleavage site of a2AP.

Methods: Epitope mapping of TC 3AP was performed using commercially available a2AP (Calbiochem). After enzymatic digestion, peptide fragments were immunoprecipitated using TC 3AP-loaded Dynabeads® Protein G. Bound peptide fragments were eluted and analyzed by mass spectrometry (MS). To localize the C-terminal cleavage site, PB- and NPB-a2AP were purified from plasma by affinity chromatography using a polyclonal anti-a2AP antibody and analyzed by MS after enzymatic digestion.

Results: We found that the epitope of TC 3AP is located between Phe429 and Asp439. The first MS data from plasma-purified a2AP showed that NPB-a2AP likely results from cleavage between Glu421 and Asp422. This fits with the epitope of TC 3AP being C-terminal of the cleavage site.

Conclusions: We mapped the epitope of TC 3AP and preliminarily localized the C-terminal cleavage site of plasma-purified a2AP. This knowledge will be used to identify the protease responsible for C-terminal cleavage of a2AP, a potential regulator of a2AP activity.

PB 167 | PAI-1 Inhibition with MDI-2001: Towards a Safer and More Effective Treatment for Deep Vein Thrombosis

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Background: Anticoagulation as treatment of deep vein thrombosis (VT) carries a significant risk of bleeding. The 33% mortality rate, 30-50% incidence of post thrombotic syndrome, and 10-30% recurrence emphasize the need for improved therapeutic options. Stimulation of the fibrinolytic system is a potentially safer option for thrombosis treatment, with plasminogen activator inhibitor-1 (PAI-1) considered a key therapeutic target. However, efforts to develop PAI-1 inhibitors have been unsuccessful due in part to complex formation of PAI-1 with its stabilizing cofactor vitronectin.

Aims: Using a proprietary screening method, we identified a first-in-class small molecule, MDI-2001, that inhibited the PAI-1 vitronectin complex. Our aim was to profile its efficacy in a murine model of VT.

Methods: VT was induced via the electrolytic inferior vena cava model in 12 week-old C57BL/6 mice. After induction, mice received either 3 mg/Kg/ MDI-2001 or Low Molecular Weight Heparin (LMWH), or vehicle IP 3x/day. Thrombi were harvested 2 days following VT induction, and thrombus weight (TW) was recorded. Bleeding risk was assessed in another cohort of mice for the same treatment groups.

Results: All mice survived until harvest and no bleeding complications or behavioral changes were observed. MDI-2001 was associated with 62% decrease in TW compared to controls ($p < 0.05$). The reduction TW on LMWH was identical compared to MDI-2001 (62% vs 62%). Importantly, MDI-2001 did not change the bleeding time, whereas LMWH demonstrated significantly prolonged bleeding times compared to the other groups.

Conclusions: This work demonstrated that MDI-2001 represents a first-in-class PAI-1 inhibitor that is as effective as LMWH in reducing TW, however without any increase in bleeding risk. Ongoing investigations will evaluate synergism between the standard of care and stimulation of the fibrinolytic system with MDI-2001. Our data suggests that fibrinolytic stimulation with MDI-2001 is a promising avenue for improving VT treatment.

PB 168 | A Phase 2/3, Open-label, Repeat-Dose Study of the Pharmacokinetics, Efficacy, and Safety of Prometic Plasminogen Intravenous Infusion in Subjects with Hypoplasminogenemia (HPLG)

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Background: Plasminogen (PLG) is converted to form plasmin, which is responsible for the lysis of clots and clearance of extravasated fibrin (Collen 1991). Type 1 plasminogen deficiency caused by mutations in the *PLG* gene is a rare disorder (HPLG) that leads to clinical manifestations due to extravascular fibrinous deposits on mucous membranes with disruption of organ function (Tefs 2006; Mehta 2008). ProMetic has developed an intravenous (IV) PLG product (Glu-PLG) derived from human plasma (ProMetic's PLG) to treat HPLG.

Aims: To determine whether ProMetic's PLG is safe and effective in subjects with HPLG.

Methods: Fourteen subjects aged 2 to 80 years with HPLG were enrolled in an open-label study to receive multiple IV doses of 6.6 mg/kg ProMetic's PLG for 48 weeks. An interim analysis was performed after 10 subjects completed 12 weeks of treatment. Subjects were dosed every 2, 3, or 4 days based upon a pharmacokinetic (PK) profile to maintain target PLG activity trough levels $\geq$ 10% (absolute) above baseline values. Trough levels were measured every 2 weeks for 12 weeks. Primary endpoint success was defined as $\geq$80% of subjects achieving target PLG trough levels for at least 3 measurements over 12 weeks; efficacy and safety were also assessed.

Results: Subject baseline characteristics are included in Table 1. Target PLG trough activity levels were achieved in 10 subjects (100%) after 12 weeks (Table 2). Baseline pharmacokinetic parameters were unchanged at 12 weeks. Six of 10 subjects had 11 visible lesions. After 8 weeks, 10 of 11 lesions (90.9%) completely resolved; the remaining lesion shrank to an unmeasurable size after 12 weeks (Table 2). There were no deaths, serious adverse events (AEs), or AEs resulting in study drug discontinuation. The most frequent AE was nasopharyngitis.

Conclusions: Repeated doses of 6.6 mg/kg ProMetic's PLG achieved target PLG activity trough levels in subjects with HPLG and demonstrated excellent efficacy after 12 weeks of treatment, with no safety concerns.

TABLE 1 Demographics and Baseline Characteristics

Baseline Characteristics	Total (N=10)
Mean Age - Years (SD)	22.7 (10.8)
Age Range Min, Max	5,39
Age 2-17	4
Age > 17	6
Gender Female	8
Gender Male	2
Mean Weight -Kg (SD)	65.2 (22.4)
BL Plasminogen Activity % Mean (SD)	24.0 (10.6)
BL Plasminogen Activity % Min, Max	<5, 43

TABLE 2 Pharmacokinetic and Efficacy Endpoints

	Baseline (BL)	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Target Plg Activity Achieved > 3 visits
Subjects with Plg Activity Levels > 10% Above BL (N=10)	---	10	10	10	10	9	10	10 (100%)
Visible Lesions	11		2		1		1	
New Lesions	---		0		0		0	
Lesion Resolved (%)	---		9 (81.8)		10 (90.9)		10 (90.9)	
Measureable Visible Lesions	6		2		1		0	
> 75% Resolved (%)	---		6 (100)		6 (100)		6 (100)	

PB 169 | Colistin Dampens Fibrinolysis and Endothelial Activation during Endotoxemia: A Randomized, Double Blind Trial

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Background: Colistin electrostatically interacts with lipopolysaccharides (LPS) located at the outer membrane of bacterial membranes. Pre-clinical studies demonstrated beneficial effects of colistin on LPS-induced coagulation and fibrinolysis.

Aims: The objective of this trial was to investigate the effects of colistin during experimental endotoxemia.

Methods: A randomized, double-blind, placebo-controlled, crossover trial in healthy volunteers was conducted. Healthy volunteers received a 2ng/kg LPS bolus after infusion of 2.5 million IU colistin or placebo. Plasma levels of F1+2 prothrombin fragments, thrombin-antithrombin complexes (TAT), von Willebrand factor antigen levels (vWF), E-selectin, plasmin-antiplasmin complexes (PAP), tissue-type plasminogen activator (tPA) antigen and activity, plasminogen activator inhibitor-1 (PAI-1) were measured.

Results: Infusion of colistin significantly reduced peak concentrations of PAP complexes by 70%, t-PA antigen levels by 63%, t-PA activity by 48% and PAI-1 levels by 63%. Two hours after the LPS bolus F1+2 levels and TAT complexes were slightly reduced in the colistin period, but peak concentrations were similar in both periods. Colistin blunted the LPS induced four-fold increase in soluble E-Selectin levels by ~50% and the two-fold increase in vWF antigen levels by ~70%.

Conclusions: The LPS-scavenging actions of colistin significantly reduce endothelial activation and fibrinolytic response in the human endotoxemia model, while the activation of the coagulation system remains largely unaffected. Thus, in patients with gram-negative sepsis colistin may exert beneficial effects, by endothelial protection in patients with overshooting immune response, independent of its antimicrobial activity.

PB 170 | A Novel Fluorescent Assay for the Measurement of Activated Thrombin-activatable Fibrinolysis Inhibitor (TAFIa) Activity

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Background: Thrombin-activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase present in plasma that acts as a molecular connection between the coagulation and fibrinolysis pathways. TAFI is activated to form TAFIa through proteolytic cleavage by thrombin, thrombin in complex with thrombomodulin, or plasmin. Several assays are available to measure the carboxypeptidase activity of TAFIa; however, these methods suffer one or more disadvantages including low sensitivity, labour intensivity and complexity.

Aims: To develop a novel fluorescence-based assay for the measurement of the basic carboxypeptidase activity of TAFIa.

Methods: A synthetic peptide substrate was designed composed of four amino acids (Gly-Ala-Gly-Arg) modified with an amino-terminal tetramethylrhodamine (TAMRA) fluorophore. An anionic quencher interacts electrostatically with the positively charged carboxyl-terminal arginine side chain in the substrate allowing quenching of the TAMRA fluorescence. The cleavage of the carboxyl-terminal arginine by TAFIa results in the release of the substrate causing an increase in fluorescence.

Results: The fluorescent quencher dye Evans Blue was determined to be the optimal quencher for the TAMRA-GAGR synthetic peptide substrate. The interaction between the substrate and quencher was unaffected by salt concentration. The sensitivity of the TAMRA-GAGR substrate is superior to that of commonly employed direct carboxypeptidase B substrates, with a detection limit for TAFIa of 100 pM. Interestingly, pancreatic carboxypeptidase B was even more active against the substrate, with a detection limit of 6.1 pM. The assay is capable of monitoring the rate of activation of TAFI *in vitro* in a single-stage assay.

Conclusions: The assay could be used to measure TAFIa activity and TAFI activation in situ in turbid or high-protein solutions, potentially including plasma and fibrin clots, in a manner analogous to how the endogenous thrombin potential is determined.

PB 171 | Global Assay of Fibrinolysis in Sickle Cell Disease: Contrasting Results in Plasma and Whole Blood

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Background: Sickle cell disease (SCD) is caused by a $Glu_6 \rightarrow Val$ mutation in the β-globin gene that results in hemoglobin polymerization in hypoxic erythrocytes. Few studies have evaluated fibrinolysis in SCD, or its contribution to vascular complications, such as the increased risk of venous thromboembolism.

Aims: To evaluate resistance to t-PA-mediated fibrinolysis in platelet-free plasma (PFP) and whole blood (WB) of patients with SCD.

Methods: A] *Global Assay in PFP*. Tissue factor (TF, 1pM), $CaCl_2$ and t-PA (2.5, 1.25 or 0.625nM) were added to citrated PFP from 13 patients with SCD at baseline and 9 race-, sex- and age-matched controls. Turbidity was read spectrophotometrically, and clot lysis time (CLT-the time from half clotting to half lysis) was used to evaluate fibrinolysis.

B] *Global Assay in WB*. TF, $CaCl_2$ and t-PA (0.625, 1.25, 2.5, 5 and 10nM) were added to citrated whole blood in transparent tubes from 15 patients with SCD and 11 matched controls. When the clot was formed, a steel ball (d=2mm, w=0.13g) was placed on its surface. The time from coagulation/fibrinolysis activation to the ball reaching the bottom of the tube was measured by capturing time-lapse video.

Sample collection was approved by IRB. Informed consents were obtained.

Results: In the t-PA challenged turbidity assay in PFP, CLT in SCD samples was significantly shorter than the AA group at all three t-PA concentrations.

TABLE 1 Clot Lysis in PFP

t-PA concentration (nM)	CLT mean±SEM (min)		p value
	SS	AA	
2.5	60.7±2.7	136.7±12.3	p<0.00001
1.25	92.9±5.9	182.7±9.2	p<0.00001
0.625	138.5±12.9	226.7±0.7	p<0.0001

In the WB ball sedimentation assay, no significant difference was noted between AA and SS clots at high t-PA concentrations (2.5-10nM). However, SS clots challenged with lower t-PA concentrations (0.6-1.2nM) were more resistant to fibrinolysis.

TABLE 2 Clot Lysis in WB

t-PA concentration (nM)	CLT mean±SEM (min)		p value
	SS	AA	
10	21.4±1.2	22.4±1.7	p=0.63
5	34.9±2.0	33.8±2.0	p=0.71
2.5	157.1±59.4	51.0±2.3	p=0.14
1.25	402.0±59.3	157.9±57.9	p=0.0085
0.625	651.5±33.9	369.8±75.4	p=0.001

Conclusions: Plasma clots from SCD patients are more susceptible to t-PA challenge compared to healthy controls. In contrast, SCD whole blood samples are more resistant to fibrinolysis at low t-PA concentrations. These findings imply an important role for the cellular components of blood in resisting t-PA mediated fibrinolysis in SCD.

PB 174 | Generation and Characterization of Monoclonal Antibodies against the N-terminus of Alpha-2-antiplasmin

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Background: ~70% of circulating alpha-2-antiplasmin (a2AP), the main natural inhibitor of plasmin, is N-terminally cleaved by antiplasmin-cleaving enzyme between residues Pro12 and Asn13, converting native Met-a2AP into the more potent fibrinolysis inhibitor Asn-a2AP. The Arg6Trp (R6W) polymorphism affects the N-terminal cleavage rate of Met-a2AP, with ~8-fold faster conversion of Met(R6)-a2AP (R6-a2AP) compared to Met(W6)-a2AP (W6-a2AP). To date, methods to determine N-terminal variation of a2AP in plasma have been limited to ELISAs that can only measure R6-a2AP.

Aims: To generate and characterize monoclonal antibodies (mAbs) towards R6-a2AP, W6-a2AP, and one towards all a2AP forms (total-a2AP), and to develop specific R6-a2AP and W6-a2AP ELISAs.

Methods: R6-a2AP, W6-a2AP and Asn-a2AP were recombinantly expressed in Drosophila S2 cells. Using hybridoma technology, a panel of 25 mAbs was generated towards a mixture of recombinant R6-a2AP plus W6-a2AP. All mAbs were evaluated for their specific reactivity using purified native a2AP and the three recombinant a2APs in one-site noncompetitive ELISAs. Three selected mAbs (one for R6-a2AP, one for W6-a2AP and one for total-a2AP) were further evaluated in sandwich-type ELISAs. The mAb towards total-a2AP was HRP-conjugated to function as detection antibody.

Results: Two mAbs were selected, MA-AP37E2 and MA-AP34C4 (both IgG1 kappa isotype), that showed selective reactivities towards R6-a2AP and W6-a2AP respectively (cross-reactivities were 0.6% and 0.05%, respectively). MA-AP15D7 (IgG2b kappa isotype) was selected for its reactivity towards total-a2AP. MA-AP37E2 strongly

responded to pooled plasma of R6 homozygotes, but did not respond to pooled plasma of W6 homozygotes, whereas MA-AP34C4 strongly responded to pooled plasma of W6 homozygotes, but did not respond significantly to pooled plasma of R6 homozygotes.

Conclusions: We developed two specific ELISAs for R6-a2AP and W6-a2AP in plasma. This will enable us to determine N-terminal cleavage of a2AP in plasma samples.

PB 175 | Stretching Fibrin Fibers Hampers their Lysis

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Background: Fibrinolysis is controlled by numerous chemical and enzymatic factors, and it is also influenced by the fibrin network architecture and fibrin fiber structure. It may also be affected by mechanical strain exerted on fibrin fibers by blood flow and clot retraction forces.

Aims: Our goal was to test the hypothesis that straining single fibrin fibers affects their lysis.

Methods: We developed a transparent, striated and highly stretchable substrate to investigate how strain affects lysis of single, suspended fibrin fibers. Lysis was observed by optical microscopy.

Results: In this suspended fiber assay, lysis manifested itself by considerable fiber elongation (up to 25%), fraying and collapse. Stretching single fibrin fibers significantly hampered their lysis. This effect was seen in uncrosslinked and crosslinked fibers. Crosslinking (without stretching) also hampered single fiber lysis.

Conclusions: Our data suggest that strain is a novel mechanosensitive factor that regulates blood clot dissolution (fibrinolysis) at the single fiber level. At the molecular level of single fibrin molecules, strain may distort, or hinder access to, plasmin cleavage sites and thereby hamper lysis.

PB 176 | Performance and Determinants of whole Blood and Plasma Fibrinolysis Assays in Patients with Mild Bleeding Symptoms

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Background: Clot stability and resistance to fibrinolysis are important for adequate haemostasis. Although enhanced clot lysis is associated with bleeding, a reliable functional lysis assay is not yet available.

Aims: We assessed whether the tissue Plasminogen Activator-Thromboelastometry (tPA-ROTEM), the Turbidity lysis assay and the Euglobulin lysis time (ELT) could find enhanced lysis capacity in patients who report bleeding symptoms, but are not diagnosed with bleeding disorders. We also gained insight in determinants of the lysis assays in these patients.

Methods: In this study, 240 patients with and 95 patients without reported bleeding symptoms on a preoperative questionnaire were included. The whole blood tPA-ROTEM (35 pM tissue factor) and plasma Turbidity assay (0.5 U/ml thrombin) were performed with addition of 125 ng/mL and 85 ng/ml tPA, respectively, and lysis times and speed were recorded. Blood count, coagulation and fibrinolysis factor activity levels were determined. Data were analysed with multiple linear regression models. Informed consent was obtained and the ethics committee approved the study.

Results: The median ELT did not differ between patients with and without reported bleeding symptoms (105 (IQR 85-120) vs. 105 (IQR 80-120) min, p=0.58), but surprisingly, the former group showed signs of *prolonged* and *slower* lysis in the tPA-ROTEM and Turbidity lysis assays (Tables 1, 2). In patients reporting bleeding symptoms, FII, plasminogen, antiplasmin, plasminogen activator inhibitor and thrombin activatable fibrinolysis inhibitor (TAFI) levels were independent

TABLE 1 tPA-ROTEM lysis in patients reporting bleeding symptoms vs. patients not reporting bleeding symptoms (subjects with all data available)

	tPA-ROTEM: Time from start to 90% lysis (n=327), Beta in SD (95%CI)	p	tPA-ROTEM: Lysis speed (%/min from 15% to 90% clot lysis) (n=320), Beta in SD (95%CI)	p
Patients reporting bleeding symptoms (vs. patients without bleeding symptoms)	0.29 (0.042 to 0.53)	0.022	-0.35 (-0.60 to -0.10)	0.007
Male	-0.026 (-0.26 to 0.20)	0.82	-0.054 (-0.29 to 0.18)	0.65
Age (SD)	0.15 (0.039 to 0.26)	0.008	-0.083 (-0.19 to 0.027)	0.14
Body Mass Index (BMI) (SD)	0.18 (0.074 to 0.29)	0.001	-0.16 (-0.27 to -0.044)	0.006

TABLE 2 Turbidity lysis in patients reporting bleeding symptoms vs. patients not reporting bleeding symptoms (subjects with all data available)

	Time from Max Optical Density to 90% lysis (n=326), Beta in SD (95%CI)	p	Lysis speed (%/min from 15% to 90% clot lysis) (n=326), Beta in SD (95%CI)	p
Patients reporting bleeding symptoms (vs. patients without bleeding symptoms)	0.23 (-0.014 to 0.46)	0.065	-0.15 (-0.40 to 0.11)	0.26
Male	0.17 (-0.048 to 0.40)	0.12	-0.24 (-0.47 to -0.005)	0.045
Age (SD)	0.15 (0.041 to 0.25)	0.007	-0.049 (-0.16 to 0.062)	0.39
Body Mass Index (BMI) (SD)	0.30 (0.20 to 0.40)	<0.001	-0.15 (-0.26 to -0.042)	0.007

determinants of the tPA-ROTEM results, while fibrinogen, antiplasmin, TAFI, gender and BMI independently influenced Turbidity lysis.

Conclusions: Patients reporting bleeding symptoms showed signs of *diminished* fibrinolysis in the tPA-ROTEM and Turbidity lysis assays compared to those without bleeding symptoms. This might be a compensation mechanism for impaired clot formation; once a clot is formed, slower lysis could help to maintain it.

PB 177 | Characterisation of the Different Pathways of Neutrophil Extracellular Trap (NET) Formation Triggered by Treatment with phorbol-12-myristate-13-acetate (PMA) or Calcium Ionophores

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Background: Neutrophils (PML) are pivotal players in host immune defence and deploy a number of mechanisms to combat invading pathogens including extrusion of chromatin and enzymes, termed NETosis. In vitro, PMA or Ca^{2+} ionophores (e.g. ionomycin) initiate NETosis. PML also respond to soluble ligands of the promiscuous receptor Mac-1 (alpha M beta 2).

Aims: We investigated differences between PMA and ionomycin as NET triggers and modulation by Mac-1 ligands fibrinogen (Fg) and fibrin.

Methods: NET formation by human PML was followed by confocal video microscopy after treatment with PMA or ionomycin, in the presence of Fg or multivalent, cross-linked fibrin degradation products (FDP). Observations were supported by biochemical studies.

Results: PMA and ionomycin both trigger chromatin release from 90 min under our conditions, but with morphological differences. Using blue Syto41 to follow intracellular DNA, green Fluo4 for intracellular Ca^{2+} and red propidium iodide to stain emerging extracellular DNA, we observed different patterns of intracellular Ca^{2+} accumulation and of nuclear DNA breakdown and release. The average area of NET DNA per NETosed cell was 1.9-fold higher with PMA than ionomycin. Adding Alexa fluor488 labelled-Fg slowed NET formation. With ionomycin, but not PMA, almost all PML bound Fg with surface clustering. In contrast, FDP bound to PML after PMA treatment, but not ionomycin. Quantitative Sytox Green staining confirmed that Fg reduced release of DNA after ionomycin treatment by more than 50%. Ionomycin also resulted in more proteolysis of cell associated Fg (alpha and gamma chains) than PMA.

Conclusions: PMA and ionomycin are not interchangeable and mimic different natural responses to pathogens, which are further modulated by cell environment. By studying triggers and regulation of NET formation we can understand how NETs switch from microbial defence into a risk factor for thrombotic and inflammatory disease and identify opportunities for therapeutic interventions.

PB 178 | Effect of Alpha-2 Plasmin Inhibitor p.Arg6Trp Polymorphism and Antigen Level on the Risk of Myocardial Infarction in Young Patients

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Background: Alpha2-plasmin inhibitor (a2-PI) is the primary inhibitor of plasmin. Its increased levels have been associated with increased thrombotic risk. A2-PI in the plasma undergoes both N- and C-terminal cleavages, which significantly modify its activities. About 70% of circulating a2-PI is amino-terminally shortened (by 12 amino acids) by antiplasmin cleaving enzyme (APCE). This isoform is cross-linked more effectively to fibrin alpha-chain by activated factor XIII; thereby the ratio of N-terminal isoforms may influence the fibrinolytic resistance of the fibrin clot. The p.Arg6Trp polymorphism affects the rate of this cleavage as APCE cleaves the Arg6 form 8-fold faster than the Trp6 form.

Aims: In this case-control study we investigated the effect of a2-PI p.Arg6Trp polymorphism and a2-PI antigen concentration on the risk of myocardial infarction (MI) in young patients.

Methods: 116 patients who had ST elevation MI below 40 years of age (MI) and age-matched healthy controls (HC, n=120) were recruited into the study. Total a2-PI antigen levels were determined by

a sandwich type ELISA, a2-PI Arg6Trp genotype was determined by RT-PCR using LightCycler® 480.

Results: Genotype distribution was consistent with the Hardy-Weinberg distribution in both study groups. The minor allele frequency did not differ significantly between HC and AMI (0.20 and 0.22 respectively; p=0.225). Possession of the Trp allele did not influence the risk for MI (OR: 1.104, 95% CI: 0.595-2.046). Total a2-PI antigen levels were significantly elevated in MI patients compared to HC (MI: 74.2±6.0 mg/L versus HC: 67.1±8.5 mg/L). Elevated a2-PI level (above 71.0 mg/L) increased the risk of MI (OR: 4.923, 95%CI: 2.72-8.909, p< 0.0001), which remained significant after adjustment to other cardiovascular risk factors.

Conclusions: In our study, the a2-PI p.Arg6Trp polymorphism had no effect on the risk of MI in young patients, however a2-PI levels in the upper tertile resulted in a significant risk enhancement.

PB 179 | Effect of PAI-1 on Vascular Smooth Muscle Cells Phenotype

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Background: Pharmacological administration of recombinant plasminogen activator inhibitor-1 (PAI-1) blocks vascular smooth muscle cell (VSMC) proliferation and migration in cell culture and neointimal formation following vascular injury in vivo. However, the role of PAI-1 in VSMC phenotype transitions remain unclear.

Aims: The purpose of this study was to determine molecular effects of PAI-1 on VSMCs.

Methods: Primary cultured human umbilical artery smooth muscle cells (HUASMC) were treated with recombinant PAI-1 mutants, and real-time reverse transcription-PCR was used to detect the gene expression.

Results: We show that PAI-1(10μg/mL) up-regulated the expression of serum response factor (*SRF*), a transcription factor in regulating VSMCs phenotypic modulation. Both the VSMCs differentiation marker genes smooth muscle α-actin (*SMA*), smoothelin (*SMTN*) and synthetic markers genes cyclin D1, 2 are all up-regulated by PAI-1 in VSMCs, whereas using other recombinant PAI-1 mutants with selective loss-of-function mutation we demonstrated that binding of PAI-1 to VN down-regulates the VSMCs synthetic markers genes (cyclin D1, 2, p< 0.05), and has no effect on the VSMC differentiation marker genes(SMA, SMTN). PAI-1 mutant with markedly reduced binding capacity for LDL receptor family members abrogate the effects of PAI-1 in the regulation VSMCs phenotype. Furthermore, pretreatment of RAP (receptor associated protein) and anti-LRP1 (LDL receptor-related protein 1) inhibited the *phenotypic* modulation *effects* of PAI-1, suggesting receptor family is required for PAI-1 regulation of VSMCs modulation.

Conclusions: Altogether, these data show diverse roles of PAI-1-VN binding and LDL receptor family in the control of the differentiated properties of VSMCs and suggest that the binding of VN is a key process for PAI-1 regulating phenotypic switch of VSMCs.

PB 180 | Study of Fibrinolytic Factors along with Thrombophilia Screening Gives a Comprehensive Picture of the Cause of Venous Thrombosis in Indian Patients

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Background: Many venous thrombosis cases, irrespective of a strong family history, recurrence or unusual site of thrombosis, remain unexplained as thrombophilia account for only about 1/3rd cases. Hypofibrinolysis is being reported to increase risk of thrombosis.

Aims: To study the fibrinolytic pathway defects along with conventional thrombophilia in venous thrombosis patients.

Methods: Study: 224 venous thrombosis cases; 104 CVT, 95 DVT, 17 PVT & 8 RVO. Protein C, Protein S, Antithrombin, Tissue plasminogen activator, Plasminogen activator inhibitor-1, Plasminogen & α-2-antiplasmin were measured by ELISA. Factor V Leiden mutation & PAI-1 4G/5G promoter polymorphism were studied by PCR-RFLP & ASA-PCR.

Results: 7.6% cases were FVL heterozygous. 4.9% cases had PC deficiency. 2.2% cases had PS deficiency. 0.9% cases had a combined PC & PS deficiency. 0.4% cases had AT deficiency. Conventional thrombophilia accounted for 16% cases. 16.5% cases had high PAI-1 levels (170.7±89.9ng/ml) against 2% in controls (p=0.0268, OR=9.6952). High PAI-1 level was significantly associated with CVT (p=0.0182, OR=11.67) & DVT (p=0.0344, OR=9.19). PAI-1 4G/5G polymorphism was associated with PAI-1 level (p=0.0065). 4G/4G (104.5±97.6ng/ml) had significantly high PAI-1 levels against 4G/5G (63±41.6ng/ml) & 5G/5G (55.9±42.5ng/ml). 23.6% cases had 4G/4G against 16.2% in controls (p=0.0953) with no significant difference in CVT (22.2%), DVT (22%), RVO (25%) cases. 4G/4G genotype had strong association (53%) (p=0.0015,OR=5.82) with PVT. Reduced TPA levels were observed in 3.1% cases (0.65±0.08ng/ml). PLG deficiency (237.5±32.7μg/ml) was observed in 1.3% cases. High AP levels (273.4±39.1ng/ml) were observed in 2.7% cases. 5.8% (13 cases) had both thrombophilia & fibrinolytic markers. Thus, 33% (74 cases) had at least one of these markers.

Conclusions: Hypofibrinolysis has significant role in venous thrombosis, and its investigation along with thrombophilia will facilitate in a comprehensive explanation of the cause of venous thrombosis.

TABLE 1 Thrombophilia and fibrinolysis markers in venous thrombosis

	Cerebral Vein Thrombosis (CVT=104)	Deep Vein Thrombosis (DVT=95)	Portal Vein Thrombosis (PVT=17)	Retinal Vein Occlusion (RVO=8)	Venous thrombosis cases (Total=224)
Protein C (PC) deficiency	2 (1.9%)	6 (6.3%)	3 (17.6%)	-	11 (4.9%)
Protein S (PS) deficiency	2 (1.9%)	3 (3.2%)	-	-	5 (2.2%)
Combined PS+PS deficiency	-	2 (2.1%)	-	-	2 (0.9%)
Antithrombin (AT) deficiency	-	1 (1%)	-	-	1 (0.4%)
Factor V Leiden (FVL) mutation	5 (4.8%)	11 (11.6%)	-	1 (12.5%)	17 (7.6%)
High Plasminogen activator inhibitor-1 (PAI-1) levels	20 (19.2%)	15 (15.8%)	2 (11.8%)	-	37 (16.5%)
Reduced Tissue Plasminogen activator (TPA) levels	2 (1.9%)	3 (3.2%)	-	2 (25%)	7 (3.1%)
Plasminogen (PLG) deficiency	1 (0.4%)	1 (1%)	1 (5.9%)	-	3 (1.3%)
High Antiplasmin (AP) levels	3 (2.9%)	3 (3.2%)	-	-	6 (2.7%)

PB 181 | Impaired Plasminogen Binding in Patients with Venous Thromboembolism: Association with Protein Carbonylation

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Background: Venous thromboembolism (VTE) is associated with hypofibrinolysis. Its mechanisms are poorly understood.

Aims: The aim of the current study was to investigate plasminogen-fibrin interaction and its impact on plasmin generation, fibrinolysis and the extent of protein oxidation/carbonylation in VTE patients.

Methods: Plasma-purified plasmin(ogen) functional activity was evaluated together with surface plasmon resonance employed for fibrin-plasminogen interactions individually in healthy controls and patients following VTE. We also assessed plasma fibrin clot permeability (K_s), clot lysis time (CLT), activators and inhibitors of fibrinolysis together with oxidation/carbonylation markers, including thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC), and total protein carbonyl (total PC).

Results: VTE patients had impaired plasminogen binding to fibrin compared with controls (Kd, +265%, p=0.006), mainly related to reduced plasmin protease activity (-20.5%, p=0.04). VTE patients had longer CLT (+17%, p=0.002) but K_s remained unaffected when compared to controls. Of note, CLT was associated with Kd (r=0.44, p=0.03), while Kd correlated with plasmin activity (r=-0.65, p=0.002), PAI-1 (r=0.43, p=0.046), and tPA-PAI-1 complex (r=0.52, p=0.03). Among oxidation/carbonylation markers, VTE patients presented higher TBARS (+50%, P< 0.001) and total PC (+49%, p< 0.001), but lower TAC (-25%, p< 0.001) compared with healthy controls. TBARS (r=0.61, p=0.003), TAC (r=-0.47, p=0.03), and total PC (r=0.56, p=0.009) correlated with CLT. Moreover, associations between Kd and TBARS (r=0.44, p=0.04) or PC (r=0.45, p=0.03) were observed in the whole group.

Conclusions: The fibrin-plasminogen binding affinity as well as plasmin activity are impaired in VTE patients. We showed for the first time an increased carbonylation of plasma proteins in VTE patients and its association with prolonged clot lysis indicating novel antifibrinolytic mechanisms in VTE.

PB 182 | Ligneous Gingivitis due to Severe Plasminogen Deficiency: Results of a Prophylactic Protocol for Dental Care

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Background: Severe plasminogen (PLG) deficiency causes a rare disease, known as ligneous conjunctivitis, characterized by the growth of fibrin rich pseudomembranes on mucosal surfaces. Besides of conjunctiva, gums are often involved, leading to ligneous gingivitis (LG). Specific therapy for LG is not established.

Aims: We describe a prophylactic protocol with enoxaparin and fresh frozen plasma (FFP) for dental invasive procedures in a patient with LG due to PLG deficiency.

Methods: A 43 years old female with LG was referred to us in 2009. She had ligneous conjunctivitis in childhood and ligneous cervicitis refractory to conization. PLG antigen and activity levels, and PLG genetic analysis were carried out. In order to prevent LG recurrence after dental invasive procedures, a prophylactic treatment including 10 ml/kg bw FFP before and the day after the intervention and enoxaparin 100 U/kg bw od for 20 days was given in addition to proper mini-invasive dentistry techniques and implant surgery.

Results: The patient's PLG antigen and activity levels were 25 ug/ml and 27%, respectively, and genetic analysis showed a c.112A>G p.K38E mutation in exon 2 and c.1256+1G>A substitution at donor site of exon 10, each in heterozygous level. Right tooth root extraction with gum suture was performed in 2009, left molar tooth extraction

and contralateral implant positioning in 2012, and right molar tooth extraction in 2015. PLG activity levels raised to about 46% two hours after FFP transfusion and returned to baseline after 48 hours. Small gingival pseudomembranes developed soon after interventions and disappeared within one week; no bleeding complications were seen. The patient denied FFP transfusion in 2015.

Conclusions: In our patient with LG, the adoption of combined hematological and dentistry protocols appeared to be safe and effective in preventing abnormal gingival pseudomembranes growth after dental interventions, maintaining a good parodontal condition.

PB 183 | Effect of Polyamidoamine (PAMAM) Dendrimers on the Activity of the Fibrinolytic System in vitro

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Background: Due to their tree-like structure, the presence of internal cavities and the multitude of surface groups, dendrimers are used in medicine as drug delivery, imaging, antiviral and transfecting agents. PAMAM dendrimers with different surface charges interact with blood components and can affect the activity of enzymes.

Aims: Studying the effect of the surface charge density and concentration of anionic and cationic PAMAM dendrimers on activity of fibrinolytic system *in vitro*.

Methods: The effect of concentration of amine-terminated (G1, G2 and G3) and carboxy-terminated (G1.5, G2.5 and G3.5) PAMAM dendrimers on amidolytic, plasminogen(Pg)-activator and thrombolytic activities of tPA and uPA was studied.

Results: Cationic dendrimers G1, G2 and G3 (0.4 mM) increased in tPA activity against S-2228 by 10-30% and have not affect on uPA activity against S-2444. Anionic dendrimers G1.5, G2.5 and G3.5 (0.4 mM) reduced amidolytic activity of tPA by 5-15% and uPA by 30, 40 and 100%, respectively. The sharp decline in Pg-activator activity of tPA was observed with increasing concentrations of both cationic and anionic dendrimers. In the case of uPA, anionic dendrimers strongly reduced, while cationic dendrimers significantly increased the Pg activation rate. Nonetheless, anionic, and, to a lesser extent, cationic dendrimers lowered the lysis rate of plasma clot induced by tPA and uPA. The thrombolytic activity of tPA and uPA reduced stronger with increasing concentration and generation of dendrimers. The possible sites of binding of cationic and anionic PAMAM dendrimers to plasminogen, tPA, uPA and fibrin surface explaining the observed effects were considered.

Conclusions: Anionic PAMAM dendrimers stronger lower the activity of the fibrinolytic system than cationic dendrimers and this effect enhanced with increasing the surface charge density and concentration of dendrimers. This should be considered when using higher generations of PAMAM dendrimers for drug delivery.

PB 184 | Fibrinolytic Markers in Patients Undergoing Total Hip Replacement with and without Administration of Tranexamic Acid

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Background: Tranexamic acid (TA) inhibits plasminogen activation and interferes with fibrinolysis. TA administration is effective in decreasing blood loss without increasing complications in surgery.

Aims: To evaluate the effect of TA on the fibrinolysis markers plasminogen (Pg) and plasminogen activator inhibitor type 1 (PAI-1) after total hip replacement (THR).

Methods: Ninety-four patients admitted for THR were enrolled in a sequential series study. The no-TA group comprised 48 patients (51±10 years, 26 men) and the TA group had 46 patients (53±13 years, 20 men). TA was administered intravenously at 30 min prior to the THR and at 6 hours after the first dose. Pg and PAI-1 were measured before the THR, and at 30 min, 1, 3, 7, and 14 days after. Total blood loss and number of symptomatic deep vein thromboses (DVT) were determined. Statistical differences between groups were analyzed by the Mann Whitney U test. All participants gave written informed consent. The study was approved by the local research ethics committee.

Results: Blood loss in the TA group was 780±293 mL and 1337±490 mL in the no-TA group. After surgery Pg was lower in the TA compared to the no-TA group: 30 min (median [interquartile range]) (65.5 [55.4, 79.1] and 72.0 [63.7, 88.5]%, $p< 0.05$), day 1 (64.3 [56.3, 75.4] and 73.3 [64.0, 85.8]%, $p< 0.01$), day 7 (111.3 [104.6, 118.7] and 123.2 [108.2, 133.9]%, $p< 0.05$), respectively. PAI-1 was higher in the TA compared to the no-TA group: 30 min, (5.4 [3.0, 8.8] and 1.55 [0.40, 5.0] U/ml, $p< 0.05$), day 1 (14.7 [8.8, 28.5] and 6.2 [2.5, 14.8] U/ml, $p< 0.01$), day 3 (10.1 [3.7, 18.7] and 5.2 [2.5, 9.6] U/ml, $p< 0.05$), respectively. Symptomatic DVT was detected in 2 patients in the TA group and in 3 patients in the no-TA group.

Conclusions: Administering TA before and after THR reduced Pg and increased PAI-1 levels in the blood serum. Blood loss was reduced by TA. Symptomatic DVT remained unchanged.

PB 185 | Hyperfibrinolysis during Liver Transplantation Detected by Classical Parameters and Thromboelastometry

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Background: Low coagulation and hyperfibrinolysis are the major causes of excessive bleeding during orthotopic liver transplantation (OLT). However, the impact of hyperfibrinolysis in haemorrhagic complications complicating OLT remains a matter of debate.

Aims: To dynamically evaluate the presence of hyperfibrinolysis during OLT and the role of thromboelastometry to detect hyperfibrinolysis and to predict bleeding loss.

Methods: Thirty consecutive liver-transplanted recipients (M/F 22/8, mean(±SD) age 56(±12) years) who underwent OLT were included. Blood samples were drawn at serial time points: T0, preoperatively; T1, anhepatic phase; T2, 15 min after the reperfusion of the graft; T3, postoperatively. Fibrinolysis parameters (plasminogen, α2- antiplasmin, tissue plasminogen activator (tPA), thrombin-activatable *fibrinolysis* inhibitor, factor XIII, D-dimer, plasminogen activator inhibitor antigen) and thromboelastometry parameters (by ROTEM) were measured.

Results: Mean levels of antifibrinolytic factors were significantly lower during T1 than T0 and T2/T3, and higher in T2 than T3. TPA was significantly higher during T1 than T0 and T2/T3. Fibrinolysis ROTEM parameters (maximum lysis (ML) and lysis at 30 min (LI30) exactly mirrored these findings; in particular ML and LI were significantly higher in T1 compared to the other time-points both in the intrinsic-INTEM and in the extrinsic-EXTEM assays. Fourteen (46%) patients had blood loss >2,000 mL during OLT. Those patients showed significantly higher ML (53%) than patients with blood loss < 2,000 mL (ML 12%, p=0.018) both in INTEM and in EXTEM assay in T1.

Conclusions: Hyperfibrinolysis seemed to be related only to the anhepatic phase of the OLT and it was mainly determined by a significant reduction of antifibrinolytic proteins and increased tPa. Thromboelastometry parameters of fibrinolysis could identify patients with hyperfibrinolysis. Hyperfinrinolysis detected by ROTEM during the anhepatic phase could identify patients at higher risk of bleeding.

PB 186 | The Use of Antiplasmin-Specific Affimer as a Tool to Modulate Fibrin Clot Properties and Thrombosis Risk

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Background: Fibrin clot lysis, which limits thrombus formation, is controlled by various factors including incorporation of anti-fibrinolytic proteins into the clot, most importantly Plasmin Inhibitor (PI). Affimers are small proteins composed of a scaffold constraining two random conformational 9AA peptides that are easily produced in *E. coli*.

Aims: Identify the role of Affimers in modulating PI-specific prolongation in clot lysis.

Methods: A library of Affimers, containing $3x10^{10}$ different combinations of random peptides, was screened for PI binding using a phage display system. After 3 rounds of panning, high affinity PI-binding Affimers were tested for modulation of fibrin clot lysis. Validated turbidimetric assays were employed to assess fibrin clot structure/lysis, using both purified and plasma systems.

Results: A total of 167 high affinity PI-binding Affimers were isolated, of which 22 had distinct sequences and these were subsequently expressed in *E. coli*. One Affimer (termed A68) consistently inhibited the prolongation of PI-induced clot lysis in a purified system in a dose dependent manner with an effect observed at Affimer/PI molar concentration of 2/1. Clots formed from pooled plasma showed a reduction in lysis time from 650±23 sec with the addition of scaffold only Affimer to 420±21 sec in the presence of A68 (35% reduction in lysis time, p< 0.001). Addition of A68 or scaffold showed no effect on clot final turbidity (0.211 and 0.213 AU respectively, p>0.1), suggesting that A68 has no significant effect on clot structure. Tested in plasma from patients with diabetes, A68 reduced clot lysis time in 11 of the 12 samples analysed by an average of 25% (range 7-47%; p< 0.01).

Conclusions: The work so far provides proof of concept that PI-binding Affimers represent a viable tool to modulate fibrin clot lysis and may help to identify novel therapeutic targets to reduce thrombosis risk.

PB 187 | Application of the CloFAL Assay, a Global Assay which Evaluates Coagulation and Fibrinolysis in a Cohort of Venous Thromboembolism (VTE)

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Background: The Clot Formation and Lysis (CloFAL) assay, that simultaneously measures coagulation and fibrinolysis, appears as an interesting method, easy to standardize with a low cost.

Aims: To evaluate the CloFAL assay in patients with VTE and evaluate alterations associated to the hypercoagulable state and rethrombosis.

Methods: A hundred-eight patients (median age of 46 years; 22-80 years; 71 female/37male) who were attended at the outpatient clinic of Hemocentro de Campinas, UNICAMP, from January 2013 to November 2016, were enrolled. Fifty-five (50.9%) patients presented spontaneous DVT. The median time after the first VTE was 3 years. The history of recurrent thrombosis was present in 12 (11.1%) patients. The control group included 20 healthy individuals with a median age of 28.85 years (13 female/7 male). In the CloFAL assay, a buffered reactant solution containing trace amounts of calcium, tissue factor and tissue-type plasminogen activator were added to plasma samples on a 96-well microplate in a spectrophotometer at 37°C. The coagulation index (CI) and various fibrinolytic index (FI) were calculated.

Results: The CI showed similar results when patients and controls were compared, with a median of 90% (49-235%) and 80% (4-892%), respectively. FI median was markedly decreased in patients (98%, 47-313%) in comparison to controls (144.5%, 69-296%), p< 0.001. No correlation was found between CI and FI. Patients who presented

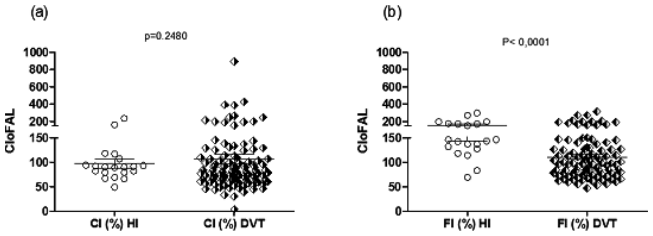

FIGURE 1 CloFAL analyses from healthy individuals (HI) and patients with Deep Venous Trombosis (DVT)

provoked DVT showed lower FI when compared with those with spontaneous DVT (98% *vs.* 76%, P= 0,0031).

Conclusions: The CloFAL global assay appears to be analytically sensitive to several key components of the coagulation and fibrinolytic systems, as well as to physiologic alterations in hemostasis. A study with a higher number of patients with DVT would be important to validate these results, and to investigate which fibrinolytic parameters are involved in the observed hypofibrinolysis. These results highlights that other fibrinolytic pathways can play a role on venous thrombotic disease.

PB 188 | Simultaneous Thrombin Plasmin Generation Assay in Haemorragic and Non Haemorragic Caesarean Section: Pilot Study

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Background: TRACES-trial is a multicenter randomized double-blind placebo-controlled therapeutic and pharmaco-biological dose-ranging study supported by French ministry of health (NCT02797119). Simultaneous Thrombin and Plasmin Generation Assay (STPGA) described by Van Geffen has been developed and validated in a preliminary study.

Aims: To evaluate interest of STPGA for the follow-up of patients undergoing either haemorragic (>800ml) (H) or non-haemorragic (< 800ml) (NH) ceasarean section (CS) compared to non-pregnant woman (NP).

Methods: The pilot study included 15, 10 and 9 patients in H, NH and NP group respectively. Eleven patients from H group were treated by Tranexamic Acid (TXA). For the 3 groups, STPGA was realized at baseline. A kinetic study was realized, after CS, for NH and H treated by TXA. Thrombin and plasmin AUCs at T0 in NH and H were compared

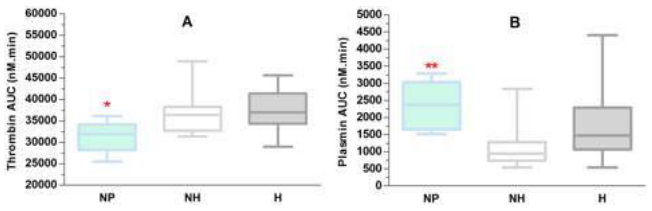

FIGURE 1 Thrombin (A) and plasmin (B) AUCs measured in STPGA at T0 on NH and H groups compared to NP group

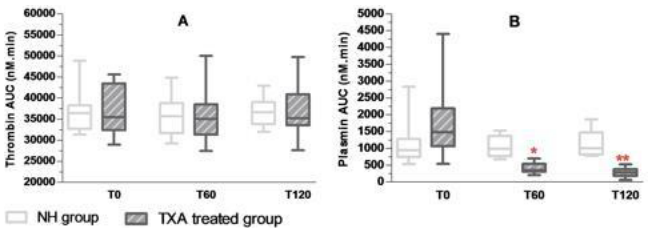

FIGURE 2 Thrombin and plasmin (B) AUCs measured in STPGA at T0 T60 and T120 in NH and TXA-treated groups

to NP group. Thrombin and plasmin AUCs were compared at T0 T60 and T120 (after CS) between NH and H treated by TXA.

Results: At T0, thrombin AUC (figure 1A) is lower in NP than in NH and H group whereas plasmin AUC (figure 1B) is higher in NP group than in NH and H group. During the follow-up, for NH and H treated by TXA, no statistical difference was noted at any time in thrombine AUC (figure 2 A). Plasmin AUC were statistically lower in the TXA-treated group at T60 (p=0.0002) and T120 (p< 0.0001) (Figure 2 B) *(*Thrombin AUC in NP vs NH group: p=0.02; **Plasmin AUC in NP vs NH group: p=0.002).*

*(Plasmin AUC in TXA-treated vs NH group at *T60: p=0.0002 and **T120: p< 0.0001).*

Conclusions: STPGA shows hypercoagulable state in late pregnancy and fibrinolysis capacity following placental separation, and is able to evaluate fibrinolysis inhibition by TXA during haemorragic CS. These primilarly results must be confirmed in the TRACES-study.

PB 189 | Simultaneous Thrombin Plasmin Generation Assay: Effect of Tranexamic Acid Concentration in vitro

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Background: In order to explore coagulation, fibrinolysis and interplay, a Simultaneous Thrombin-Plasmin Generation Assay (STPGA) has been developed recently in our laboratory.

Aims: To evaluate the sensivity of the STPGA to in vitro effect of Tranexamic Acid (TXA) , spiking of different concentration of TXA was realized in a normal pooled plasma.

Methods: Successive dilutions from a tranexamic acid (TA) solution for injection (tranexamic acid concentration :100 mg/mL) were realized , in order to obtain a final TA plasmatic concentration in each well ranged from 0 to 8 mg/L, to assess the effect of different TA concentrations *in vitro* on STPGA in a normal pooled plasma. Briefly, STPGA is realized with 2 non-interfering fluorogenic thrombin and plasmin specific substrates. In a black microtiter well, were added 80 µl plasma, 2µl cephalin, 2 µl TF (0.28pM approximately), 4µ µl fluorogenic thrombin substrate, 2µl fluorogenic plasmin substrate

and TBS. After adding starting reagent (Cacl2 and t-PA), fluorescence was measured and convert to thrombin and plasmin concentration.

Results: The STPGA profiles of differents TA concentration are presented separately for thrombin (figure 1) and plasmin (figure 2).

As expected, plasmin generation decreased with rising TA concentrations, whereas thrombin generation was unaffected by the TA concentration variations.

Conclusions: *In vitro*, STPGA permitted to show the dose effect relationship between TA and plasmin generation. The next step will be the *in vivo* evaluation of TA treated patient.

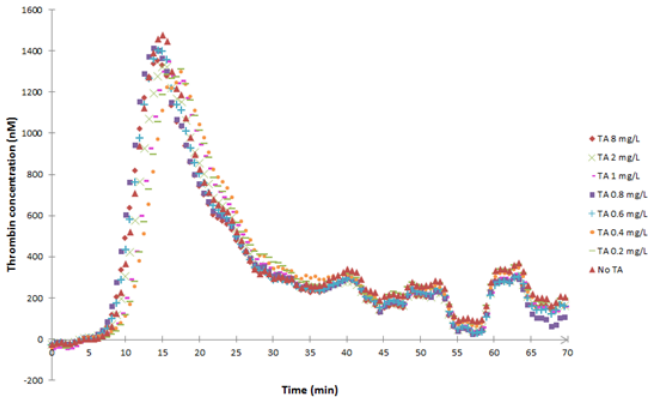

FIGURE 1 Thrombin generation profile in the STPGA of different tranexamic plasmatic concentrations

PB 190 | Association of Fibrinolytic Parameters with Coagulation Activity and Fibrin Clot Permeability in Patients with Hemophilia A

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Background: Hemophilia A has been considered as a bleeding disorder that is not only a consequence of the defect in the coagulation system, but also of the impaired down-regulation of the fibrinolytic system.

Aims: To examine correlation between activities and/or concentrations of most important fibrinolytic parameters with overall hemostasis potential (OHP) and fibrin clot permeability (FCP) in hemophilia A patients.

Methods: Activities of FXIII, plasminogen, plasmin inhibitor, plasminogen activator inhibitor-1 (PAI-1) (Siemens Healthcare Diagnostics, Germany) and thrombin-activatable fibrinolysis inhibitor (TAFI) (Diagnostica Stago, France), as well as activated/inactivated TAFI antigen (TAFIa/ai) (Diagnostica Stago) and prothrombin fragment F1+2 concentrations (PF1+2) (Siemens) were tested in plasma samples of 63 hemophilia A patients (30 severe, 33 non-severe). OHP method,

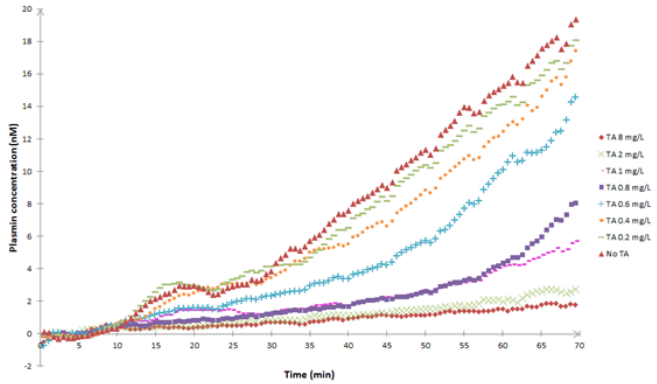

FIGURE 2 Plasmin generation profilein the STGPA of different tranexamic plasmatic concentrations

based on repeated spectrophotometric registration of fibrin-aggregation in plasma, after addition of small amounts of exogenous thrombin, tissue plasminogen activator and calcium, gave beside OHP parameter (area under the fibrin aggregation curve) 3 supplementary parameters: overall coagulation potential (OCP), overall fibrinolytic potential (OFP) and clot lysis time (CLT). Permeability coefficients (Ks), providing information on fibrin network porosity, were obtained by FCP method, flow measurement technique along with visualisation by electron microscopy scanning.

Results: Among fibrinolytic and OHP parameters significant but weak correlation (P< 0.05) was found only for OCP with TAFI and PF1+2 (r=0.252 and 0.266, respectively). Regarding FCP, significant correlation of Ks was found with FXIII, plasmin inhibitor and PF1+2 (r=-0.466, -0.432 and -0.599, respectively). There was no correlation between Ks and OHP parameters.

Conclusions: Obtained correlation between fibrinolytic parameters and parameters that assess coagulation activity and fibrin network porosity confirmed the association between these two processes in patients with hemophilia A.

PB 191 | Consequences of Tranexamic Acid on Fibrinolysis in Patients with Hereditary Bleeding Disorders Compared with Healthy Controls

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Background: Tranexamic acid (TXA) is an anti-fibrinolytic used to prevent bleeding in haemophilia patients who undergo minor procedures such as dental extractions. Despite a number of studies reporting avoidance of additional factor replacement using this strategy, the impact of TXA on fibrinolysis in this setting is yet to be elucidated.

Aims: To use the clot lysis assay to evaluate the impact of TXA on fibrinolysis in haemophilia patients undergoing dental procedures.

Methods: Ten healthy volunteers and ten patients with haemophilia were recruited after approval by the hospital research ethics board. Blood was

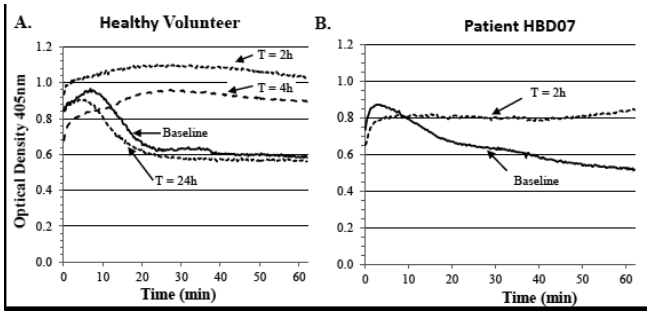

FIGURE 1 Represenative Clot lysis profiles. A. Healthy Volunteer, B. Patient HBD07

TABLE 1 Patient Demographics

Patient	Age	Bleeding Disorder	Factor Level (%)	Dental Procedure	Post-procedural Bleeding and Management
HBD01	57	Haemophilia A	15	1x Tooth extraction	Severe: Hospital admission for factor replacement
HBD02	29	Haemophilia A	4	1x Tooth extraction	Nil
HBD03	41	Haemophilia A	<1	1x Tooth extraction	Nil
HBD04	53	Haemophilia A	3	1x Tooth extraction	Nil
HBD05	56	Haemophilia A	10	1x Tooth extraction	Nil
HBD06	77	Haemophilia A	33	1x Tooth extraction	Nil
HBD07	59	Haemophilia A	28	1x Tooth extraction	Nil
HBD08	49	Haemophilia A	11	1x Tooth extraction	Nil
HBD09	80	Haemophilia B	15	1x Tooth extraction	Moderate: Extended treatment with TxA
HBD10	68	Haemophilia B	3	3x Tooth extraction	Nil

taken from the healthy volunteers at baseline, then at 2, 4 and 24 hours after ingestion of 1 gram of TXA. Haemophilia patients had blood collected on the day of their procedure, at baseline and 2-4 hours post ingestion of TXA. The Clot lysis assay was performed as described (Niego et al 2008 Blood Coag Fibrinol 19:322-324). Any procedural bleeding was retrospectively evaluated by accessing patient medical records.

Results: In the healthy volunteers, typical normal clot formation and lysis profiles were observed at baseline and TXA showed complete blockade of fibrinolysis at 2 and 4 hours but returned back to normal at 24hours (Figure 1A: representative profile of one volunteer). Ten haemophilia patients underwent tooth extractions under local anaesthetic. Patient details are outlined in Table 1. Six patients had comparable clot formation and lysis profiles at baseline and all patients had completely inhibited fibrinolysis at 2-4 hours post TXA ingestion (Figure 1B: representative profile of one patient). Unexpectedly, one patient with mild haemophilia experienced post-procedural bleeding requiring factor replacement.

Conclusions: Similar to healthy volunteers, TXA is effective at blocking t-PA mediated fibrinolysis in patients with haemophilia undergoing dental procedures and further supports its use as prophylaxis in the absence of factor concentrate.

PB 192 | Association of TAFI Polymorphisms and Fibrinolytic Status with Obesity in Subjects Suspected for Coronary Artery Disease

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Background: Obesity is a risk factor for coronary artery disease (CAD), which is associated with impaired fibrinolysis. Plasminogen activator inhibitor-1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) are inhibitors in fibrinolysis. Their levels were genetically controlled by gene polymorphisms.

Aims: This study aimed to assess the association of *PAI-1* and *TAFI* polymorphisms with obesity and fibrinolysis status in subjects suspected for CAD.

Methods: A of total of 327 subjects suspected for CAD and underwent coronary angiogram were recruited and individuals with body mass index ≥25 kg/m^2 were classified as obesity. The studied polymorphisms included *PAI-1* -675 4G/5G, *TAFI* 505G/A, 1040C/T, +1542C/G and +1583T/A. All polymorphisms were genotyped by allele-specific polymerase chain reaction (ASPCR) except for *TAFI* 1040C/T which was genotyped by PCR-restriction fragment length polymorphism. The global fibrinolytic activity was measured by euglobulin clot lysis assay while PAI-1 antigen was investigated by enzyme-linked immunosorbent assay.

Results: There was no difference in genotype distribution of -675 4G/5G in obesity compared to non-obesity group. Significantly increased level of PAI-1 was found in obesity compared to non-obesity group (7.8±2.1 vs 5.7±2.3, p=0.018). The T allele carrier of 1040C/T and G allele carrier of +1542C/G were associated with obesity after adjustment for age and sex [adjusted OR (95% CI) 0.62 (0.40, 0.97), and 0.57 (0.36, 0.89), respectively]. Global fibrinolytic activity was not significantly different among genotypes of the studied polymorphisms. Moreover, the polymorphisms were not associated with CAD.

Conclusions: This study suggested that the *TAFI* 1040C/T, +1542C/G may affect fibrinolysis in obesity. The tendency of hypofibrinolysis in subjects suspected CAD may in part due to increased PAI-1. However, TAFI level was not determined in this study, thus it should be further measured to clarify these results.

PB 193 | Nattokinase Easily Hydrolyzed Fish Elastin and Collagen, and Produced Anti-platelet Aggregation Activity

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Background: *Bacillus subtilis natto* is well known as one of the safest bacteria in the world. Nattokinase has a single-stranded polypeptide structure with 275 amino acid residues linked together, at pI 8.7 with a molecular weight of 27,274.

This enzyme also hydrolyzes elastin (Sumi *et al*., XXIII Congress of ISTH, P-TU-269, Kyoto, 2011).

Aims: There were two aims to this study: first, to hydrolyze fish elastin and collagen, and, second, to show each anti-platelet aggregation activity.
Methods: Nattokinase was supplied by Organo Foodteck Co. Ltd. and lumbrokinase was supplied by Well Stone Ltd.. Elastin, trypsin, chymotrypsin and synthetic substrates were purchased from Sigma-Aldrich Co. Ltd.. Several fish elastins were kindly supplied Hayashikane Sangyo Co. Ltd., and fish collagen was kindly supplied by Yamaki Co. Ltd..
The fibrin plate method (Sumi *et al*., *Experientia* 43:1110, 1987), elastin plate method (Sbarra *et al*., *Nature*, 188:322, 1960) and platelet aggregation activity (Sumi, *J. Home Econ. Jpn*., 50:683, 1999) was tested elsewhere.
Results: It was observed that nattokinase has strong lysis activity for elastin (< 2.5mg/ml, elastin plate method), which is a characteristic not observed at all under the same conditions for trypsin (10mg/ml), chymotrypsin (10mg/ml), or lumbrokinase (50mg/ml).
Nattokinase also easily hydrolyzed fish elastin (sea jack and sea bream). The addition of fish collagen rather than fish elastin, resulted in the same anti-platelet aggregation activity.
Conclusions: Nattokinase easily hydrolyzed fish elastin and collagen, and each anti-platelet aggregation activity was produced.

PB 195 | A New Functional Food "Coix-Natto" for Fibrinolysis

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Background: Coix (*Coix lacryma-jobi var. ma-yuen*) is a well known Chinese medicine which increases peripheral cytotoxic T and natural killer cells (Hidaka *et al*., *Biotherapy*, 5:2013, 1992). On the other hand, natto is a common Japanese food made of fermented soybeans, and nattokinase (NK) is a fibrinolytic enzyme generated from *Bacillus subtilis natto*. NK (M.W. 27,724, pI. 8.7) has been determined as a single polypeptide molecular structure linking 275 amino acid residues (Sumi *et al*., *Experientia*, 43:1110, 1987; Sumi *et al*., 22nd ISFP, P8, Marseille, 2014). NK has t-PA releasing activity from human endotherial cells (Yatagai *et al*., *Pathophysiol. Haemost. Thromb*., 36:227, 2009).
Aims: Coix (*Coicis Semen*) and Bacillus (*Bacillus subtilis* (*natto*)) were first conjugated. This study compared Coix-Natto and normal natto products by employing an evaluation method.
Methods: *Bacillus subtilis natto* (A and B) and *Bacillus subtilis* (C) were used in solid culture. Determinations of fibrinolysis (Sumi *et al*., *Experientia*, 43:1110), polyamine (Saito *et al*., *Anal. Sci*., 8: 675, 1992), vitamin K_2(Sumi, *Food Sci. Technol. Res*., 5, 48, 1999) and synthetic substrate hydrolysis were reported elsewhere. Double immnodiffusion was performed using anti-serum against highly purified NK (Sumi *et al*., 23rd ISTH, P-TU-268, Kyoto, 2011).
Results: The maximal fibirinolysis "B" (>1,000mm^2/30µl) was detected in the presence of glycerol (1.0-5.0%) at 37°C for 90hr culture. The "B" hydrolyzed synthetic substrate II (Suc-Ala-Ala-Pro-Phe-*p*NA) at a rate less than half of synthetic substrate I (Bz-Ile-Glu-(OR)-Gly-Arg-*p*NA). The "B and C" also produced high concentration of vitamin K_2 (1,400-1,530µg/100g wet wt.) and polyamine (4.81-5.01mg/100g wet wt.). All NK and NK-like activities were very stable in dry powder conditions.
Conclusions: Coix-Natto was not sticky and offered a good taste. The vitamin K_2 concentration was no less than that of general soybean natto (Sumi *et al*., 25th ISTH, PO462-TUE, Toront, 2015; *New Food Industry*, 59:23, 2017).

PB 197 | Fibrinolytic Effects of Regulatory Leucine-containing Peptides in the Organism

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Background: Previously it is established that the products of hydrolysis of structural proteins -collagen and elastin- proline-containing peptides have the ability to inhibit the polymerization of fibrin . It is shown the interaction of amino acid leucine with fibrinogen.
Aims: The aim of this work was to study the effects of two leucine-containing peptides Leu-Pro-Gly-Pro(LPGP) and Pro-Gly-Pro-Leu (PGPL) on the parameters of enzymatic fibrinolysis and polymerization of fibrin, in the blood of animals in vivo.
Methods: Experiments were conducted on more than 50 white laboratory rats weighing 200-230 g in accordance with the ethical principles of the Helsinki Declaration. Experienced rats intranasally daily for 5-7 days injected each peptide in a volume of 20 µl (dose: 200 µ/kg of the rat body weight), a control - 0.85% saline. In blood plasma the level of fibrinogen, fibrindepolymerizing activity (FDPA), total enzymatic fibrinolytic activity (TEFA) and activity of tissue plasminogen activator (t-PA) was determined by the standard methods 20 hours after the last administration of peptides.
Results: It was found that the peptides LPGP and PGPL increased TEFA on 55 - 90%, t-PA on 120-140%, FDPA on 70- 110%, respectively. Fibrinogen concentration decreased under the action of both peptides by 12%. The highest fibrinolytic and fibrindepolymerizing effects has been installed after the introduction of the PGPL.
Conclusions: We showed that addition of leucine to the C - or N-ends of the peptide Pro-Gly-Pro increased fibrinolytic activity of blood plasma. Thus, leucine-containing glyprolines introduced into the organism promoted depolymerization of fibrin, increased the enzymatic properties of blood plasma, reduced the concentration of fibrinogen. Therefore they can be considered effective fibrinolytic factors, which prevented the polymerization of fibrin in the organism.

PB 198 | Very High Production of Nattokinase by D-amino Acids

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Background: Nattokinase (NK) is a single-stranded polypeptide structure with 275 amino acid residues linked together at pI 8.7 with

a molecular weight of 27,274. NK has also t-PA releasing effect on human endotherial cells (Yatagai *et al*., *Pathophysiol. Haemost. Throm*b., 36:227, 2009).

NK production is controlled by dipicolic acid (Ohsugi *et al*., *J. Food. Biochem*., 35:370, 2011) and isoflavone (genistein) (Yatagai *et al*., *Pathophysiol. Haemost. Thromb*., 36:298, 2009).

Aims: Relation of D-amino acid NK production was first reported.

Methods: D-amino acids and synthetic substrates were purchased from Sigma-Aldrich Co Ltd.. Additional amino acid test was performed for 7days at 41°C (2% High- polypeptone S-3% glycerol).

Determinations of fibrinolysis (Sumi *et al*., *Experientia*, 43:1110), polyamine (Saito *et al*., *Anal. Sci*., 8: 675, 1992), vitamin K_2(Sumi, *Food Sci. Technol. Res*., 5, 48, 1999) and synthetic substrate hydrolysis were reported elsewhere. Double immnodiffusion was performed using anti-serum against highly purified NK (Sumi *et al*., 23rd ISTH, P-TU-268, Kyoto, 2011).

Results: Addition of 1% D-amino acids (D-Phe, D-Lys, D-Pro, D-Asp etc.) in *Bacillus subtilis natto* (Miyagino) culture system produced very high NK activity 8 times more than the control (which contained no amino acid).

An increase of NK at 3.5 times higher than the control was also confirmed using synthetic substrate (Bz-Ile-Glu-(OR)-Gly-Arg-*p*NA) hydrolysis.

Production of vitamin K_2 was confirmed to be like that of NK.

Conclusions: It was proven that very high production of NK occurred with the addition of several D-amino acids.

PB 906 | Thrombin-activable Fibrinolysis Inhibitor (Procarboxypeptidase B2) Deficiency Protects from Carbon Tetrachloride-induced Liver Fibrosis

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Background: Thrombin-activatable fibrinolysis inhibitor (TAFI; procarboxypeptidase B2, [proCPB2]) is produced by several cells including hepatocytes, macrophages, and megakaryocytes. Activated TAFI induces inactivation of proteins or peptides by removing the arginine and lysine residues from the carboxy-terminus. TAFI can be activated by the thrombin/thrombomodulin complex on the surface of endothelial cells leading to inhibition of fibrinolysis and inflammation. TAFI gene deficiency was reported to ameliorate bleomycin-induced lung fibrosis in a murine model but contrarily it was reported that TAFI gene deficiency worsens liver damage in a carbon tetrachloride (CCl4)-induced liver failure in mice.

Aims: In this study, we examined the effect of TAFI gene deficiency in a murine model of CCl4-induced liver fibrosis.

Methods: To induce liver fibrosis CCl4 was injected intraperitoneally twice a week for eight weeks in wild type and TAFI deficient mice both under C57BL/6 background. The experimental protocol was approved by the Institutional Committee for Animal Investigation and followed approved international guidelines.

Results: Liver damage was ameliorated in TAFI deficient mice after chronic CCl4 administration. TAFI deficient mice showed decreased plasma levels of aspartate aminotransferase and alanine aminotransferase compared to wild type mice. Hepatic fibrosis was also ameliorated in TAFI deficient mice. Collagen deposition assessed by Sirius Red staining and hydroxyproline content in the liver was significantly lower in TAFI deficient mice.

Conclusions: These data suggest that TAF play a detrimental role in the fibrotic phase of liver injury.

PB 907 | Transcriptional Regulation of the Tissue Factor Gene is Mediated by the Androgen Receptor

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Background: Prostate cancer is one of the leading causes of cancer death in men. Advanced prostate cancer is usually treated by androgen deprivation therapy (ADT), which aims at diminishing circulating testosterone to reduce cancer growth. There is growing evidence that ADT increases the rate of VTE in prostate cancer patients, suggesting that low levels of testosterone can induce a hypercoagulable state. One of the most important mediators of extrinsic coagulation is tissue factor (TF). Despite a potential role for TF in ADT, an analysis of androgen receptor (AR) mediated regulation of TF has not been performed.

Aims: To characterize androgen receptor mediated regulation of tissue factor expression.

Methods: We stimulated two AR dependent prostate cancer cell lines with dihydrotestosterone (DHT) and analysed TF expression by qPCR and FACS. Furthermore, we cloned the TF promoter into a luciferase reporter vector and created serial deletions of known transcription factor binding sites. In addition, we use castration experiments in mice to characterize AR mediated TF regulation *in vivo*.

Results: We show that TF expression is regulated by DHT in two androgen dependent prostate cancer cell lines LNCAP and VCAP. This DHT mediated TF regulation is AR dependent as it could be blocked by the addition of the AR antagonist bicalutamide. Using luciferase reporter assays, we show that TF regulation is mediated through the NF-κB and EGR1 in LNCAP as well as the AP-1 and EGR1 binding site(s) in VCAP cells, respectively. Finally, we provide evidence that TF expression is also regulated through AR *in vivo*.

Conclusions: In summary, we provide evidence that TF expression in prostate epithelial cells is mediated through AR *in vitro* and *in vivo*. These findings are especially important in the context of ADT. We

provide a molecular basis for the underlying signaling events, which could contribute to better treatment options for androgen deprived patients.

PB 908 | Factor V Expression in Aggressive Breast Cancer Modifies Patient Survival, Tumor Cell Growth and Response to Treatment

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Background: The hypercoagulable state in cancer patients contributes to tumor progression. Coagulation factor V (FV) is an essential co-factor of the tissue factor pathway. We have shown that genetic variants in *F5* are associated with breast cancer, yet, it is unknown how FV affects cancer processes.

Aims: To clinically and functionally characterize the role of *F5* expression in cancer pathogenesis and response to therapy.

Methods: The endogenous and doxorubicin-responsive expression of *F5* was characterized in breast, ovarian, colon and liver cancer cell lines. Tumor-expressed *F5* was related to clinical data, survival and biological function in a Scandinavian breast cancer material (n=152) and a merged breast cancer dataset (n=1881). *F5* was overexpressed *in vitro* in breast cancer cells and growth (WST-1), apoptosis (DNA fragmentation), and cancer signaling pathways (Cignal Finder Rep. Array) were assessed. Gene/protein expression was determined by qRT-PCR/ELISA. CRISPR-Cas9 will be used to activate/repress endogenous *F5* cell expression.

Results: *F5* was expressed in all cancer cell lines tested, and the expression was further induced by doxorubicin in *TP53* wt cells. In breast cancer patients, tumor-expressed *F5* was increased in aggressive subtypes; estrogen receptor negative, human epidermal growth factor receptor 2 positive and basal tumors. High *F5* expression was associated with improved survival in these patient groups. Consistently, *F5* overexpression reduced *in vitro* cell growth and induced apoptosis, likely through a p53-dependent mechanism. *F5* correlated with the expression of immune response genes in the tumors, and *in vitroF5* overexpression induced the gene and protein expression of multiple cytokines.

Conclusions: *F5* expression in aggressive breast tumors was a positive predictor of survival. FV was shown to inhibit cancer growth and to be a possible mediator of treatment response. In conclusion, we demonstrate a novel function of FV as a possible tumor suppressor gene.

PB 909 | Low Phosphatase Activity Mediates L-selectin Shedding in B-CLL

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Background: Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in adults and has a variable clinical outcome that is dependent on several factors including cellular trafficking.

Aims: We investigated L-selectin surface expression on CLL cells from untreated patients and explored the mechanism that lead to L-selectin shedding.

Methods: In 51 untreated CLL patients, surface and soluble L-selectin expression was investigated by flow cytometry and ELISA respectively. Magnetically isolated B-cells from 33 untreated CLL patients and 20 healthy subjects were included in our study. Protein phosphatase activity was determined both for total and PP2 activity (PP2A) by using a ^{32}P-labeled substrate. Flow cytometry was utilized for the determination of intracellular phosphorylated p38MAPK and surface ADAM17 expression as well as to study the effect of specific inhibitors on ADAM17 and L-selectin expression.

Results: In CLL cells, both total and PP2 activities were significantly lower (p< 0,0001 and p=0,009), conversely intracellular phosphorylated p38MAPK level was higher (p=0,023) compared to normal B-cells. In case of CLL patients, increased soluble L-selectin levels were detected with decreased surface L-selectin and ADAM-17 expression. During in vitro experiments, normal and CLL-B cells were treated by the phosphatase inhibitor Calyculin A (CLA). Malignant B cells showed an elevated phosphorylated p38MAPK level and reduced surface L-selectin and ADAM17 expression compared to normal B-cells. When normal B-cells were preincubated with the p38MAPK inhibitor (SB203580) before CLA treatment, p38MAPK phosphorylation was blocked and CLA-induced L-selectin shedding was also attenuated.

Conclusions: The decreased phosphatase activity detectable in CLL, results in a downstream signaling cascade with subsequent reduction of surface L-selectin expression and we suggest that this effect is mediated by enhanced phosphorylation of p38MAPK and altered ADAM17 expression.

PB 910 | Platelet-dependent von Willebrand Factor Activity in Acute Myeloid Leukemia Patients: Role in Haemostatic Alterations

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Background: Acute myeloid leukemia (AML) patients may suffer from bleeding complication which occurs mainly due to thrombocytopenia,

disseminated coagulopathy (DIC) and platelet dysfunction. von Willebrand Factor (VWF) defects have been implicated as contributor in bleeding complication.

Aims: The study aimed to investigate the vWF-Ristocetin cofactor (vWF-RCO) activity pattern in newly diagnosed AML patients and its relation to therapy, to explore its potential role in the prevalent phenomenon of bleeding in AML patients, to correlate between vWF-RCO activity and other hemostatic parameters and known prognostic factors in AML.

Methods: Thirty newly diagnosed patients with AML and twenty healthy age and sex-matched subjects (control group) were studied. The vWF-RCO activity using platelet agglutination method was performed on plasma samples of both patients and controls.

Results: Our results showed a significantly reduced vWF-RCO activity in AML patients at diagnosis compared to control group (mean value of 80.5%±12.6% and 99.5%±10.5% respectively) ($p< 0.001$), meanwhile, vWF-RCO activity significantly increased after two weeks post-treatment. In addition, vWF-RCO activity showed a statistically significant lower mean value among patients presenting with bleeding manifestations (76.0 ±11.7%) in relation to those without bleeding (86.5±11.4%)($p=0.02$). Also, on grading the bleeding manifestation, patients with grade (3) had the lowest mean value of vWF-RCO activity (61.1% ±5.2%), Our study proposes the following cut-offs for vWF-RCO :"≤ 85.2%" for predicting the AML patients prone to bleeding manifestations and „≤82.6%" for the prediction of bad outcome.

Conclusions: In AML, vWF-RCO activity at diagnosis, represents a valuable prognostic marker for predicting bleeding complication and bad outcome, and the provided cut-offs for vWF-RCO activity represent first steps for its use as a bleeding predictor and prognostic marker.

PB 911 | Fibronectin Supports Glioma Invasion and Colonization of Fibrin in Co-operation with Slug/Snail2

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Background: High grade gliomas are brain tumors that are characterized by diffuse growth and resistance to most if not all therapies. Typically, these aggressive tumors possess high angiogenic activity, which causes vascular leakage and fibrin-rich edema.

Aims: To determine if fibrin promotes invasion and colonization of malignant brain tumors and whether this process is supported by factors of epithelial to mesenchymal transition (EMT) such as fibronectin and Slug/Snail2.

Methods: Primary tumor cells from patients with high-grade glioma (WHO grade III-IV) or benign meningioma (grade I-II) were embedded in a 3-dimensional matrix of clotted plasma, fibrin, fibrin-fibronectin or Matrigel™ and scored for invadopodia formation as well as proliferation using phase contrast microscopy. Expression of fibronectin and Slug/Snail2 was analyzed using quantitative PCR, western blotting and fluorescence microscopy. The role of fibronectin and Slug/Snail2 for invasion/proliferation in 3D matrices was further assessed by transfecting U87MG glioma cells with siRNA.

Results: Glioma cells invaded extensively in clotted plasma and fibrin while lagging behind in matrigel. Benign meningioma cells, on the other hand, were considerably less clot invasive. Glioma invasion was associated with strong expression of the transcription factor Slug/Snail2 and the adhesion protein fibronectin, which worked together to promote invadopodia formation in fibrin as well as matrigel. Moreover, knocking down Slug/Snail2 or fibronectin reduced colonization of gliomas in 3D, suggesting that both proteins are involved in the self-renewal of tumor-initiating cells.

Conclusions: Our data show that clotted plasma, which is present in the fibrin-rich edema of the tumor extracellular matrix, strongly promotes invasion as well as colonization of high-grade glioma. Mechanistically, we found that this process is promoted by the EMT factors fibronectin and Slug/Snail2, which both are upregulated in aggressive glioma.

PB 912 | Altered Fibrin Clot Properties in Advanced Lung Cancer: Impact of Chemotherapy

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Background: The impact of cancer, a well-established risk factor for thromboembolism, on clot properties remains unclear. Faster formation of dense and poorly lysable fibrin networks has been previously reported in patients with gastrointestinal cancer.

Aims: To investigate alterations of plasma fibrin clot features in lung cancer patients before and after chemotherapy.

Methods: Plasma fibrin clot permeability (K_s), clot formation (lag phase and maximum absorbance at 405 nm, ΔAbs), and lysability (clot lysis time, CLT) were investigated before chemotherapy and during the third or fourth cycle of standard chemotherapy adjusted to the histopathological type of cancer and co-morbidities in a cohort of 150 consecutive patients aged 46-82 years with advanced inoperable lung cancer, including 61 subjects with the small-cell lung carcinoma (SCLC) and 89 with the non-small-cell lung carcinoma (NSCLC). Healthy subjects (n=90) matched for age and sex served as controls.

Results: At baseline lung cancer patients had 27.9% higher fibrinogen, 27.2% lower K_s, 8.1% shorter lag time, and 26.5% longer CLT than healthy controls. ΔAbs did not differ between both groups. Chemotherapy was associated with increased K_s and lag phase (+7.5% and +9.5%, respectively; both $p< 0.05$), shorter CLT (-4.8%, $p=0.053$), and unaltered fibrinogen and ΔAbs. Differences in clot properties remained significant after adjustment for fibrinogen. No differences in fibrin characteristics were found between lung cancer stages as well as between SCLC and NSCLC at baseline and after chemotherapy. As few as 19 (12.7%) patients survived during a median follow-up of 10.4 (5.8-18.6) months. The Kaplan-Meier analysis showed that the lag phase ≤37s (1st quartile) was the predictor for death (log-rank test, $p=0.039$).

Conclusions: Advanced lung cancer unfavorably alters plasma clot properties, including faster formation of more compact clots displaying impaired lysability regardless of cancer histology and disease stage but chemotherapy improves these properties.

PB 913 | The Effect of Neoadjuvant Chemotherapy on the Activated Protein C (aPC) Pathway in High Grade Serous Ovarian Cancer Patients

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Background: Ovarian cancer and chemotherapy treatment are risk factors for cancer-associated thrombosis. However, the mechanisms are not understood. We have previously shown that the aPC pathway is dysregulated in ovarian cancer.
Aims: To determine the effect of chemotherapy on the aPC pathway in ovarian cancer and to elucidate the role of aPC in increased thrombin generation in ovarian cancer patients.
Methods: Ovarian cancer patients (benign n=23, chemotherapy naïve n = 23, neoadjuvant treated n=23) gave full and informed consent. Venous blood samples were obtained prior to surgery. Thrombin generation was determined +/- thrombomodulin (TM) (10 nM). Plasma levels of sTM and soluble Endothelial protein C receptor (EPCR) were determined by ELISA. OAW42 (ovarian) and EA.hy926 (endothelial) cells were treated with paclitaxel or carboplatin for 24 h. mRNA expression of TM and EPCR were determined by TaqMan PCR.
Results: Neoadjuvant patients had lower endogenous thrombin potential (ETP) compared with the benign and chemo naïve groups($p< 0.001$). Lag time and peak thrombin were increased compared with benign and neoadjuvant groups($p< 0.001$). Following addition of TM, neoadjuvant chemotherapy increased ETP, peak thrombin and decreased lag time compared with benign and chemo naïve groups($p< 0.01$). Neoadjuvant patients also had decreased levels of sEPCR in plasma compared with benign and chemo naïve groups($p< 0.01$). sTM levels were unchanged. Neither paclitaxel nor carboplatin altered TM or EPCR mRNA expression in EA.hy926. Conversely, carboplatin exposure decreased TM expression in OAW42 cells($p< 0.008$), while paclitaxel exposure decreased EPCR expression($p< 0.009$).
Conclusions: Neoadjuvant chemotherapy alters thrombin production in ovarian cancer patients. The increased resistance to the aPC pathway maybe mediated by reduced levels of sEPCR which may originate from the tumour. This data highlights that neoadjuvant chemotherapy alters tumour expression of aPC proteins which may impact the increased VTE risk in these patients.

PB 914 | Inhibition of Tumour Cell-induced Platelet Aggregation by a Novel Stat3 Inhibitor in Podoplanin Positive Cancer Cells

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Background: Cancer cells acquire the ability to penetrate the walls of lymphatic and/or blood vessels for escape from the primary site. Podopalanin (PDPN) was involved in cancer development, lymphatic metastasis and poor prognosis in a wide variety of cancer types. By binding to platelet C-type lectin-like receptor 2 (CLEC-2), PDPN stimulates tumor cell-induced platelet aggregation (TCIPA) and facilitates cancer cell metastasis. Furthermore, the Signal Transducer and Activator of Transcription 3 (STAT3) plays an important role in megakaryopoiesis and serves as a transcription factor activated by cytokine-induced intracellular signals. Recent study indicated that stat3 inhibitors selectively inhibited collagen-induced aggregation of human platelets.
Aims: To investigate a novel stat3 inhibitor whether elicit functional blockage of PDPN-stimulated CLEC-2 activation and TCIPA.
Methods: TCIPA was induced in human platelets by mixing with two PDPN-overexpressed osteosarcoma cancer cells, MG63 and HOS cells.
Results: Our results shown that the a novel stat3 inhibitor consistently inhibited tumour cell-induced platelet aggregation (TCIPA) in response to all two PDPN-overexpressed osteosarcoma cancer cells, MG63 and HOS cell lines. The PDPN- or collagen-induced platelet aggregation was significantly decreased in a dose-dependent manner of the novel stat3 inhibitor treatment. The Western blot analysis shown that treatment with PDPN produced marked Syk phosphorylation and treatment with collagen produced PLCγ2 and Syk phosphorylation. We noted that a novel stat3 inhibitor inhibited PDPN-induced Syk phosphorylation, collagen-induced PLCγ2 and Syk phosphorylation.
Conclusions: Our results show that a novel stat3 inhibitor was an effective inhibitor of TCIPA and inhibited PDPN- or collagen-induced Syk and PLCγ2 phosphorylation. The findings are valuable for future application of novel stat3 inhibitor in the prevention and treatment of cancer metastasis.

PB 915 | Cancer Stromal Targeting Therapy Using MMAE Conjugated Anti-insoluble Fibrin Antibody

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Background: Most invasive cancers including pancreatic cancer possess abundant stroma that hinders the distribution of high molecular

weight agents including antibody drug conjugate (ADC). We report here a successful new strategy that overcomes the above drawbacks by ADC against particular components of tumor stroma.

The fibrin clot (Fbn) formation in cancer stroma lasts for as long as the cancer cells survive in the body, unlike non-malignant conditions such as cardiac infarction. Anti-human monoclonal antibody (mAb) against insoluble fibrin recognizes an unexplored hole that is uncovered only when a fibrin clot forms.

Aims: In this study, we show a new concept of ADC that conjugated anti-insoluble fibrin mAb to monomethyl auristatin E (MMAE) for targeting stroma.

Methods: Antibody and ADCs: Anti-insoluble fibrin IgG (Fbn-ADC) and Control (Control-ADC) were prepared in our laboratory.

In vivo experiments: Bulb/c nude mice were subcutaneously inoculated with 5×10^5 5-11 cells on the back and used in the experiments when the tumor volume reached approximately 200 mm^3. Mice received PBS, 0.3 mg/kg MMAE, 20mg/kg ADCs (0.3 mg/kg, MMAE equivalent) twice a week, 12 times in total.

8 weeks-old spontaneous pancreatic tumor-bearing KPC mice were treated with PBS and ADCs at same regimen of subcutaneous model.

Results: In the subcutaneous tumor model, Fbn-ADC significantly inhibited tumor growth compared with PBS, MMAE and Control-ADC ($P< 0.01$). In the KPC mice, Fbn-ADC significantly improved survival compared with PBS and Control-ADC ($P< 0.05$).

Conclusions: Our strategic concept of cancer stromal targeting (CAST) therapy is unique as follows.

1) The ADC can extravasate from the leaky tumor vessels selectively, and forms a scaffold as it is captured by tumor stromal network.

2) The ADC allows the effective sustained release of anti-cancer agent from the scaffold, and the released payload is distributed throughout the tumor resulting in high antitumor effect by damaging tumor cells and tumor vessels.

PB 916 | A Linkage between ERBB2 Expression and the Pro-coagulant Properties of Breast Cancer Cells is Mediated by the PI3k/Akt Signaling Pathways

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Background: The overexpression of ERBB2 in primary tumours and corresponding metastases are associated with certain aggressive types of breast cancer. As regards cancer, the occurrence of thrombosis is often the first indicator of an underlying malignancy and a close association has been established between tumour progression and the development of a procoagulant profile, suggesting that the activation of the blood coagulation system contributes to tumour biology.

Aims: To investigate a possible modulatory role of ERBB2 in the procoagulant status of breast cancer cells.

Methods: A line of ERBB2 overexpressing human breast cancer MCF-7 cells (MCF-7 ERBB2+) and human normal mammary MCF-12a cells (MCF-12a ERBB2+) respectively, were established by transfection with an ERBB22 plasmid. The effect of ERBB overexpression on the synthesis and activities of selected blood coagulation proteins were analysed as well as relevant signalling pathways.

Results: Overexpression of ERBB2 induced by gene transfection was associated with a significant increase in the levels of p-ERK / p-Akt - central components of key signalling pathways for tumorigenesis - as well as enhanced expression of pro-tumorigenic proteins. In addition, Western Blot showed that the expression levels of the majority of the coagulation proteins corresponded with the level of ERBB2 when tested in three native breast cancer cell lines. The overexpression of ERBB also altered the level of each of these blood coagulation proteins in MCF-7-ERBB2+ and MCF-12a-ERBB2+ cells. The modulation of coagulation proteins, as mediated by the level of ERBB2 in breast cancer cells, was dependent on the PI3k/Akt pathway. ERBB2 also modulated the procoagulant activity including tissue factor and thrombomodulin, again, in a PI3K/Akt pathway manner.

Conclusions: The level of ERBB2 correlates positively with the procoagulant properties of breast cancer cells, an effect which is mediated by PI3k/Akt pathways.

PB 917 | Exosomes from Heparin-treated Cancer Cells Exhibit Transferable Anti-tumorigenic Activity

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Background: We have shown previously that heparin treatment of human breast cancer cells induces a less tumorigenic genotype and phenotype. Recently, interest has been engendered in the role of exosomes, extracellular vesicles released from cells, which are stable concentrated carriers of genetic and proteomic information, capable of altering the properties of target cells and thereby acting as intercellular messengers.

Aims: To investigate *whether* the exosomes released from heparin treated cells exhibit a similar anti-tumorigenic effect as heparin, itself.

Methods: The anti-tumorigenic properties of exosomes prepared from heparin treated breast cancer cells (Exo-HT) were compared to those from untreated cells (Exo-Ctrl) as well as to those separated from conditioned medium following the termination of treatment (heparin discontinued, Exo-HD) in terms of the profile of pro-tumorigenic proteins and cytological characteristics. Also, confocal microscopy and flow cytometry were used to determine if heparin was bound to the purified exosomes.

Results:

1) The numerous anti-tumorigenic effects of heparin treatment on human breast cancer cells were reversed after heparin was removed from the cultures.

2) Similar anti-tumorigenic effects, in terms of *pro-tumorigenic and* cell cycle regulatory proteins as well as signalling *activities were observed* in breast cancer cells treated with Exo-HT and Exo-HD.

3) A less tumorigenic cytological profile was also observed following treatment of MCF-7 and MDA-MB231 cells with Exo-HT and Exo-HD.

4) Heparin was observed to be bound to exosomes and heparin-loaded exosomes could, therefore, act as a longer lived reservoir of heparin activity in the circulation.

Conclusions: The anti-tumorigenic effect of heparin treatment on cancer cells was also observed in treatment with heparin derived exosomes - whether the occurrence of such heparin derived exosomes in the circulation is of clinical significance awaits investigation.

PB 918 | Sonic Estimation of Elasticity via Resonance (SEER) Sonorheometry to Identify Hypercoagulability in Patients with Cancer

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Background: Venous thromboembolic (VTE) disease is associated with significant morbidity and mortality and is one of the leading causes of death in patients with cancer. The actual reported incidence of VTE in patients with cancer varies greatly and depends on the type and stage of cancer, type of treatment, and patient-specific factors. Currently no specific biomarkers have been shown to reliably predict VTE risk.

Aims: To assess the efficacy of Sonic Estimation of Elasticity via Resonance (SEER) Sonorheometry in identifying hypercoagulability in cancer patients.

Methods: SEER Sonorheometry was implemented in an earlier research version of the Quantra, (HemoSonics) a novel cartridge-based viscoelastic analyzer. Twelve cancer patients and 30 controls were enrolled in this study. Treatment naïve patients with a new diagnosis of a solid tumor were eligible for inclusion. Baseline conventional measures of hemostasis (D-Dimer, Fibrinogen, Thrombin time, PT, PTT, and Platelet count) were measured within the first cycle of chemotherapy and at 6 months for all patients. Quantra measurements were simultaneously obtained and included Clot Time (CT), Clot Stiffness (CS), Fibrinogen (FCS) and Platelet (PCS) Contributions to clot stiffness.

Results: Seven patients with lung cancer, 4 patients with squamous cell carcinoma of the head and neck, and 1 patient with gastric adenocarcinoma were included in the analysis. A summary of Quantra parameters is shown in Figure 1. CS, FCS, and PCS increased by over 80% from baseline to follow-up, indicating a higher than normal state as compared to normal healthy subjects. At the initial and follow-up time points, the mean values for PT, PTT, Thrombin time, and Platelet count were normal. The mean D-Dimer and mean Fibrinogen were elevated at both time points. The D-Dimer decreased by 7.1% over the 6 months while Fibrinogen increased by 7.7% over the same period.

Conclusions: SEER measurements showed a trend towards increasing coagulation, suggesting a potential hypercoagulable state.

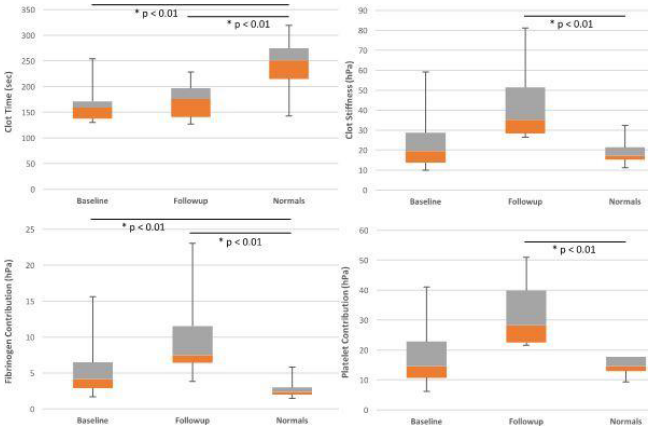

FIGURE 1 Box and whiskers plots of Quantra parameters: cancer patients at baseline, cancer patients at 6 month follow up, and normal controls

PB 920 | Direct Oral Anticoagulants Do Not Inhibit Growth and Metastasis of Orthotopically Growing Claudin-low MDA-MB-231 Human Breast Cancer Cells in Immunodeficient mice

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Background: Cancer is associated with a 4-6-fold increased risk for venous thromboembolism (VTE). It has recently been suggested that coagulation could drive cancer progression as well. However, beneficial effects of prophylactic treatment with Low Molecular Weight Heparin on survival in cancer patients were not consistently found. Recently, direct oral anticoagulants (DOACs) have shown a superior safety profile and are at least as efficient in inhibiting VTE recurrence in patients without cancer.

Aims: We tested whether the DOACs dabigatran and rivaroxaban exert anti-tumorigenic effects in claudin-low breast cancer cells *in vivo* as well as *in vitro*, as powerful anticancer strategies for claudin-low breast cancer are lacking.

Methods: Seven-week-old NOD/SCID/$\gamma_C^{-/-}$ (NSG) mice were continuously (day-2 onwards) fed a custom-made chow diet, which included dabigatran etexilate (DE) (10mg/g) or rivaroxaban (0.4mg/g). All mice were orthotopically inoculated with 500,000 MDA-MB-231 claudin-low breast cancer cells and tumor size was weekly measured with a caliper. Human-specific qPCR allowed for sensitive detection of metastatic cells in the liver and lungs at the end (11 weeks) of the experiment.

Results: DE and rivaroxaban increased significantly the coagulation time when compared to vehicle mice as measured by aPTT and PT assay, respectively. In addition, FXa activitiy was inhibited in the rivaroxaban mice using a chromogenic assay. However, both DE and

rivaroxaban did not result in significant inhibition of tumor growth at primary or metastastic site.

Furthermore, the coagulant factors thrombin (10-50nM) and FXa (2-20nM) did not induce migration, proliferation or stemness in the MDA-MB-231 cells *in vitro*.

Conclusions: This study showed that DOACs could not inhibit cancer progression in claudin-low breast cancer. However, it remains to be investigated whether DOACs exert anti-tumorigenic effects in other types of breast cancer, as well as other malignancies such as pancreatic cancer.

PB 921 | Does Remote Ischemic Preconditioning Increase Fibr inolysis in Head and Neck Cancer Surgery? Preliminary Results from a Randomized Controlled Trial

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Background: Cancer induces abnormal coagulation and fibrinolysis leading to an increased risk of thromboembolism. Large head and neck tumors are primarily treated by surgical resection and reconstruction of the defect by transfer of the patient's own tissue. Thrombosis in the transferred tissue or the patient's circulation is a life-threatening complication in head and neck cancer surgery. Remote ischemic preconditioning (RIPC) attenuates ischemia-reperfusion injury and reduces subsequent thrombotic events in cardiovascular procedures.

Aims: The aim of the trial is to investigate if RIPC increases fibrinolysis in head and neck cancer patients undergoing surgery.

Methods: The local Ethics Committee has approved this trial, and informed consent is obtained before study inclusion. We aim to randomize 60 patients undergoing head and neck cancer surgery to RIPC or sham intervention. RIPC is induced with an inflatable tourniquet and administered intraoperatively by four 5-minute cycles of upper extremity ischemia and reperfusion. Blood samples are collected preoperatively, intraoperatively, and in the postoperative phase. An in-house clot lysis assay, tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), and fibrin D-dimer will be measured.

Results: Until now, 35 patients have been included in the trial. Preliminary results show reduced clot lysis area under the curve and 50% lysis time, reduced PAI-1, and increased tPA measured postoperatively in the RIPC group. No difference is found in postoperative fibrin D-dimer between the groups.

Conclusions: Preliminary results indicate that RIPC increases fibrinolysis in head and neck cancer patients undergoing surgery, which may reduce the risk of postoperative thromboembolism. Despite increased fibrinolysis, postoperative fibrin D-dimer was similar in the two groups.

We expect to present data from at least 40 patients at the congress.

PB 922 | The Role of asTF in Glioblastoma Multiforme

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Background: Full-length Tissue Factor (flTF) in complex with its ligand, protease FVIIa, is the initiator of the coagulation cascade and a signaling receptor involved in inflammation and angiogenesis. It is also known to be overexpressed in Glioblastoma Multiforme (GBM). This aberrant expression results in microenvironmental changes including recruitment of proinflammatory cells that affect the cancer cell genome and epigenome . Alternatively spliced Tissue Factor (asTF), the soluble isoform of flTF, has been reported to contribute to cell proliferation, angiogenesis and tumor growth in vivo. However, the exact role of asTF in GBM has not been determined.

Aims: Here we aim to unravel the influence of asTF in GBM and to distinguish between the functions of flTF and asTF.

Methods: Stable cell lines expressing flTF or asTF, and control cells that express neither isoform, were generated through lentiviral transduction of low TF expressing U373 human glioma cells. These cell lines were used to characterize the role of asTF in GBM by examining cell proliferation, migration, invasion and gene expression levels.

Results: Interestingly, no differences in proliferation were found in U373 flTF and asTF cells compared to control cells. flTF cells, unlike asTF cells, showed upregulation of the typical TF-dependent pro-inflammatory gene CCL-2 in response to FVIIa. In contrast, migration and invasion rates were found to be increased in asTF cells in comparison to control and flTF glioma cells.

Conclusions: flTF in GBM is associated with pro-inflammatory signaling, while asTF is associated with cell movement and invasion.

PB 923 | Tumoral Factor VII Expression Changes TGF-β-mediated Intercellular Communication in Breast Cancer

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Background: It is known that cancer patients have an increased risk for developing thromboembolic events compared to healthy people. The incidence of venous thrombosis in cancer patients is even further enhanced upon metastatic disease, suggesting a correlation between tumor cell spread and coagulation. Recently, our group found that a subset of breast tumors expresses coagulation factor VII (fVII), a protein normally solely produced by the liver. In an orthotopic mouse model for breast cancer, MDA-MB-231 cells expressing fVII proved to be more metastatic, when compared to the control cell line. One of the major regulators of tumor metastasis is the tumor

stroma, which consists of endothelial and immune cells, extracellular matrix and cancer-associated fibroblasts (CAFs). Previously, we have shown that interactions between CAFs and tumor cells are of crucial importance for tumor invasion and metastasis and that Transforming Growth Factor-β (TGF-β) is a key player in this process.

Aims: The aim of this study was to assess the effect of tumoral fVII expression on TGF-β-mediated cell-cell interactions in breast cancer.

Methods: In order to assess the effects of cell-cell interactions on TGF-β-mediated signaling we used RNA expression analysis by qPCR and western blot for protein regulation and modification. Moreover, direct and indirect co-culture experiments were performed to allow for bidirectional paracrine interactions and their effects.

Results: Preliminary data showed that the TGF-β signaling route is activated in breast cancer CAFs upon stimulation with conditioned medium (CM) from either fVII-expressing or control MDA-MB-231 cells. Additionally, various TGF-β receptors appeared to be upregulated to a higher extent after stimulation with fVII CM than after control CM.

Conclusions: Based on these data, we conclude that the TGF-β-mediated interactions between CAFs and tumor cells are altered when tumor cells express fVII.

PB 924 | Chemotherapy as a Factor in the Pathogenesis of Thrombosis in Patients with Ovarian Cancer

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Background: Patients with ovarian cancer represent group of the highest risk of thrombotic and hemorrhage complications, especially during chemotherapy. Chemotherapy stipulates for endothelium damage, direct platelet activation and reduction of fibrinolytic activity.

Aims: To determine necessary range of laboratory tests for a high-grade estimation of haemostasis state in ovarian cancer patients undergoing chemotherapy.

Methods: 209 patients with ovarian cancer undergoing chemotherapy were divides at random on 2 groups: **Igroup** 109 has received LMWH 0,4 ml (4000 IU) before each chemotherapy curse and **II group** 100 has not received any anticoagulant prophylaxis during chemotherapy.

Laboratory tests: Platelet aggregation tests with different stimulators: Adrenaline, Ristocetin and ADP in various concentrations, platelet factor 4 (PF4). DIC and thrombophilia marker tests: D-dimer, TAT complexes, F1+2 prothrombin fragments. Fibrinolytic activity tests: determine PAI level, Protein C and S levels.

Results: We have detected the sign of thrombofilia and DIC in more than 90% patient during chemotherapy. The rate of the sub-compensated forms of DIC was 30%, decompensated 23%. It was observed damage of fibrinolytic activity due to iatrogenic effects of chemotherapy: reduction in proteins C and S levels, increase PAI concentration, platelets hyperaggregation in ristotetin presence.

In **I group** normalization of lab test results was detected during 2-3 days after chemotherapy course in comparison with **II group** normalisation was in 5-7 days in 22% and in 7-12 day in 58%, in 20% was not registered spontaneous normalisation.

Conclusions: Due to endothelium protection activity LMWH in ovarian cancer patients during chemotherapy significantly reduce intensity of thrombofilia and DIC. 85-90% patients with ovarian cancer required permanent preventive anticoagulant prophylaxis.

PB 925 | Preoperative Panel Scores of CA 19-9, FVIII and D-dimer Predict Postoperative Survival in Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-associated deaths worldwide. It is often diagnosed at a late incurable stage, and survival after surgery even with local PDAC is poor. CA 19-9 is the most used tumor marker for PDAC, however its sensitivity and specificity are not optimal. PDAC is associated with signs of increased coagulation activity.

Aims: The aim of this study was to find out whether a preoperative panel of CA 19-9, FVIII, D-dimer and thrombin time (TT) predicts cancer diagnosis and survival outcomes after pancreatic surgery.

Methods: Patients (n=121) were operated during 2010-2015 for PDAC in Helsinki. CA 19-9, FVIII, TT and D-dimer were analyzed 1-3 days preoperatively. Patients were divided into groups based on the disease stage: local (n=92) and metastasized (n=29). Neo-adjuvant treatments (NT) were recorded. The results were analyzed with a 10-point preoperative panel score. The median (range) follow-up for was 1.62 (0.01-1) years for local and 0.81 (0.16-2.29) years for metastasized PDAC. Survival data was gathered for each patient. A Kaplan-Meier survival analysis was made.

Results: Of the 92 local PDAC patients, 48 were alive at follow-up. All patients with a metastasized disease were deceased at follow-up. The median (range) panel score was 7 (4-10) for local and 8 (6-10) for metastasized PDAC. In local PDAC 75% had values of 7 or higher and it predicted worse survival in local PDAC vs lower scores ($p< 0.01$), regardless of NT. CA 19-9 alone associated with worse survival when over 340 kU/L (n=31, $p< 0.05$). When CA 19-9 was below 340 kU/L, panel scores of 7 or more predicted worse survival in local PDAC ($p< 0.05$). Panel score values did not predict survival in metastasized disease.

Conclusions: Preoperative screening of CA 19-9 combined with FVIII, D-dimer and TT associates with survival after PDAC surgery in local disease. A larger study is needed to verify whether the panel could assist in the determination of surgical suitability.

PB 926 | Expression of Coagulation Proteases from the Activated Protein C Pathway in Ovarian Tumours

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Background: Ovarian cancer is the leading cause of death from gynaecological malignancy. Coagulation proteases have been implicated in the pathogenesis of ovarian cancer. Previous studies by our group have shown that ovarian cancer is associated with altered gene expression of coagulation proteases particularly those associated with the anticoagulant activated protein C pathway (aPC).

Aims: The aims of this study were to characterise the expression of the aPC pathway in ovarian cancers using cell line models and ovarian cancer tissue.

Methods: mRNA expression of 15 coagulation genes was measured in ovarian cancer cells lines (HIO-80 (benign characteristics), OAW42, SKOV3 and 59M). Protein expression of thrombomodulin(TM) was determined by Western blot and ELISA. TM and Factor V(FV) expression was localised in benign, high grade serous (HGS), clear cell and endometrioid tumour tissue using immunohistochemistry.

Results: OAW42 had the most similar pattern of expression to that found in our previous study of tumour tissue with reduced expression of Protein S(PS), TM and Factor VIII, and a slight reduction in EPCR. Factor V and Protein C were not expressed. TaqMan PCR confirmed the reduced TM mRNA expression in OAW42 cell lines compared to HIO-80(benign model)($p < 0.001$). Weak to moderate staining of TM expression was observed in tumour cells from malignant biopsies compared with strong staining in cells from benign serous tumours. Strong staining for FV was also found in HGS tumours compared with benign tissue which was localised to tumour cells.

Conclusions: OAW42 cell line showed reduced levels of expression of genes and proteins involved in the activation of protein C compared with the benign cell line which concurs with previous findings in tumour tissue. To the author's knowledge, this is the first report of FV expression in ovarian cancer tissue. As the plasticity of the tumour milieu is crucial for ovarian cancer, components of the aPC may be responsible not only regulating coagulation but also driving oncogenesis.

PB 927 | Mucin1 Acts as a Potential Mediator of the Pro-coagulant Properties of Breast Cancer Cells by Altering the Activity of Key Phosphokinase Pathways

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Background: An aberrantly glycosylated form of cell membrane tethered Mucin 1 (MUC1) is overexpressed in a variety of epithelial cancers in which it participates in intracellular signalling thereby regulating the expression of a wide range of genes. Likewise, clinical data indicate that the activation of the blood coagulation system contributes to tumour aggressiveness and vice versa. Whether there is a link between the level of expression of the cancer biomarker MUC1 and the procoagulant activity of cancer cells is unknown.

Aims: To investigate if there is a link between the level of MUC1 in breast cancer cells and procoagulant activity.

Methods: A *shRNA-mediated MUC1 gene knockdown* was carried out in human breast cancer cell line MCF-7 establishing the shMUC1-MCF7 cell line which was investigated antigenically by WB for the level of kinase activity in selected key signalling pathways and compared to cells treated with a blank shRNA and to untreated MCF-7 cells. The level of phosphorylated proteins on the cell surface was also analysed by flow cytometry. The effect of MUC1 knockdown on the procoagulant properties was investigated by activity assays and the expression of selected coagulation factors was determined by WB.

Results: Compared to shCtrol-MCF7 cells, the phosphokinase activity of the MAPK, PI3K/Akt, TGFβ receptor, ERBB, and PKC signalling pathways were significantly reduced in shMUC1-MCF7 cells. Clotting activity and tissue factor activity were also reduced in shMUC1-MCF7 cell as well as levels of factors Xa and XIa as determined by WB.

Conclusions: MUC1 gene knockdown in breast cancer cells diminishes the activities of wide range of phosphokinase pathways as well as procoagulant activity and coagulation factor expression. Aberrantly glycosylated MUC1 in breast cancer cells may act as a potential mediator for pro-coagulant properties via the intensification of signalling activity.

PB 928 | Coagulation Factor Xa Promotes Melanoma Metastasis

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Background: A hypercoagulable state and complications from metastasis are the principal reasons for cancer-related mortality. Accumulate evidence suggest that the coagulation system plays a role in metastasis, and anticoagulants have been reported to be beneficial in prolonging cancer patient survival. However, the contribution of distinct proteins of coagulation cascade to cancer progression is still understood. The coagulation factor Xa (FXa), an effector protease of coagulation cascade, has a pivotal role among distinct cells types, such as: endothelial cells, fibroblast, mesangial cells and vascular smooth muscle cells. Nevertheless, the contribution of FXa to cancer cell biology and tumor progression is poorly described.

Aims: The principal aim of this study is to elucidate the contribution of FXa to cancer cell proliferation, migration, invasion (*in vitro*), and the contribution to cancer cell metastasis (*in vivo*)

Methods: The potential role of FXa upon metastasis was assessed using tail-vein injections of melanoma in the B16F10-C57BL/6 metastasis model. The effect of FXa upon the biological properties of cancer cells was assessed *in vitro*, using boyden chamber (invasion assay), scratch assay (migration) and flow cytometry (cell cycle analysis).

Results: FXa significantly increased B16F10 melanoma lung metastasis in mice, but FXa did not alter melanoma cell proliferation, migration or invasion capabilities in *in vitro* assays

Conclusions: Despite having no detectable effect upon the melanoma cells in culture, FXa can notably increase metastasis foci. FXa may promote metastasis by modulating the microenvironment of the metastatic niche. Preliminary data suggest that FXa promote the adhesion of melanoma cells to endothelial cells monolayer. Furthermore, FXa change Vascular endothelial cadherin (VE-cad) levels and distribution, suggesting an increase of endothelial permeability. These data support the idea that FXa may promote metastasis through changes in the tumor microenvironment, specifically the endothelium.

PB 929 | Identification of Novel Molecular Targets of the Kunitz-type Molecule Amblyomin-X Using Immuno-fishing Interactomics Approach

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Background: Amblyomin-X is a Kunitz-type protein, identified from a cDNA library of the salivary glands from the tick *Amblyomma cajennense* that has shown great potential to combat variety of cancer cells including skin melanoma, murine renal cell carcinoma and pancreatic cancer. In contrast, Amblyomin-X has not showed any cytotoxic effects on normal human fibroblasts.

Aims: The aim of the work was the identification of novel molecular targets inside cells and deducing mechanism of action of Amblyomin-X.

Methods: Proteins that bind to Amblyomin-X was identified using Amblyomin-X- Immuno-fishing column followed by mass spectrometry. For identification and evaluation of the pathways, we employed STRINGS, METACORE, flow cytometry and confocal microscopy.

Results: Proteins from several pathways such as endocytosis, apoptosis, cell cycle, blood coagulation and proteasomal degradation were found to interact with Amblyomin-X. Amblyomin-X was confirmed to enter the tumor cells via endocytoses and induced death in tumor cells via those pathways from which the proteins were identified to interact with this molecule.

Conclusions: The interactomics profile and mechanism of action was construed for Amblyomin-X. This is extremely effective model of interactomics, which can provide valuable clues for the pathways that could be effected by a particular protein/peptide drug.

PB 930 | Dissolution of Platelet-rich Thrombus by Perfusion of N-acetyl Cysteine

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Background: Both stroke and heart attack arise from thrombotic occlusion of an artery. These thrombi can be either fibrinous or platelet-rich depending on the mechanism of formation. Current thrombolytics typically target clots composed of fibrin. The effects of these agents on platelet-rich arterial thrombi are not known.

Aims: We have developed an *in vitro* system to study the efficacy of known and proposed thrombolytic agents on platelet-rich thrombi under flow. Specifically, we test anti-vWF agents as an alternative to tPA.

Methods: Whole porcine blood was perfused through a collagen-coated glass stenosis at a shear rate of 3500 s^{-1} to create platelet-rich thrombus. Then an agent was perfused at 1 mL/min for an hour. The agents tested include tissue plasminogen activator (tPA) (n=5), N-acetyl cysteine (NAC) (n=5), ADAMTS-13 (n=2) and abciximab (n=2). Saline was used as a negative control (n=3).

Results: Larger concentrations of NAC completely dissolved platelet thrombi within 10 min (Fig 1). Lower doses of NAC also showed dissolution within 1 hr perfusion, with average reduction in surface area of 96.8% ± 6.0% (avg ± s.d.). This was significantly different than tPA and the control ($p << 0.001$). TPA perfusion reduced thrombus surface

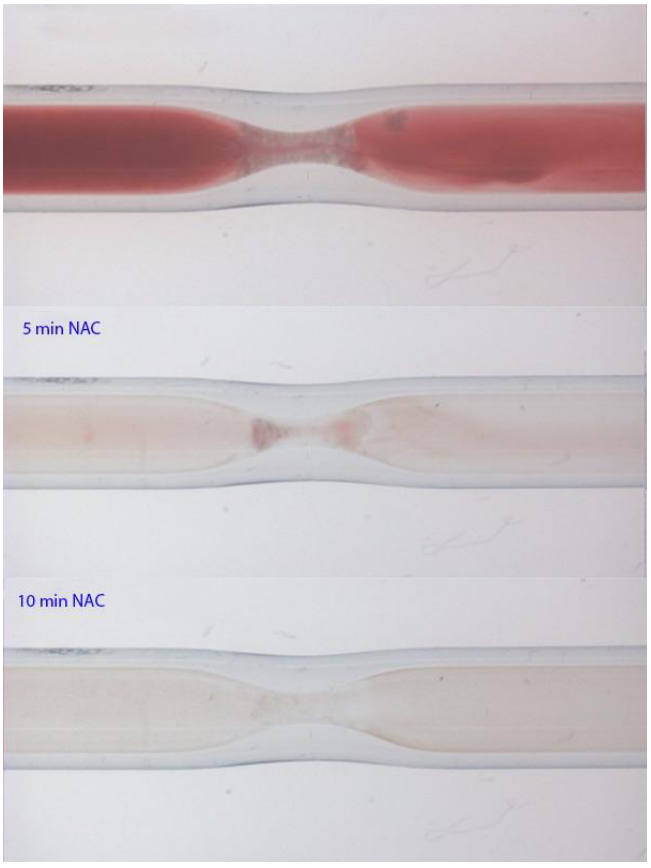

FIGURE 1 Platelet thrombus created under high shear conditions dissolves before 10 mins of perfusion with NAC.

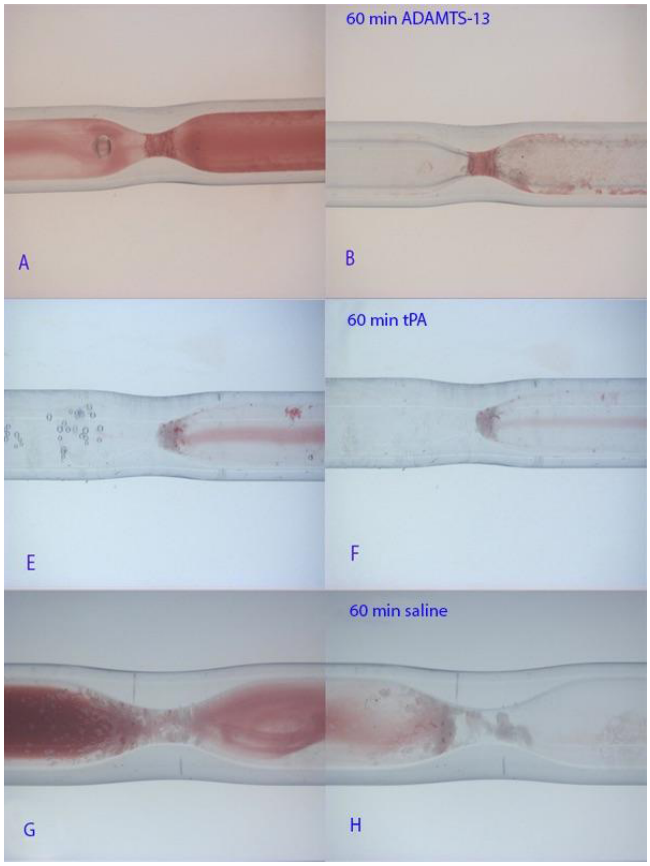

FIGURE 2 Platelet thrombus after A,B: perfusion of ADAMTS-13; E,F: perfusion of tPA; and G,H: perfusion of saline.

area by 25.2% ± 8.6%, and control by 7.4% ± 0.8%, and these were significantly different (p=0.008). ADAMTS-13 perfusion reduced thrombus surface area by 14.0% ± 12%, and abciximab by 13.9% ± 6.1%, which are significantly different than NAC (p<< 0.001 for both agents). ADAMTS-13, tPA, and saline post-perfusion thrombi are shown in Fig 2.

Conclusions: NAC demonstrated the ability to completely dissolve a platelet-rich thrombus under flow. Other agents such as tPA and ADAMSTS-13 demonstrated limited ability to achieve platelet thrombus dissolution. These results lead to a novel use for NAC as an effective thrombolytic agent against platelet-rich arterial occlusions.

PB 931 | Thrombolysis by rt-PA Combinated with Anticoagulant and Antiaggregant Molecules under Flow Conditions

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Background: We have developed a flow chamber model to study fibrinolysis under arterial flow conditions allowing to evaluate the thrombolytic efficacy of rt-PA.

Aims: To compare in whole blood the efficacy of rt-PA alone or in association with anticoagulant or antiplatelet drugs on thrombolysis.

Methods: Fluorescent fibrin-rich thrombi were obtained by perfusing recalcified citrated whole blood containing A467-coupled fibrinogen inside collagen- and tissue factor-coated flow chambers. Next step consisted in the perfusion of whole blood in which platelets were labeled by DIOC6 and supplemented with rt-PA (15 µg/mL) associated or not with anticoagulants [Heparin or Hirudin (20 U/mL), Argatroban
(1 µg/mL)], or with antiplatelets [Abciximab (3 µg/mL), Ticagrelor or a blocking anti-GPVI Fab (50 µg/mL)] over selected preformed fibrin rich thrombi. Real-time measurement of the fluorescence associated to fibrin and platelets permited quantifying fibrinolysis and platelet accumulation.

Results: A progressive lysis of the fibrin network was observed in the presence of rt-PA alone reaching up to 70% in 10 min as compared to the control in absence of rt-PA (p = 0.0026). However, and unexpectedly, a persistant accumulation of new platelets on the thombi was observed during lysis with an up to 500 fold increase in the platelet associated fluorescence. Combining anticoagulants with rt-PA did not modify the recruitment of platelets, but Hirudin increased the rate of fibrinolysis as well as its extent (+ 50%). All antiplatelet molecules combined to rt-PA, decreased the recruitment of platelets by up to 50%; additionaly the anti-GPVI Fab accelerated fibrinolysis.

Conclusions: These results strongly suggest that platelet accumulation by a thrombus during lysis could limit the therapeutic efficacy of rt-PA and evidence the relevance to associate rt-PA with antiplatelet molecules to optimize the thrombolysis efficacy.

PB 932 | Systematic Review and Meta-analysis of Treatments for Massive and Submassive Pulmonary Embolism

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Background: The standard treatment for acute pulmonary embolism (PE) is anticoagulation. Patients with massive or submassive PE may require other interventions including systemic thrombolysis (STL) or catheter-directed thrombolysis (CDT). In the absence of randomized trials comparing STL and CDT, the benefits and harms of these therapies are unclear.

Aims: To evaluate the risks and benefits of STL and CDT in patients with massive and submassive PE.

Methods: Systematic literature search of MEDLINE, Embase, Cochrane Central Register of Controlled Trials was performed. Pooled proportions and 95% confidence intervals (CI) for recurrent PE, fatal PE, major bleeding, intracranial hemorrhage (ICH), and mortality were calculated.

Results: 62 studies were included for analysis: 28 evaluated STL; 19 used CDT; 5 used the Angiojet system; and 10 studies used ultrasound-assisted CDT.

TABLE 1 Pooled Proportions for Outcomes

Outcome % (95% CI)	Heparin Therapies (16 Trials)	All Systemic thrombolytics (28 Trials)	Catheter directed thrombolytics (19 Studies)	AngioJet System (5 Studies)	Ultrasound assisted CDT (10 Studies)
Recurrent PE	6.91 (3.06 - 12.16)	3.15 (1.95 - 4.63)	2.68 (1.33 - 4.47)	5.57 (1.84 - 11.15)	1.35 (0.46 - 2.68)
Fatal recurrent PE	2.28 (0.97 - 4.14)	0.63 (0.34 - 1.02)	1.54 (0.54 - 3.04)	5.57 (1.84 - 11.15)	1.16 (0.34 - 2.47)
Major Bleeding	4.90 (2.55 - 7.95)	9.74 (7.15 - 12.70)	5.46 (2.47 - 9.53)	11.26 (1.03 - 30.27)	4.56 (2.19 - 7.74)
ICH	0.48 (0.15 - 0.98)	1.17 (0.76 - 1.68)	1.29 (0.49 - 2.45)	2.44 (0.30 - 6.56)	0.61 (0.10 - 1.55)
Mortality	5.48 (3.60 - 7.73)	4.16 (3.21 - 5.23)	9.47 (5.95 - 13.70)	12.62 (5.32 - 22.45)	4.48 (1.80 - 8.29)

TABLE 2 Pooled Proportions for Outcomes according to Systemic Thrombolytic Agent

Outcome % (95% CI)	Low-dose t-PA (5 Trials)	Standard-dose t-PA (12 Trials)	Streptokinase (8 Trials)	Urokinase (8 Trials)	Tenecteplase (3 Trials)
Recurrent PE	2.16 (0.21 - 6.10)	3.45 (1.69 - 5.81)	4.31 (2.11 - 7.25)	3.65 (1.19 - 7.38)	1.75 (0.015 - 6.26)
Fatal recurrent PE	0 (0 - 1.94)	1.48 (0.58 - 2.80)	0.98 (0.13 - 2.62)	0.84 (0.26 - 1.74)	0 (0 - 0.50)
Major Bleeding	3.29 (0.57 - 8.13)	10.74 (5.92 - 16.77)	14.25 (5.38 - 26.45)	10.55 (5.91 - 16.34)	8.67 (4.08 - 14.78)
ICH	0 (0 - 2.02)	1.23 (0.44 - 2.49)	0 (0 - 2.15)	0.73 (0.21 - 1.57)	2.28 (1.23 - 3.66)
Mortality	2.54 (0.85 - 5.11)	4.70 (2.59 - 7.39)	4.54 (2.21 - 7.65)	5.48 (3.80 - 7.45)	2.44 (1.34- 3.85)

Recurrent PE rates were lowest with STL [3.15% (95% CI: 1.95-4.63%)] and ultrasound assisted CDT [1.35% (0.46-2.68%)] (Table 1). Major bleeding rates were highest in the STL treated group: 9.74% (7.15-12.70%); however, mortality in the STL group was lowest at 4.16% (3.21-5.23%). Rates of ICH were lowest in the heparin group [0.48% (0.15-0.98)] and ultrasound assisted CDT [0.61% (0.10-1.55%)].

Outcomes according to systemic thrombolytic agent used are provided in Table 2. Low-dose t-PA was associated with the lowest major bleeding rates [3.29% (95% CI: 0.57-8.13%)] and ICHs [0% (0-2.02%)]. Mortality rates were lowest in the low-dose t-PA [2.54% (0.85-5.11%)] and tenecteplase groups [2.44% (1.34-3.85%)].

Conclusions: In patients with massive or submassive PE, mortality rates are similar between STL and ultrasound-assisted CDT, and lower than other therapies. Major bleeding rates were lowest with heparins and CDT options, with the exception of the Angiojet device. Low-dose t-PA was associated with the safest risk profile. Randomized trials comparing STL and CDT are needed to determine the optimal therapies in patients with massive and submassive PE.

PB 933 | Structure and Function of Trypsin-loaded Fibrinolytic Liposomes

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Background: Protease encapsulation and its local release in thrombi by external targeting may contribute to the reduction of haemorrhagic complications of thrombolysis.

Aims: To prepare sterically stabilized trypsin-loaded liposomes (SSL_T) and characterize their structure and in vitro fibrinolytic efficiency.

Methods: Hydrogenated soybean phosphatidylcholine-based SSL_T was prepared and their structure was studied by transmission electron microscopy combined with freeze fracture (FF-TEM), Fourier transform infrared spectroscopy (FT-IR) and small angle X-ray scattering (SAXS). Fibrinolytic activity of SSL_T was examined at 45 and 37 or 24°C on fibrin or plasma clots under static conditions with turbidimetric assay and in a dynamic, permeation-driven lysis assay measuring the soluble protein content released from the permeated clots. The stability of the construct was monitored daily by measuring the activity of the encapsulated trypsin on a small peptide substrate.

Results: Trypsin is attached to the inner surface of vesicles (according to SAXS and FF-TEM) close to the lipid hydrophilic/hydrophobic interface (as revealed by FT-IR). According to the trypsin activity measurements, following an initial drop of 30% during the first 3 days, enzymatic activity of the SSL_T preparation remained unchanged for more than one week. Thermosensitivity of SSL_T was observed by enhanced fibrinolysis at 45˚C:

(i) time to reduce the maximal turbidity to 20% decreased by 8.6% compared to 37˚C and

(ii) the soluble fibrin degradation products in the early stage of the permeation lysis assay was 7-fold higher than at 24˚C. SSL_T exerted its fibrinolytic action on fibrin clots under both static and dynamic conditions, whereas plasma clot dissolution was observed only in the permeation-driven assay.

Conclusions: The improved fibrinolytic efficiency of SSL_T under dynamic conditions suggests that they may serve as a novel therapeutic candidate for dissolution of intravascular thrombi, which are always exposed to permeation forces.

PB 934 | Changes in Treatments and Outcomes after Creation of a Pulmonary Embolism Response Team (PERT)

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Background: Multidisciplinary pulmonary embolism response teams (PERTs) are being implemented to improve care of patients (pts) with life-threatening PE.

Aims: To determine how creation of PERT affects treatment and outcomes of PE pts.

Methods: Study compared PE patients before and after implementation of PERT. Consecutive sample of ER patients with PE and one high-risk feature were included in pre-PERT group. Activations from ED were included in post-PERT group. Primary outcomes included types of treatment, major bleeding and 30-day mortality. Means were used to summarize continuous variables; percentages summarized categorical variables. T-tests, Chi-square tests and logistic regression compared outcomes. To further control for differences, matched subgroups of pts pre- and post-PERT were analyzed. Data were divided into mutually exclusive six-month time periods and an interrupted time-series design examined slopes and change points pre- and post-PERT. SAS® was used for analysis and two-sided p-value < 0.05 was considered significant.

Results: Total of 212 pre-PERT pts and 228 post-PERT pts were analyzed. Patient demographics were generally similar, though pre-PERT, PE were more likely to be low-risk (37% vs. 19%) while post-PERT, PE were more likely to be submassive (32% vs. 49%), p< 0.0001. After adjusting for severity, more pts underwent catheter directed thrombolysis (1% vs. 14%, p=< 0.0001) post-PERT and there was trend towards use of any advanced therapy post-PERT (9% vs. 19%, p=0.011). Results of matched analysis were similar. There were no differences in major bleeding or mortality pre- and post-PERT. However, the interrupted time-series analysis suggested a downtrend in both bleeding and death post-PERT.

Conclusions: Creation of PERT affects treatment and outcomes of pts with life-threatening PE. Advanced therapies, particularly catheter-directed thrombolysis, increased after creation of PERT, especially in pts with submassive PE. In contrast, PERT appears to be associated with lower bleeding and mortality.

PB 935 | Comparing Emergency Department Patients to Inpatients Receiving a Pulmonary Embolism Response Team (PERT) Activation

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Background: Pulmonary Embolism Response Teams (PERTs) that provide multidisciplinary care to patients with severe PE are increasingly common. Most PERT activations originate from the Emergency Department (ED).

Aims: To describe how PERT activations originating from the ED differ from those originating from inpatient floors or intensive care units (ICU).

Methods: We enrolled a consecutive cohort of patients for whom PERT was activated at an urban teaching hospital. We compared PERT activations based on whether the activation originated from the ED, ICU or an inpatient floor. We compared groups in terms of the proportion of PERT activations that occurred during day, evening or weekend hours, and the proportion of confirmed PE. We compared PE severity, treatment and outcomes across locations. We tested differences using Fisher exact tests, with a two-tailed p-value < .05 considered significant.

Results: We enrolled 565 patients; including 449 (79%) with confirmed PE. The mean age was 61±16 years, and 241 (54%) were male. Activations from the ED (n=286, 89%) or floor (n=100, 75%) were more likely to be for confirmed PE than activations from the ICU (n=63, 58%), p< 0.0001. There were no statistical differences in the time of day of PERT activation based on location. Most activations for massive PE originated from the ICU (n=41, 65.1%), followed by the ED (n=82, 28.7%) and inpatient floors (n=22, 22.0%), p< 0.0001. Most activations from the ED (n=157, 54.9%) and floors (n=55, 55.0%) were for submassive PE. The use of thrombolysis or thrombectomy was most common among ICU patients (n=18, 31.6%), and more common among ED patients (n=52, 18.4%) than floor patients (n=6, 6.0%). Mortality and major bleeding events were most common among ICU patients, and similar among ED and floor patients.

Conclusions: PERT activations from different clinical locations differ in terms of patient presentation, PE confirmation, treatments and outcomes. PERTs should be designed and prepared to support the different needs of each clinical area.

PB 936 | Indications and Results of Treatment with Ultrasound Assisted Cathether Thrombolysis in Patients with Pulmonary Embolism

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Background: The precise role of ultrasound assisted thrombolysis (USAT) in the treatment of pulmonary embolism (PE) is not defined.
Aims: To investigate safety and efficacy of USAT in special subgroups of PE patients.
Methods: During the period of 3 years, 163 patients with PE were treated in the tertiary emergency clinic. Among 75 patients categorized as intermediate-risk, 17 patients were eligible for USAT-EkoSonic endovascular system (age 58±12 y, 14 men) and 58 patients (age 59±16 y, 30 men) were treated with slow systemic thrombolysis. The indications for USAT were: 2 patients had severe refractory pneumonia in the irrigation of the occluded pulmonary artery, 10 had high risk for bleeding (recent major surgery or major bleeding) and 5 were in NYHA-3 stage of heart failure (HF) between 7-45 days from the onset of PE symptoms. All of them received infusion of 2 mg/h tissue plasminogen activator (t-PA) via EKOS with the total dose of 50 mg. Intrahospital mortality, major bleeding, right ventricle dysfunction improvement measured by the change of right ventricle systolic pressure (RSVP) by echocardiography and duration of hospitalization were compared between two groups.
Results: The inserting of EkoSonic endovascular device was successful in all patients without complications. Intrahospital mortality were 12.1% vs 0% and major bleeding were 13.8% vs 11.8%, in systemic thrombolysis and USAT group, respectively. The median duration of hospitalisation was 8 days for both groups. There were no significant differences between admission (53.7 vs 58.4 mmHg, p=0.39) and discharge (28.3 vs 36.7mmHg, p=0.08) RVSP between two groups. Only two patients with the duration of PE symptoms between 30-45 days treated with USAT because of the NYHA-3 stage HF had no significant reduction of RVSP during follow-up.
Conclusions: USAT therapy is efficious and safe in intermediate-high risk PE patients with high risk for bleeding, severe pneumonia behind the occluded artery and less efficious in patients with longer duration of symptoms.

PB 937 | In vitro Thrombolytic Action of New Fibrinolytic Enzyme Complexes - Strictoliase and Lilasyn

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Background: Modern medicine has made its choice in a favor of thrombolytic agents - plasminogen activators. Such preparations implement the lysis of blood clots by activating the patient's own system of thrombolysis and greatly reduce the number of conventional medical treatment complications: bleeding and rethrombosis. A very promising source for finding new therapeutic thrombolytic agents are new plasminogen activating proteinases, produced by microspore fungi.
Aims: Studying the properties of thrombolytic enzyme complexes strictoliase and lilasyn with fibrinolytic activity.
Methods: Strictoliase and lilasyn were obtained from the culture fluid of *Sarocladium strictum* and *Purpureocillum lilacinum*, respectively. Fibrinolytic activity was determined by Atrup- Müllertz method, destruction of a thrombus *in vitro*, and with the chromogenic peptide substrates (CPS).
Results: The test on the fibrin plates demonstrated the presence of fibrinolytic activity in both studied preparations. For the strictoliase the value of the fibrinolytic activity was 651 U/ml and plasminogen activator activity 674,3 U/ml, but for the lilasyn these values were significantly lower and were 36,1 and 9,4 U/ml respectively. Nevertheless, both preparations demonstrated the high possibility to thrombolysis. The adding of the preparation of a single active purified protein from the lilasyn complex resulted in the thrombus weight reduction of 25% after 30-minutes incubation at 37°C, %94 after 45 minutes and whole reduction after 1 hour. The thrombolytic activity of the four purified protein fractions of the strictoliase preparation after 90 minutes was 57.1 ,36.8 ,25.6 ,38 %, respectively. Activity determination with CPS showed that, strictolyase has urokinase activity and lilasyn has tissue plasminogen activator-like activity.
Conclusions: Thus, was demonstrated the trombolytic and plasminogen activator activity of the obtained enzyme complexes, thanks to which may be possible their use for treatment of thrombophlebitis and phlebothrombosis.

PB 938 | A Case Series of Low Dose Alteplase for Submassive Pulmonary Embolism (PE)

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Background: Anticoagulation is standard therapy for PE. Fibrinolytic therapy with alteplase is reserved for massive PE with hemodynamic collapse, by administering 100 mg over < 1 hr (AHA/AT9 grade 2C recommendations). Alteplase doses of 50 mg to 100 mg have been studied for submassive PE, but the benefits of clot lysis are offset by increased bleeding risk. We have previously shown that lysis of massive deep venous thrombi can be achieved with catheter-directed alteplase using daily doses of ≤10 mg. We describe treatment of 9

TABLE 1 Clinical Summary of Patients Treated with Low Dose Alteplase for Submassive PE

Case	Alteplase Rx	Outcome
42 y/o with thymoma, s/p pneumonectomy	6 mg/6 hr, daily, x 2	Filling defect of R PA improved on CTA at 72 hr.
86 y/o with heparin-induced thrombocytopenia	6 mg/6 hr, daily, x 3	Improved perfusion RUL and LPIL on V/Q at 72 hr.
75 y/o with metastatic endometrial cancer	6 mg/6 hr, daily, x 2	Left PA and smaller clots improved on CTA at 72 hr.
41 y/o sickle cell anemia, chronic PE	6 mg/6 hr, daily, x 2	No improvement on CT at 72 hr.
46 y/o von Hippel Lindau s/p VP shunt surgery	2 mg each PA, then 6 mg/6 hr x 1	Improved RV function on echocardiogram at 24 hr.
78 y/o prostate cancer	2 mg each PA, then 6 mg/6 hr daily x 1; subsequent low dose tPA of leg DVT	Marked decrease right PA thrombus and decrease in L PA thrombus on CTA at 72 hr.
65 y/o with sickle cell anemia, chronic PE	6 mg/6 hr, daily, x 3	Bilateral decreased perfusion abnormalities on V/Q at 72 hr.
52 y/o with Cushing´s disease, s/p adrenalectomy	2 mg each PA, then 6 mg/6 hr x 1; subsequent low dose tPA of leg DVT	Improved RV function and decreased PA pressures on echocardiogram at 24 hr.
29 y/o s/p nephrectomy & retroperitoneal lymph node dissection for renal cancer	2 mg in R PA, then 4 mg/4 hr, x 1, then 6 mg/6 hrs x 1	Hemorrhage into operative field requiring re-exploration. Improved PA pressures and RV function on echocardiogram at 24 hr.

patients with submassive PE with low dose alteplase infused through central venous catheters.

Aims: To inform the design of randomized clinical trials to test the safety and efficacy of low dose alteplase for submassive PE.

Methods: Nine patients (29-86 yrs) enrolled in clinical research studies at the NIH Clinical Center under IRB-approved protocols were treated with one to three alteplase infusions (1 mg/hr for 6 hours daily) via central venous catheters; four were treated with 2 mg boluses of alteplase into the affected pulmonary arteries before the continuous infusion. All patients received concomitant anticoagulation.

Results: Eight patients had evidence of clot lysis within 72 hours by CT angiogram, V/Q scan and/or echocardiogram; one without immediate lysis had normalization of the CT angiogram after one month. One patient with recent abdominal surgery developed bleeding that required re-exploration.

Conclusions: Low dose (≤10 mg/day) alteplase may provide benefit compared with anticoagulation alone in the setting of submassive PE, particularly in patients that merit more aggressive therapy than anticoagulation alone. A randomized trial comparing anticoagulation with and without low dose alteplase could determine the relative risks and benefits. Patients with recent invasive procedures involving non-compressible structures should be excluded from any such trial.

PB 939 | Successful Thrombolysis of Huge Right Atrial Thrombus in a Patient with Primary Repair of Atrial Septal Defect

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Background: Surgical repair of an atrial septal defect (ASD) is a standard procedure with very low morbidity and mortality. Thrombus formation after patch-based repair, albeit a recognized complication, is very rare after the primary repair of an ASD.

Aims: Herein, we report a case of huge right atrial thrombous formation 6 months after repairs of ASD which treated nonsurgically.

Methods: A 23-year-old man underwent primary surgical closure of an ASD via a right lateral minithoracotomy. Defect was closed directly by 4-0 polypropylene. Six months postoperatively, however, although he was asymptomatic, routine follow-up echocardiography revealed a partially mobile mass (measuring 45 mm × 32 mm) in the right atrium. Intravenous infusion of low dose of Streptokinase (250,000 U bolus followed by 10,000 U/ hour) for 48 hours resulted in complete resolution of the thrombus. Heparin treatment was continued after fibrinolytic therapy. Follow-up at 3 month, 6 month, 1 year, 2 year, and 3 year after treatment of the clot demonstrated no evidence of intra-cardiac thrombus recurrence.

Results: Fibrinolysis is generally efficient but exposes the patient to risk of migration of the intra-cavity thrombus. Between surgery and thrombolysis, we tend to opt for thrombolytic therapy because of the association of surgery with high rates of morbidity and mortality.

Conclusions: We recommend periodic follow-up echocardiography and short-term (three months) of postoperative anticoagulation administration in patients undergoing primary ASD closure.

PB 940 | Thrombolytic Properties of Longolytin - Proteinase Complex from Impertfect Fungi Arthrobotrys Longa - in Dependence on its Fibrinolytic Activity

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Background: Proteinase complex from imperfect fungi Arthrobotrys longa -longolytin- possesses of high fibrinolytic and thrombolytic activities, has significant affinity to thrombi, and is studied as perspective thrombolytic preparation. Longolytin fibrinolytic activity (F.A.) is very sensitive to rather variable fungi cultivation conditions. It is noted, the preparations with more high plasminogen activator activity (P.A.A.) as part of its general F.A. have more essential thrombolytic activity.

Aims: The aim- retrospectively to evaluate thrombolytic properties of different longolytin preparations in dependence of its P.A.A. in model of thrombi dissolving in thrombosis of marginal rabbit's ear vein.

Methods: There were analyzed 3 groups of longolytin preparations with different P.A.A.:25-32%-9 rabbits, 39-42%-8 rabbits, 44-48%-13 rabbits, compared to control group, containing correspondingly 5-4-7 rabbits. There were compared velocity of thrombi dissolving by longolytin in dependence on different P.A.A. of its general F.A.

Results: Velocity of thrombi dissolution was greater in experimental thrombi - 0,04-0.28 mm^2/min, where thrombi dissolved by longolytin, compared to control 0,02-0,12 mm^2/min.with glycerol as dissolving material. It depended on thrombi size and was greater in large thrombi, compared to small ones as in experiment and in control. However large thrombi (20-40 mm^2) in most big experimental group with P.A.A. 44-48% were dissolved with velocity 0,05-0,34 mm^2/min, and in group wth P.A.A.25-32 % with velocity 0,02-0,17 mm^2/min. ($p< 0,05$) This difference was significantly less in control:0,03-0,16 mm^2/min. -P.A.A. 44-48%, 0,02-0,14 mm^2/min -P.A.A. 25-32%.

Conclusions: Longolytin thrombolytic activity depended on level of P.A.A. in structure of general F.A. of preparation. It is necessary to create such cultivation conditions, which stimulated greater level of P.A.A. in synthesized longolytin.

PB 941 | Sub-massive PE - A Role for Low Dose Thrombolysis?

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Background: The use of thrombolysis in sub-massive PE is controversial (1). Studies to date have been heterogeneous, making it difficult to draw conclusions (2). The MOPETT trial (3) showed that an intermediate dose of t-PA was safe and effective and resulted in a significant reduction in pulmonary artery pressure but not in death and recurrent PE. The PEITHO study (4) found that fibrinolytic therapy reduced haemodynamic decompensation but increased the risk of major haemorrhage and stroke. The American College of Chest Physicians suggest consideration of systemic thrombolysis in selected patients with acute PE who deteriorate after starting anticoagulant therapy but have yet to develop hypotension and who have a low bleeding risk (5).

Aims: To review the outcomes of patients with sub-massive PE who presented to a tertiary referral centre.

Methods: A retrospective review of the records of patients between January 2014 and December 2016.

Results: 24 patients were included, their characteristic are shown in table 1. They underwent either low dose systemic thrombolytic therapy using t-PA (10mg in 1 minute followed by 40mg over 2 hours) - six patients or catheter-directed thrombolysis - nine patients, or standard therapy with close observation, following multidisciplinary review (respiratory/radiology/critical care/haematology). Indications for thrombolysis included progression on anticoagulation (6), large clot burden (5) and unwell at presentation (but not meeting criteria for massive PE) (4). The group that received thrombolytic therapy had more severe disease (higher BNP, echo features of pulmonary hypertension, higher PESI scores). No major bleeding was seen.

Conclusions: Low -dose thrombolysis is an option for a selected cohort of patients with sub-massive PE. It should be considered when a patient is significantly compromised by a PE or has progressed on anticoagulation, in a clinical setting where experienced clinicians are available to guide treatment.

TABLE 1 Characteristics of patients with sub-massive PE

Table 1. Characteristics of patients with sub-massive PE

	Sub-massive PEs thrombolysed	Sub-massive PEs not thrombolysed
N	15	9
Median (IQR) age in years	51 (43-67)	50 (46-70)
Provoked/Unprovoked	13/2	5/4
DVTs	5 /11 (4 had no doppler)	3/9
Baseline echo features of pulmonary hypertension	14/15	5/9
Right heart strain on CTPA	13/15 (2 had extensive clot burden but no right ventricular strain)	8/9 (1 had V/Q scan only – with extensive clot burden)
Median (IQR) BNP (ng/L)	2553 (672-4504) (14/15 had BNP measured)	410 (120-1746)
Median (IQR) Troponin (ng/L)	28 (17-93)	86 (45-131)
Median (IQR) lowest SBP (mmHg)	118 (95-121)	109.5 (106-113)
Median (IQR) PESI	102 (97-130)	75 (70-96.5)
Systemic thrombolysis/Catheter-directed thrombolysis	6/9	Not applicable
Median (IQR) Length of hospital Stay (days)	16 (6-22.5)	5 (5-9)
Median (IQR) Length of critical care admission (days)	5 (2-12)	0 (0-0)
Bleeding risk factors	3 (menorrhagia, pregnancy, recent surgery)	4 menorrhagia, pregnancy, recent fracture, recent surgery)
Complications of thrombolysis	5 (bleeding at puncture sites)	Not applicable
Deceased at time of data censure	4 (2 catheter directed, 2 systemic thrombolysis)	0

TABLE 2 References

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PB 942 | A Complex Non-invasive Assessment of the Effect of Hirudotherapy on Structural and Functional State of Vascular Bed

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Background: A successfully curable dysfunction of vascular endothelium is an initial stage of atherosclerotic vascular lesions that underlies the majority of cardiovascular diseases. The use of hirudotherapy (HT) was considered helpful in this case.

Aims: A non-invasive assessment of the HT effect on the various levels of vascular bed is topical, and it was the aim of the present work.

Methods: Patients with arterial hypertension, dysfunction in micro- and macrocirculation in endothelium and practically healthy people underwent a course HT (8 sessions). To this end, 7 leeches were stated to them once a week. The vasoactive function of vascular endothelium was measured using a certified device of «Angioscan-01» at various diagnostic regimes. A contour analysis of volume pulse waves parameters before and after the sublingual introduction of trinitroglycerol (0.5 mg) was performed. The state of artherial capillaries was observed using videocapillaroscopy (VC).

Results: The vasodilating effect of HT was partly preserved in time, and it increased with each session. The volume pulse waves parameters that are a function of the vasoactive NO-dependant endothelium activity equally differed from their pathologically changed initial values after a course of HT and a single dose of nitroglycerol. Those parameters approached (either increasing or reducing) the parameters characteristic of healthy people. The VC showed that the HT increased the number of functioning capillaries, improved the rate of blood circulation in them and increased the size of perifocal zone (vessel-tissue).

Conclusions: For the first time, using the non-invasive method of photoplethysmography, it was established in vivo and confirmed by VC that the course of HT reliably improved the values of structural and functional characteristics of various vascular bed levels in patients with cardiovascular diseases even making a few of those characteristics approach the normal values.

PB 1742 | FXIII and EACA Increase Thrombus Stability and Decrease Pulmonary Embolism in an in vivo Mouse Model of Venous Thromboembolism, but EACA Increases Thrombus Size

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Background: Clinical factors determine if a patient presents with deep vein thrombosis (DVT) or pulmonary embolism (PE). Thus, the mechanisms underlying DVT stability and the progression of DVT to PE are unknown. Anticoagulation is contraindicated in patients with DVT and severe bleeding. Inferior vena cava filters are used but are associated with morbidity. An ideal treatment would stabilize clots, minimize embolization and PE burden, without worsening bleeding. Using a mouse model of venous thromboembolism, we showed that thrombin-mediated factor XIII (FXIII) activation stabilizes DVT and limits PE.

Aims: Compare the effects of epsilon-aminocaproic acid (EACA) and FXIII supplementation on DVT stability and PE burden in this mouse model.

Methods: Platelets were labelled using Alexa Fluor 488 conjugated α-CD41 Fab fragments. DVT was induced in the femoral veins of C57BL/6 female mice by application of ferric chloride. Mice received intravenous saline, dalteparin (0.2 U/g), dabigatran (33 mg/g), or EACA (1 mg/g) alone, or FXIII (1 mg/kg) in combination with anticoagulants starting 12 minutes after thrombus formation. Using intravital videomicroscopy, the thrombus sizes, and the size and number of emboli dislodging from the thrombus were quantified for 2 hours. Lungs were then harvested, sectioned and stained to detect PE.

Results: FXIII has little effect on thrombus size, whereas EACA increased thrombus size by 3-fold. Both EACA and FXIII, given with saline, dalteparin, or dabigatran, reduced the total number of embolic events. As well, the number of large emboli were reduced following treatment with FXIII and EACA, and this correlated with reduced PE burden.

Conclusions: Both FXIII and EACA increase DVT stability and decrease PE burden in this model. However, EACA increases DVT size, whereas FXIII has little effect. Therefore, supplemental FXIII may be useful to stabilize DVT and reduce the risk of PE in patients who cannot be anticoagulated.

PB 1743 | Fibrinogen May Possess a Second Factor XIII Cross-linking Site for α2-AP in Fibrin

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Background: The fibrin clot is subject to challenges such as shear stress from blood flow, and fibrinolysis. A key regulator of fibrinolysis is α_2-antiplasmin (α_2-AP), which is cross-linked to the fibrin α-chain by activated FXIII, at residue Lys303.

Aims: The aim of this study was to generate a fibrinogen (FGN) mutant lacking the cross-linking site for α_2-AP, and investigate the contribution of α_2-AP cross-linking to the inhibition of fibrinolysis.

Methods: FGN wild-type (WT) and mutant (αK303R) were produced in CHO cells and purified by IF-1 affinity chromatography. The effect

of αK303R and WT on clot formation and lysis in the absence and presence of α_2-AP was analysed by turbidity. A permeation set-up was also used to study clot lysis for both variants. Cross-linking of α_2-AP to αK303R and WT was investigated in a plate-based α_2-AP incorporation assay.

Results: In the absence of α_2-AP, the turbidity and lysis profiles were similar for both αK303R and WT, in both the absence and presence of FXIII, indicating that this mutation intrinsically does not affect fibrin polymerisation and lysis. When α_2-AP was added, the difference in time to half-lysis between presence and absence of FXIII was significantly reduced (-38.8%) for αK303R (33.5±4.5 min) compared to WT (54.7±3.3min). However, mutation of the α_2-AP cross-linking site did not completely abolish the effects of α_2-AP on clot lysis. Furthermore, in agreement with the effects on clot lysis, analysis of α_2-AP incorporation into fibrin showed decreased (but not completely abolished) cross-linking by 42.7±4.8% for αK303R compared to WT.

Conclusions: The lack of full inhibition of α_2-AP reduction in lysis and of α_2-AP incorporation onto fibrin indicate that FGN likely possesses a second FXIII cross-linking site for α_2-AP. Further studies are required to identify this potential second site and investigate its physiological relevance.

PB 1744 | Fibrin-derived Bß$_{15\text{-}42}$ Peptide Is a Gatekeeper of Thrombus Resolution

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Background: Thrombus resolution is driven by leukocyte recruitment and thrombus angiogenesis. An effective inhibition of leukocyte transmigration *in vitro* is mediated by naturally occurring peptide Bß$_{15\text{-}42}$, which is a competitive inhibitor of the interaction between N-terminus of the fibrin beta chain and vascular endothelial cell cadherin (VE-cadherin). Bß$_{15\text{-}42}$ consists of 28 amino acids corresponding to the N-terminal sequence of the ß-chain of fibrin.

Aims: Fibrin clots from patients with chronic thromboembolic pulmonary hypertension (CTEPH) are resistant to fibrinolysis. Therefore, we explored the role of peptide Bß$_{15\text{-}42}$, a plasmin-generated cleavage product of fibrin in thrombus resolution.

Methods: Study groups of 8-12 weeks old C57BL/6 mice were injected i.p. over various time periods with Bß$_{15\text{-}42}$ or random peptide after thrombus had been induced by subtotal inferior vena cava (IVC) ligation. Thrombi from each animal were collected for further Immunohistochemistry (IHC) and RT-PCR analysis. Pulmonary endarterectomy (PEA) specimens were collected during surgery from randomly selected patients with CTEPH. Anti- Bß$_{15\text{-}42}$ immunoblots of human red (erythrocyte-rich) CTEPH thrombi and white (collagenous) CTEPH thrombi were compared.

Results: Bß$_{15\text{-}42}$ delayed thrombus resolution after IVC ligation. Thrombi of all treated groups were larger than controls. We observed a significant decrease of thrombus macrophages and microvessel density. Measurements of Bß$_{15\text{-}42}$ in red clot of human cases of chronic thrombosis indicated higher concentrations compared with controls.

Conclusions: Our data suggest that excess of the fibrin fragment Bß$_{15\text{-}42}$ misguides thrombus resolution, presumably by inhibiting VE-cadherin mediated leukocyte migration.

PB 1745 | Theoretical Model for Thrombin-FXIII-A2 Complex and the Effect of Ca^{2+} Ions on the Dynamic Behavior of FXIII-A$^{2'}$

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Background: The normal physiological activation of FXIII-A_2 requires the concerted action of thrombin and Ca^{2+} ions. Unfortunately, neither the structure of the FXIII-A_2:thrombin Michaelis complex nor the driving force of its formation are known and the effect of Ca^{2+} on the structure and on the dynamics of FXIII-A_2' (the proteolitically cleaved but still inactive FXIII-A_2) is only partially understood.

Aims: Our aim was to get deeper insight into the process of FXIII-A_2:thrombin complex formation and the effect of Ca^{2+} ions on the structure and stability of FXIII-A_2'.

Methods: In order to derive a theoretical model for the FXIII-A_2:thrombin complex, protein-protein docking experiments were performed. On the complex derived this way 1 ms molecular dynamics simulation was carried out with explicit solvent model. Simulations have also been carried out both on FXIII-A_2' and FXIII-A_2 in the presence and absence of Ca^{2+} ions, respectively.

Results: From the analysis of the molecular dynamics trajectory one can conclude that the electrostatic forces between the thrombin and the first β-barrel domain of FXIII-A play fundamental roles in the FXIII-A_2:thrombin Michaelis complex formation. Although the structure we obtained have similarity to the corresponding one derived from the X-ray experiments, remarkable differences can also be observed between them. Simulations predict that the thrombin can also accommodate the activation peptide (AP) fragment conformation, which exists in the non-cleaved FXIII-A_2. The simulations on FXIII-A_2' and FXIII-A_2 systems revealed that the presence of Ca^{2+} ions results in increased flexibility and weaker interaction between the A subunits of FXIII-A_2'. Sampling snapshots from the molecular dynamics trajectory allow the localization of Ca^{2+} binding sites on the surface of FXIII-A_2'.

Conclusions: In conclusion, the atomic details of the first events of FXIII-A_2 activation outlined above agree well with the few experimental data revealed so far, and provide valuable complementary information.

PB 1747 | Platelet and Plasma Factor XIII Levels after Replacement Therapy in Severe Congenital Factor XIII Deficiency: Is there a Role for Factor XIII Uptake by Megakaryocytes?

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Background: Factor XIII (FXIII), the fibrin stabilizing factor, is present both in plasma and platelets. Megakaryocytes can synthesize FXIII but it is still unclear whether platelet FXIII may derive from endocytosis of exogenous FXIII by megakaryocytes.

Aims: To evaluate plasma and platelets FXIII levels in a patient with severe FXIII deficiency under replacement therapy with recombinant (r)FXIII. To investigate the possible *in vitro* uptake of FXIII by patient megakaryocytes.

Methods: Plasma and intraplatelets levels of FXIII were measured before and after therapy. Clot formation was evaluated by whole blood rotational thromboelastometry (ROTEM®). Synthesis and uptake of FXIII by megakaryocytes in culture were studied by immunofluorescence technique.

Results: Plasma FXIII activity and antigen levels were < 5% and < 1%, respectively. These values increased about 100% after administration of rFXIII. Subsequently, a progressive reduction of both levels up to 32% and 24%, respectively, were seen at day 13 after infusion. Platelet FXIII antigen levels were < 1% before therapy and reached 6% within 13 days after infusion.

INTEM and EXTEM assays before therapy, showed a prolonged clot formation time, reduced clot stability and early clot lysis. Normalization of ROTEM® parameters was seen after administration of rFXIII. Patient megakaryocytes were negative for the immunostaining with anti-FXIII antibody and became positive after the addition of FXIII to the culture medium.

Conclusions: Administration of rFXIII in severe FXIII deficient patients results in restoration of FXIII plasma levels and increased intraplatelet FXIII content. Coagulation profile can be monitored by ROTEM®. *In vitro* experiments on cultured patient megakaryocytes revealed that these cells, which were unable to synthesize FXIII, can endocytose exogenous FXIII and possibly produce FXIII-containing platelets, as shown with the *in vivo* data. The role of intraplatelet FXIII still remains to be fully elucidated.

PB 1748 | Investigating the Role that a Factor XIII Binding Site on Fibrinogen αC (233-425) Plays in Controlling Glutamine Substrate Specificity

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Background: Fibrinogen αC (233-425) contains three reactive glutamines (Q) that can be crosslinked by Factor XIIIa to reactive lysines (K) located on another fibrin αC molecule. Crosslinking of the αC region modifies fibrin clot structure by enhancing lateral aggregation and increasing fibrinolytic resistance. Fbg αC (233-425) also has a putative FXIII binding site (389-403) with E396 proposed to serve as the key interaction residue. Greater knowledge on the functional role of E396 within αC (389-403) will aid in better understanding what initiates and controls fibrin clot architecture.

Aims: This study was aimed at characterizing the ability of both FXIII A_2 and FXIII A_2B_2 to cross-link reactive glutamines (Q237, Q328 and Q366) in Fbg αC (233-425) to a lysine mimic. Studies were carried out with wild-type (WT) Fbg αC versus αC E396A. Results obtained would help assess if E396 influences FXIIIa substrate specificity and/or provides an anchoring site via FXIII B_2

Methods: A mass spectrometry assay was used to monitor crosslinking of reactive glutamines to a lysine mimic in αC (233-425) versus E396A variant. Assays were performed with FXIII A_2 or A_2B_2. The lysine mimic was glycine ethyl ester and its ^{15}N-labeled version was used in 2D ^{15}N-HSQC NMR studies. Assays were done in triplicate (+/- SD).

Results: The Q reactivity rankings for WT Fbg αC (233-425) and E396A were both Q237 >> Q366 ≥ Q328. Increasing FXIII concentration by 4-fold suggested Q366 could be more reactive than Q328. Reactivity of Q237 was slightly slower with FXIII A_2B_2 than with FXIIII A_2, but the overall Q rankings were still preserved for WT and E396A.

Conclusions: Glutamine reactivity rankings are the same for both WT and E396A Fbg αC (233-425). Fbg αC E396 is therefore not critical for supporting FXIIIa activity. Results also suggest FXIII A_2 and A_2B_2 do not need E396 as an anchoring site. Other residues of αC (389-403) or a nearby region may serve as a replacement for E396.

PB 1749 | Genetic and Clinical Epidemiology of Congenital Fibrinogen Disorders

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Background: Congenital fibrinogen disorders (CFDs) are rare conditions. While the molecular basis of these disorders is relatively well studied, for some world regions reports on the distribution of fibrinogen mutations and description of clinical phenotypes are scarce.

Aims: To report the distribution of fibrinogen mutations and clinical features according to the place of residence in a large cohort of CFD patients.

Methods: We conducted a retrospective study of all consecutive CFDs cases genotyped in our laboratory from 1996 to 2016. Demographic and clinical data at diagnosis were assessed by a standardised case report form.

Results: Among 303 mostly Caucasian (97%) CFDs patients, there were 81 afibrinogenemia (Afib), 59 hypofibrinogenemia (Hypo), 163 dysfibrinogenemia (Dysf) and 16 hypodysfibrinogenemia (HDysf). Mean age at diagnosis differed: 3.4, 22.1, 30.1 and 23.7 years for Afib, Hypo, Dysf and HDysf, respectively. At the time of genotyping, patients lived in Europe (83%), Asia (9.2%) or Africa (8%). FGA null mutations were highly prevalent in Afib patients (81.5%) and FGA or FGG missense mutations in Dysf (93%). Hotspot mutations were identified in patients from all continents. Almost all Afib patients had bled (98.8%) with umbilical (66.7%), muscle (40.7%), joint (29.6%) and cerebral (23.5%) bleeds described. Nearly half of Hypo, Dysf and HDysf also reported bleeding, including menorrhagia (32%) and post-surgery bleeding (32.3%). Thromboses were more frequent in qualitative diseases (19.6% Dysf and 31.3% HDysf) than in quantitative ones (8.6% Afib and 8.5% Hypo). In Afib the bleeding phenotype was similar between continents but thromboses appeared more frequent in Asia and Africa than in Europe.

Conclusions: Hotspot mutations leading to CFDs are frequent around the world. While bleeding phenotypes appear similar between different continents, thromboses in qualitative CFDs may be more frequent in Asia and Africa than in Europe. Different modalities of fibrinogen replacement may partially explain this finding.

PB 1750 | Calcium Binding Sites in Coagulation Factor XIII and Other Transglutaminases: Their Evolution, Diversity, Conservation and Functional Relevance

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Background: Calcium binding proteins have typically been characterized as EF hand family of proteins because of shared conserved structural and sequence characteristics. The enzyme family of Transglutaminase which includes the coagulation Factor XIII (FXIII) also binds to calcium and this has important consequences on their activation process. However, they do not share any similarity with the EF hand proteins.

Aims: To characterize the evolution, conservation, functional diversity and relevance of FXIIIA calcium binding sites by using phylogenetic strategies, protein modeling, docking and molecular dynamic (MD) simulation, *in vitro* mutagenesis and ITC (Isothermal titration calorimetry).

Methods: Structure and sequence based structural phylogeny analysis was performed for the calcium binding sites on the TreeFam, Consurf server and with structural alignment tools embedded in VMD and YASARA. Docking of sodium and calcium were performed alongwith MD simulation at high salt concentrations on FXIII core domain structures to explain their supportive functions. Recombinant FXIIIA subunit was mutated for gain of/ loss of function mutations and expressed in Pichia based system as well as in *HEK293t* cells and expression products analyzed. We also performed ITC based titrations of FXIIIA subunit and heterotetramer under different salt concentrations.

Results: Calcium binding site residues are in general highly conserved. Mutation of these sites usually results in unstable protein indicating that these sites are critical to the native fold of the protein. These sites first evolved in Transglutaminase like cysteine proteases family of proteins as crucial to their functional activity. The calcium binding sites in Factor XIII might also bind with very low affinity to sodium which explains their combined effect during non-proteolytic activation.

Conclusions: Our studies demonstrate the functional importance of the calcium binding sites in not only Factor XIII but also other Transglutaminases.

PB 1751 | On-demand Treatment with Fibrinogen Concentrate in Acute Bleeding and for Surgery in Patients with Congenital Fibrinogen Deficiency

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Background: Patients with congenital a- and hypofibrinogenemia experience severe bleeding episodes. Human fibrinogen concentrate (HFC) can correct the hemostatic defect and arrest bleeding in these patients. Octafibrin is a highly purified, plasma-derived, lyophilized, double virus-inactivated (using 2 dedicated virus inactivation/removal steps) HFC.

Aims: To investigate the efficacy of Octafibrin in this setting.

Methods: This was a prospective, open-label, multinational study in adult and adolescent a- or hypofibrinogenemic patients. Efficacy in treating bleeding events (BEs) was assessed using a 4-point objective scale completed by the investigator and adjudicated by an independent endpoint adjudication committee. The HFC effect was also assessed using thromboelastometry.

Results: This planned interim analysis comprises data of 13 patients (11 adult and 2 adolescent), of which 11 experienced a total of 23 minor BEs. The median (range) dose of HFC administered for BEs treatment was 58.8 mg/kg (33.9-101.7 mg/kg) per BE. The success rate (efficacy rating of excellent or good) for all BEs was 100% (90% CI: 0.88, 1.00) as adjudicated by the IDMEAC (all excellent). Maximum clot firmness (MCF) measured in plasma was also determined for the first infusions administered for BEs treatment. The mean (±SD) change in MCF from baseline (0 mm) to 1 hour after the first HFC infusion was 6.5 mm (±2.0) (95% CI: 5.65, 7.40; p< 0.0001). Four patients

underwent 4 surgeries (3 major, 1 minor). The post-operative success rate was 100% (90% CI: 0.5, 1.0). There were no related serious adverse events, no thromboembolic events, no allergic or severe hypersensitivity reactions, and no anti-fibrinogen antibodies that developed during the study.

Conclusions: This interim data showed 100% hemostatic efficacy in the treatment of bleeds and during perioperative prophylaxis and no drug related SAEs of this newly developed human fibrinogen concentrate in patients with congenital fibrinogen deficiency.

PB 1752 | Congenital FXIII Deficiency in Pakistan: An Update

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Background: Factor XIII (FXIII) deficiency is a rare bleeding disorder (RBD) with an incidence of about one in 1-2.5 million and its incidence is higher in populations with consanguineous marriages.

Aims: The aim of this study was to characterize patients and relatives from fourteen families with suspected FXIII deficiency from Pakistan and to identify the clinical characteristics and underlying mutations

Methods: FXIII deficient patients were enrolled in this study. The patients' medical histories were recorded in a questionnaire. As a first indicator of FXIII deficiency, a 5M urea clot solubility test was used. Plasma FXIII A- and B-subunit antigen levels were determined by ELISA. FXIII activity was measured with an incorporation assay. Sequencing of all exons and intron/exon boundaries of F13A was performed.

Results: We analyzed 14 families in which 23 were severe FXIII deficient with FXIII level < 1%. 19 first-degree relatives with mean FXIII level 71.19 ±21.1 are asymptomatic. Each family had a history of consanguineous marriages except one. 50% had significant family history of bleeding. Age at first presentation was ranged from birth to 18 years. In these patients, we identified 8 missense mutations, 5 Splicing mutations and 2-nonsense mutations. Bleeding after injury (78%), delayed wound healing (70%), umbilical cord bleeding (57%), hematoma, bruises (39%), abortions and menorrhagia (38%), circumcision (35%) were the main clinical manifestations followed by gum bleeding (30%), joint bleeding (26%), melena (9%) and ear bleeding (5%) and epistaxis in (4%) of patients.

Conclusions: We have analyzed a cohort of 42 individuals from 14 families in which 23 were severe FXIII deficient (homozygous or compound heterozygous) and remaining were FXIII deficient carriers (heterozygous). We identified 12 mutations in these families leading to congenital FXIII deficiency. Moreover, early and appropriate diagnosis of FXIII deficiency should be made so that prophylaxis can be initiated immediately.

PB 1753 | Pharmacokinetics of Fibrinogen Concentrate and Efficacy in Treating Acute Bleeding in Children and Adolescent Patients with Congenital Fibrinogen Deficiency

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Background: Human fibrinogen concentrate (HFC) can correct the hemostatic defect and arrest bleeding in patients with congenital fibrinogen deficiency. Octafibrin is a highly purified, plasma-derived, lyophilized, double virus-inactivated (using 2 dedicated virus inactivation/removal steps) HFC.

Aims: To describe the pharmacokinetics (PK) and efficacy of Octafibrin in pediatric patients.

Methods: Data in patients aged < 18 years from two multinational, prospective, open-label studies in afibrinogenemia patients was summarized. The studies investigated PK, surrogate efficacy and safety (study FORMA-01) after infusion of 70 mg/kg HFC, and efficacy and safety in the on-demand treatment of bleeding episodes (BE) and surgical prophylaxis (study FORMA-02). The surrogate efficacy endpoint in FORMA-01 was thromboelastometric maximum clot firmness (MCF) in plasma. Efficacy in treating bleeding in FORMA-02 was assessed by a 4-point objective scale.

Results: Seven patients aged 12-17 years were included in the completed PK study and an interim analysis of the treatment study (5 and 2 patients, respectively). The PK data showed AUC_{norm} of 1.53±0.6 h·kg·g/L/mg, clearance of 0.68±0.18 mL/h/kg, $T_{½}$ of 72.8±14.5 h, and incremental in-vivo recovery (IVR) of 1.95±0.41 mg/dl/(mg/kg). The 1-hour MCF values were 9.5±1.7 mm, from 0 mm at baseline.

Two patients in FORMA-02 received treatment with HFC for BEs. In both patients, treatment efficacy was adjudicated as excellent.

There were no related serious adverse events (SAEs), thromboembolic events, allergic or severe hypersensitivity reactions, and no anti-fibrinogen antibodies developing during the studies.

Conclusions: This analysis presents data in patients aged < 18 years from two studies with fibrinogen concentrate in afibrinogenemia patients. The safety data showed no related SAEs. A favorable PK profile was observed in the 5 patients from the FORMA-01 study and excellent treatment efficacy in the 2 patients from the FORMA-02 study.

PB 1754 | Factor XIII in Tears and its Possible Role in Corneal Wound Healing

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Background: We have reported the presence of factor XIII (FXIII) subunits in tears, but its function has not been explored.

Aims: The effect of FXIII was investigated on wound healing, in vitro on corneal epithelial cells and in vivo in tears of patients following corneal surgeries with different types of wound: phacoemulsification, penetrating keratoplasty (PKP) and photo-refractive keratectomy (PRK).

Methods: Scratch-wound assay, proliferation and migration assays were performed on immortalized corneal epithelial cells. Using a hypersensitive chemiluminescent ELISA method, developed in our laboratory, FXIII complex and subunits were detected in tears of patients before and after different surgical interventions on the cornea; post surgical angiogenesis and re-epithelization were observed.

Results: The addition of recombinant cellular FXIII (cFXIII, rFXIII-A_2) resulted in a concentration dependent faster healing of the scratch wound. rFXIII-A_2 promoted the proliferation of corneal epithelial cells, but no effect on migration was observed. After corneal surgeries FXIII complex and subunits concentrations increased in tears, then decreased reaching the normal interval at different times after the surgical intervention. After cataract surgery, FXIII concentrations correlated with the inflammation of the eye and the corneal edema. Lower FXIII concentrations associated with slower re-epithelisation of the corneal surface after PRK. Extremely high FXIII concentrations measured in a few cases after PKP was associated with neovascularization of normally avascular cornea.

Conclusions: FXIII accelerates corneal cell proliferation. Its presence in tear proteome has a beneficial effect on corneal re-epithelisation. which results in decreased period of complaints caused by the corneal erosion. FXIII might be considered as an additional therapy in the treatment of corneal erosions, but long exposition to high FXIII concentrations in tears might induce undesired angiogenesis of the cornea.

PB 1755 | A Role for Factor XIII in Clot Formation and Clot Contraction of Whole Blood

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Background: Coagulation factor XIIIa (FXIIIa) is a transglutaminase that stabilizes a clot via the formation of covalent bonds between fibrin strands and by crosslinking anti-fibrinolytic enzymes to the clot. FXIIIa does not induce visible changes in a fibrin clot as observed with scanning electron-microscopy (SEM). However, recently it was shown that clots formed from FXIII-deficient blood retained fewer red blood cells (RBCs) during contraction compared to control clots.

Aims: The aim of this study was to investigate the role of FXIIIa on clot formation and contraction in whole blood.

Methods: Citrated whole blood from healthy volunteers was clotted in the absence or presence of a transglutaminase inhibitor (T101). After contraction, the number of RBCs that were expelled from the clots and the clot mass was determined for each clot. Additionally, the contracted clots, formed in the absence or presence of T101, were visualized with SEM.

Results: Blood clots formed in the presence of T101 retained fewer RBCs during clot contraction and were significantly smaller than control clots. Electron-microscopy revealed that the contracted clots formed in the presence of T101 were encapsulated with a dense layer of fibrin. No RBCs were observable through this layer of fibrin, in contrast to control clots where RBCs were clearly visible. Additionally, only in control clots we observed fibrin branches on RBCs suggesting a direct interaction.

Conclusions: In the absence of FXIIIa, fewer RBCs were retained in a contracting clot. We hypothesize that with fewer RBCs to physically block further contraction, the clot contracts to a smaller size. The reduction of the clot volume and surface area may then lead to a concentration of fibrin fibers on the surface of the clot. This process then continues until the layer of fibrin reaches a density and pore size where RBCs cannot escape the clot anymore.

PB 1756 | Structural and Phylogenetic Analysis of Coagulation Factor XIII Explains its Uniqueness in the Transglutaminase Family

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Background: The coagulation factor XIII (FXIII) belongs to the Transglutaminase class of enzymes. While it shares a number of biochemical and biophysical properties with the other orthologues and paralogues of this family, it also possesses certain characteristics which make it a structurally and functionally unique enzyme in this family.

Aims: To use structural/sequence derived phylogenetic tools to trace the structural and functional evolution of FXIII.

Methods: Structural phylogeny was traced using reported PDB structures under the search term “Transglutaminase” (FXIIIA subunit) and “Sushi domain” (FXIIIB Subunit) in the SCOP database. For proteins/domains where no structure was available, they were modeled on the ITASSER server. Structural conservation measures were generated on

the Multiseq. Phylogeny based analysis was done based on maximum-likelihood on the MEGA6 platform and MrBayes 3.1 for nucleotide sequences. Phylogenetic trees for amino acid sequences were directly downloaded from TreeFam database. Phylogenetic measures like molecular clock, substitution rates etc. were evaluated on the MEGA6 platform for all trees and alignments.

Results: Approximately 526 sequences from 91 species relevant for A subunit and 230 sequences from 87 species for the B subunit with a taxonomic distribution limited to Metazoans were analyzed for sequence conservation. Around 60 PDB files relevant for the A subunit and 84 PDB files for the B subunit were analyzed for structural conservation.

Conclusions: The A and B subunits co-evolved simultaneously approximately 500 million years back in Coelacanths from TGM1 and CFH respectively. Characteristic structural features like activation peptide, dimeric/tetrameric interfaces, catalytic triad, calcium binding sites, surface recognition residues of A and B subunit are associated with residues that show inter/intra-clade variability (responsible for protein-protein interaction) and also highly conserved residues(functionally relevant).

PB 1757 | *In silico* Analysis of Extended Promoter Regions of *F13A1* and *F13B* Genes Indicates Putative Sites Might Underlie Regulatory Variation in Factor XIII Levels

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Background: Coagulation Factor XIII (FXIII) plasma levels show almost 3 fold variability (50-150%) in the general population. The variability can be attributed to genetic or non-genetic (age/sex) reasons. The 5´ untranslated region (UTR) of *F13A1* and *F13B* genes have not been elaborately investigated so far to detect its contribution to plasma FXIII level variability.

Aims: To *in silico* screen the 5´ UTR of *F13A1* and *F13B* genes in order to define putative regulatory genetic parameters governing FXIII levels.

Methods: The 5´ UTR of *F13A1* and *F13B* gene upstream of the transcription start site (till 5000 bp) was downloaded from ensemble database. The downloaded sequences were screened on the TRANSFAC platform to generate a list of putative regulatory transcription factor matrices corresponding to these regions. Similarly a list of polymorphisms and CpG sequences occurring in these regions were extracted by indicating the specific genomic locus on the dbSNP database. The data was combined and analyzed for relevant overlap.

Results: A total of 246 and 291 SNPs were located in the regions corresponding to *F13A1* and *F13B* genes respectively. Fifty and 44 CpG´s were found distributed across *F13A1* and *F13B* regions respectively. Fifty nine (130 binding sites) and 47 (143 binding sites) transcription factor (TF) matrices were predicted to bind in different parts of *F13A1* and *F13B* regions respectively. Amongst these TF matrices, 31 TF matrices were common to both genes. Fifty eight of the 130 *F13A1* and 32 of 143 *F13B* binding sites were located on reported variants many of them deletions and insertions. Twenty seven of *F13A1* and 24 of *F13B* CpG´s were also located on reported variants.

Conclusions: A high density of variability in this region is an indicator that it might be associated with the high level of variability in FXIII levels that is observed and reported from the general population. Common transcription factors between the two genes indicate regulatory crosstalk.

PB 1758 | Fibrinogen Caracas IX a Novel Fibrinogen Mutation: *FGA* g. 3057 C>T (p. 104 Arg>Cys) Impair Fibrinogen Secretion

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Background: Abnormal fibrinogens can be caused by clinically silent hereditary mutations. A new case was detected accidentally in an 11 year-old girl when routine pre-operative coagulation tests were performed for nasal turbinate surgery.

Aims: To characterize a new abnormal fibrinogen.

Methods: The fibrinogen genes FGA, FGG and FGB were sequenced using standard protocols. The kinetic of fibrin formation was followed by turbidity at 350 nm. Purified fibrinogen was degraded with plasmin and the degradation products analyzed by SDS/PAGE. The formation of fibrinogen-albumin complexes was analyzed by immunoblotting. Fibrin structure was observed in a Nikon Eclipse TE 2000-U laser microscope. The secretion of the variant protein was analyzed directly by reverse phase-electrospray time of flight-mass spectrometry (TOF-MS).

Results: DNA sequencing revealed a novel heterozygous g. 3057 C>T mutation in the *FGA* that causes a p. 104 Arg>Cys substitution, in the proband and her father. Interestingly, the father also had the Aα Thr312Ala fibrinogen polymorphism. Both patients were asymptomatic with a mild prolonged thrombin time in the proband (+2.5 s), and normal functional fibrinogen concentration. The proband´s plasma fibrinogen polymerization was almost normal, with a 12% decrease in the final turbidity. However, the father´s fibrin formation had a remarkable diminished slope and final turbidity (2.5x and 40%, respectively). Aα 104 Arg is located in the coiled-coil region of the molecule and is cleaved by plasmin. However, the father´s fibrinogen

plasmin degradation was normal. Although the exchanged Cys introduces an unpaired -SH, immunoblotting showed no fibrinogen-albumin complexes. Furthermore, the plasma clot structure observed by confocal microscopy appeared normal. TOF-MS revealed that only 4% of the circulating fibrinogen molecules contained the Aα 104Cys chain.

Conclusions: The mutation Aα 104 Arg>Cys is barely expressed in circulation and this could account for the patients´ clinical picture.

PB 1759 | Effect of Mutations in the Fibrinogen $\alpha R_{95}G_{96}D_{97}$ Sequence on Clot Structure and on the Interaction of Fibrinogen with Red Blood Cells

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Background: Recent data suggest a role for red blood cells (RBCs) in haemostasis and thrombosis, driven through interactions of RBCs with the clot. RBCs are shaped into polyhedrocytes in the clot, RBC retention in the clot is dependent on FXIII, and fibrinogen has been reported to bind to RBCs via $\alpha_V\beta_3$. However, the binding site for RBCs on fibrinogen is unknown.

Aims: The aim of this study is to investigate the role of the fibrinogen α-chain $R_{95}G_{96}D_{97}$ sequence, which is located in the coiled coil region, in clot structure and binding of fibrinogen to RBCs.

Methods: Three mutations in and near the RGD sequence were produced: R95Q, D97N and F98I, the latter was previously reported in a patient with recurrent miscarriage. These mutations were stably transfected into βγ-CHO cells, expressed in roller bottles and purified. The proteins were analysed by SDS-PAGE and CD-spectra for their basic structure/function compared to wild type. A plate binding assay was developed to study the binding between RBCs and fibrinogen. Turbidity and confocal microscopy was used to study the effect of the mutations on clot structure.

Results: SDS-PAGE analysis showed high integrity and purity of the proteins, but the mobility of the α-chains appeared affected, indicating differences in polypeptide folding for all mutations. CD spectra confirmed minor differences in α-helical content for each mutation. Turbidity analysis showed reduced maximum absorbency for each mutation compared to the wild type, indicative of thinner fibres. Confocal microscopy images showed thinner, shorter and denser fibres in all mutations to a different extent. RBC binding was not affected by any of the mutations.

Conclusions: Mutations in the fibrinogen $\alpha R_{95}G_{96}D_{97}$ sequence influence fibrin formation and clot structure but do not change RBC binding. Further studies are required to locate the RBC binding site on fibrinogen.

PB 1760 | Evidence for the Presence of Cross-beta Structures in Fibrin Clots

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Background: Amyloids are insoluble fibrillary assemblies that contain beta-sheets organized in a characteristic cross-beta structure. Preliminary data in literature suggests that fibrin contains similar cross-beta structures. However, these studies have not been experimentally described.

Aims: The aim of this study was to detect cross-beta structures in fibrin clots by using thioflavin T (ThT), a specific dye for amyloid-like structures.

Methods: Fibrin clots were prepared by incubating fibrinogen (3 mg/ml), ThT (25 μM) and bovine serum albumin (BSA, 0-60 mg/ml) with thrombin (1 NIH U/ml) and calcium (7.5 mM) to induce coagulation. The fluorescence of the cross-beta structure-bound ThT was measured with excitation at 425 nm and emission at 485 nm using a plate reader. Turbidity of the clots was measured at 405 nm.

Results: The purified fibrinogen and BSA preparations that were used in these experiments showed binding of ThT, suggesting that they contained some denatured protein. However, upon coagulation of fibrinogen ThT binding increased in time, in parallel with the turbidity of the clots. This indicated that clot formation was associated with the formation of cross-beta structures. In the absence of BSA the increase of ThT binding was small, but in the presence of 30 - 60 mg/ml BSA the increase was significant, indicating that BSA promoted the cross-beta structures.

Conclusions: Thioflavin T binding indicates that fibrin clot formation is associated with an increase in cross-beta structures. The promoting effect of albumin suggests that cross-beta structures are involved in the lateral aggregation of fibrin protofibrils.

PB 1761 | Prevalence of Mild Factor XIII Deficiency Phenotype: Preliminary Results of a WFH Sponsored CRGP Study

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Background: Individuals carrying the mild form of Factor XIII deficiency (FXIII) (FXIII levels in the range of 20-60%) are asymptomatic unless exposed to some kind of a trauma. This is the reason the accurate prevalence of this disorder is not known.

Aims: Under the WFH sponsored CRGP grant, we aim to screen a minimum of 1000 apparently healthy controls for their FXIII levels and their *F13A1* / *F13B* genotypes.

Methods: Peripheral blood samples are being collected from apparently healthy controls and separated into plasma and cellular component by centrifuging. Plasma samples will be evaluated for FXIII activity using photochemical and incorporation assays. The plasma samples are also being tested for FXIIIA and B subunit antigen levels as well as the $FXIIIA_2B_2$ hetero-teramer antigenic levels. Plasma samples are also being evaluated for alpha 2-antiplasmin incorporation assay and FXIIIa generation assay. In individuals detected for mild FXIII deficiency, the DNA is being extracted and sequenced to detect potential variants associated with this deficiency.

Results: At the time of submission of this abstract 360 apparently healthy controls had been collected. Amongst them 200 have been analyzed by FXIII photometric assay so far. They showed activity levels between 59.5-156%. One sample corresponded to mild FXIII deficiency level i.e. 59.5%. A total of 132 samples had also been analyzed by FXIIIa generation assay so far. The FXIII activation rates in These samples range between 26.3-87.0 (ΔRFU/Δt). The sample with mild FXIII deficiency showed an activation curve significantly different than the samples from the rest of the cohort.

Conclusions: Mild FXIII deficiency appears to have a prevalence of 1 in 200 from the currently tested cohort. FXIIIa generation assay could be useful in detecting mild FXIII deficiency. However, final conclusions can be made only after gene sequencing is done and collection as well screening is completed for all 1000 samples.

PB 1762 | Development of Fibrinogen Formulation with High Resistance to Thermal Stress

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Background: Fibrinogen concentrates are used for patients with fibrinogen deficiency to prevent bleeding episodes. Traditional formulation products are stored at 5 to 25℃, where albumin is used as a stabilizer. For distribution and patient's usability with guaranteed product quality, improved storage conditions have been required.

Aims: To ensure high-quality lyophilized fibrinogen concentrate, we developed stable formulation under harsh thermal stress.

Methods: A full factorial design was employed to evaluate effects of albumin and L-arginine on the thermal stability of fibrinogen formulation. Contents of albumin and L-arginine were tested from 0 to 500 mg/vial and 120 to 470 mg/vial, respectively. With the selected formulation, stability studies were conducted for 6 months at 40℃ and 7 days at 60℃, respectively and size exclusion chromatography was used to detect fibrinogen monomers. Pharmacokinetic (PK) studies were conducted in Sprague-Dawley rats (60 mg/kg, IV bolus injection) using Human Fibrinogen ELISA kit (AssayPro). JMP software (ver. 10.0; SAS Institute Inc., USA) was used for statistical analyses.

Results: L-arginine showed a significant effect on the thermal stability of fibrinogen formulation after 6 months at 40℃ (P=0.0406), but albumin did not (P=0.2233). Furthermore, the monomer contents were higher in the albumin-free formulation than the traditional albumin-containing formulation under the harsh thermal stress condition (60℃): for albumin-free formulation, 99.1% and 98.4% after 3 days and 7 days, respectively; for albumin-containing formulation, 93.5% after 3 days (7 day's sample did not dissolved). No significant differences were found in PK results between the albumin-free and containing formulations.

Conclusions: In conclusion, fibrinogen formulation with high resistance to thermal stress was obtained through stability studies (40 and 60℃) with varying contents of albumin and L-arginine. We will conduct new stability studies for long-term storage above 25℃.

PB 1763 | Cellular Factor XIII in Human Cornea

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Background: The presence of cellular FXIII (cFXIII), the dimer of the catalytic FXIII A subunit ($FXIII\text{-}A_2$) has been described earlier in platelets, monocytes and monocyte derived macrophages. More recently it has also been demonstrated in a few other cell types, like chondrocytes, osteoblasts, osteocytes, adipocytes. We detected FXIII subunits in tears, which raised the question if cFXIII is present in corneal cells.

Aims: The aim of the study was investigate the occurrence of cFXIII in cells of human cornea, and if such cells exist, to reveal their distribution in the cornea and to test if they actively synthesize the protein.

Methods: Human cornea specimens were obtained from cadavers or from surgical material obtained by enucleation. Tissue sections of cornea were investigated for the presence of FXIII-A and FXIII-B by immunofluorescence technique. Staining for FXIII-A was combined with immunostaining for CD34 antigen. The same double immunofluorescent stainings were also performed on isolated corneal keratocytes and evaluated by immunohistochemisty and by flow cytometry. Quantitation of FXIII-A in the corneal tissue was performed by Western blotting technique. FXIII-A mRNA was detected by RT-qPCR.

Results: In the cornea FXIII-A, but not FXIII-B was detected in stromal CD34+ keratocytes by immunfluorescence staining. Flow cytometry revealed that 84% of of isolated corneal cells were CD34+ keratocytes and two third of them were also stained for FXIII-A. The distribution of these cells in the corneal stroma was unequal; they were

particularly abundant in the subepithelial region. As opposed to the membrane localization of CD34, cFXIII was of cytoplasmic localization, occasionally it also appeared in the nucleus. 2.87 ng cFXIII/mg corneal protein was measured by quantitative Western blotting. The synthesis of cFXIII by these cells was confirmed by RT-qPCR.

Conclusions: cFXIII is present in keratocytes of human cornea and might be involved in the corneal wound healing process.

PB 1764 | Fibrin-associated ROTEM Variables and Thrombin Generation in Patients with Congenital Fibrinogen Defects

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Background: The clinical course of congenital hypo-/dysfibrinogenemia is unpredictable and cannot be evaluated by standard coagulation assays.

Aims: To better characterize a Finnish cohort of congenital hypo-/dysfibrinogenemia by genotyping and assessing thrombin generation (TG) and ROTEM.

Methods: Ten patients from 8 unrelated families (7 with homogeneous and 1 with mixed phenotypes) were studied, 3 with bleeding phenotype (BP), 3 with thrombotic phenotype (TP) and 4 asymptomatic with a familiar BP (ABP) in 1 and TP (ATP) in 3. Fibrinogen (Fg) activity (Clauss) and antigen, platelet functions by PFA-200 and Multiplate, and ROTEM in all, and TG by Calibrated Automated Thrombogram (CAT, 1 pM tissue factor, TF) in plasma of 7 patients were measured. Genotype was available in 6 patients from 6 families.

Results: According to Fg activity and antigen levels, 5 unrelated patients had hypo- (3 BP and 2 TP), and 2 unrelated (1 ABP, 1 ATP) and 3 related (1 TP, 2 ATP) patients had dysfibrinogenemia**.** 6 different causative mutations were identified, 4 (2 new variants) in the Fg gamma (2 BP, 2 ATP) and 2 in Fg alpha chain (1 BP, 1 TP). Overall platelet functions were not deficient. Fibrin polymerization capacity measured as clot firmness in FIBTEM was drastically reduced (maximum clot formation, MCF < 5 mm) in all patients with hypo-, but normal in patients with dysfibrinogenemia. MCF by FIBTEM correlated with Fg antigen, but not activity. Markedly prolonged CTs in FIBTEM were measured in 2/3 patients with BP. Baseline peak thrombin varied up to 7-fold between the patients (one hypofibrinogenemic patient with undetectable TG excluded) compared to 3-fold variation in 11 normal controls. No other TG differences were identified between the patients and controls.

Conclusions: MCF in FIBTEM detected hypo-, but not dysfibrinogenemia. TG varied widely, illustrating that TG could be affected by the abnormal fibrin structures. These findings should be confirmed in a larger patient cohort.

PB 1765 | Studies to Determine Novel Interacting Partners and Functional Roles for Factor XIII B Subunit

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Background: Coagulation Factor XIII B (FXIIIB) protein is present in plasma as a regulatory and/or protective subunit of FXIIIA. Unlike the A subunit for whom multiple interaction partners and several pleiotropic roles are known, B subunit so far is known to participate only in the hemostatic system. However, B subunit possesses sushi domains which are also frequent in complement proteins. This combined with the fact that the B subunit exists also in free form apart from being bound in a complex with the A subunit suggests that it might have other interacting partners and as yet unknown functions.

Aims: Investigating novel roles and partners of the free form of FXIIIB protein.

Methods: FXIIIB subunit was analyzed in 1) Content characterization of Fibrogammin was done to check putative interacting partners of FXIII 2) Using immobilized FXIIIB monoclonal antibodies, putative FXIIIB interacting partners were pulled down from FXIII deficient plasma and analyzed by Mass spectroscopy 3) Reconstitution experiments were performed to check the direct link of FXIIIB with complement activation by checking the rate of complement activation in the presence/absence of FXIIIB subunit.

Results: Fibrogammin P was found to be comprised of Complement factor H (CFH) and Alpha-2-macroglobulin apart from the coagulation $FXIIIA_2B_2$ heterotetramer. Mixing studies performed on CFH and FXIII deficient plasma and analyzed by FXIII generation assay suggests that both CFH/FXIIIB might additively control FXIIIA access to thrombin. However, no physiological role of CFH was observed in mixing studies analyzed for complement activation. Alpha-2-macroglobulin and C1q subunit were observed to co-immunoprecipitate with FXIIIB subunit.

Conclusions: C1q subunit/CFH from the complement system and Alpha-2-macroglobulin appear to have affinity for FXIIIB subunit/FXIIIB subunit epitopes. However, neither interaction seems to have strong physiological relevance although its relevance in disease like conditions cannot be ruled out.

PB 1766 | The Effect of Factor XIII Levels and Factor XIII B Subunit Polymorphisms on the Risk of Venous Thromboembolism

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Background: As FXIII plays an essential role in the protection of newly formed fibrin, its association with the risk of thrombotic diseases has been a subject of intensive research. However, the association of plasma FXIII level with the risk of venous thromboembolism (VTE) is still controversial and the effect of the recently discovered polymorphism in FXIII-B Intron K (F13B:c.1952+144 C>G) leading to a novel splice acceptor site and 25 new amino acids at the C-terminal end has not been investigated in this respect.

Aims: The association of FXIII levels and FXIII-B polymorphisms with the risk of VTE was explored in the study.

Methods: 227 VTE patients and 227 age and gender matched controls were enrolled in the study. There was at least three months between the acute thrombotic event and the investigation. FXIII activity, FXIII-A_2B_2 antigen, FXIII-B antigen levels and fibrinogen were measured and FXIII-B p.His95Arg and FXIII-B Intron K polymorphisms were determined.

Results: Adjusted FXIII activity and FXIII-A_2B_2 antigen levels were significantly higher in the VTE group than in controls. In contrast, FXIII-B levels were significantly lower in the control group. FXIII activity and FXIII-A_2B_2 antigen levels in the upper tertile as compared to the lowest tertile increased the risk of VTE (adjusted OR:1.61 CI:0.96-2.71 and OR: 2.19 CI:1.30-3.68, respectively). Elevated FXIII-B antigen level had a protective effect (adjusted OR:0.39 CI:0.22-0.70). The Arg95 allele was associated with moderately elevated FXIII activity, FXIII-A_2B_2 and FXIII-B antigen levels in the control but not in the VTE group. FXIII-B Intron K G allele significantly lowered the FXIII activity, FXIII-A_2B_2 and FXIII-B antigen levels in both groups. FXIII-B polymorphisms did not influence the risk of VTE.

Conclusions: Elevated FXIII levels could be considered as risk factor of VTE. The FXIII-B polymorphisms are not associated with VTE risk.

PB 1767 | Novel Immunoassay Reagent for Fibrinogen without Cross-reactivity to FgDP and XDP in Plasma

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Background: A new mouse monoclonal antibody (clone G3D4) specific to the αC domain of fibrinogen was prepared.

Aims: A novel immunolatex reagent prepared by loading this antibody onto latex particles was used to develop an immunoassay reagent for fibrinogen without interference with crosslink fibrin (XDP) and fibrinogen degradation products (FgDP) in plasma.

Methods: After mixing 140 µL of R1 reagent containing Tris buffer as a main component with 4 µL of human citric acid plasma, diluted 40-fold with a fibrinogen standard substance or physiological saline, using an automatic clinical chemistry analyzer BIOLIS 50i (TOKYO BOEKI MEDYSIS INC.), the mixture was heated at 37°C for 4 minutes. To this mixture, 50 µL of R2 reagent prepared by physically loading a mouse monoclonal IgG 1 antibody specific to the αC domain of fibrinogen onto polystyrene latex particles was added to measure absorbance (546 nm) at 30-120 seconds. Using the calibration curve of a standard substance, plasma fibrinogen levels were calculated.

Results: The immunoassay reagent for plasma fibrinogen showed excellent simultaneous reproducibility (n = 10) within the measurement range of 20-900 mg/mL: CV = 1.85% at 50 mg/dL, CV = 0.76% at 200 mg/dL, and CV = 0.95% at 800 mg/dL. In addition, a linearity was demonstrated up to about 900 mg/dL. Furthermore, no effect of coexisting substances was observed.

Regarding the reactivity of the reagent to FgDP and XDP prepared with plasmin over time, high reactivity was observed for intact fibrinogen (340-280 KD) and DesAABB fibri, while no reactivity was noted for other FgDP (X, Y, D, and E) or XDP.

Conclusions: The kit provides sensitive immunoassay for plasma fibrinogen in a wide measurement range without being affected by FgDP, XDP, and antithrombin preparations in plasma.

PB 1768 | Galactosemia Presenting with Afibrinogenemia

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Background: Fibrin is associated with cloth formation, thrombocyte aggregation and transport of factor VIII. Fibrinogen levels< 50 mg/dL may be associated with disseminated intravascular coagulation but levels < 10 mg/dL are usually associated with congenital deficiencies. Congenital fibrinogen deficiency is a rare genetic disorder usually presenting at neonatal period with umbilical bleeding. Cephal hematoma, melena, hematuria, hemarthrose, intracranial bleeding, spontaneous spleeen ruture may also be the presenting sign.

Aims: Here we report a boy presented with afibrinogenemia and diagnosed with galactosemia.

Methods: Our patients was born as the second child of a healthy mother and father who are first degree cousins. He was referred to our hospital when he was 10 days old because of cholestasis. Physical examination revealed hepatomegaly and leukocoria. Eye examination reported that he has cataract. Laboratory findings showed

ALT: 46U/L, AST: 69U/L ,total bilirubin: 16.9 mg/dl, direct bilirubin: 5.3 mg/dl, hemostatic parameters showed aPTT: 106 sec, INR: 4.5, D-Dimer>40 mcgr/ml, thrombin time: 15 sec. Antithrombin III activity 37% and fibrinogen level was found to be < 10 mg/dl so it was first thought that he had afibrinogenemia. He did not have bleeding, disseminated intravascular coagulation, thrombocytopenia or microangiopathic hemolitic finding in his blood smear. When he was evaluated for the etiology of cholestasis a homozygous mutation (Q118R) in the galactose 1 fosfate uridil transferase (GALT) gene was detected so he was diagnosis as galactosemia.

Results: Lactose free diet started and after that his fibrinogen levels increased and observed in normal limits during the follow-ups.

Conclusions: Coagulopathy may be associated with 4-9% of the patients with galactosemia. When GALT is defective afibrinogenemia may be caused by the liver damage. Our patient seems to had pseudoafibrinogenemia associated with galactosemia.

PB 1769 | Plasma Tromboelastometry (ROTEM) Could be a Complement in Study Fibrinogen Disorders

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Background: While the first dysfibrinogenemia mutation was identified as early as 1968 , still today , it may be difficult to diagnosis even in specialized laboratories.

Dysfibrinogenemia could be a bleeding or thrombotic disorder, or both.

Aims: Prove the possibility of whether functional Fibrinogen assay by Rotem (FIBTEM) in plasma can be a method for establishing the presence of dysfunctional fibrinogen.

Methods: Fibrinogen in plasma of 20 healthy volunteers, was measured, as Control Group using Clauss and Rotem Fibtem .Correlation between Clauss values and Maximum Clot Firmness (MCF) Fibtem was established.

Subsequently, the same correlation was performed in 17 individuals with confirmed or suspected dysfibrinogenemia. In both groups, correlation with Pearson Coefficient was made. Also ratio Fibrinogen(Clauss)/MCF were calculated in both group and comparisons with a T Test for independent samples were made. In all cases a significant p-value < 0.05 was considered.

Results: The control group showed a good correlation between Clauss and MCF values ($r = 0.693$; $p < 0.001$).

The analysis of the Fibrinogen / MCF ratio showed significant differences: Control: 11.75 (95% CI: 10.75-12.75) vs Pathological: 16.42 (95% CI: 12.70-20.14) ($p < 0.03$).

Conclusions: Perhaps combination of two functional assays like Clauss an Fibtem could be a complementary method to suspect dysfibrinogenemia. Allowing also to visualize the possible prothrombotic or hemorrhagic behavior of the patient.

PB 1770 | A Fibrinogen Activity Method on Photo-optical Coagulation Analyzers without Interference from Hydroxyethyl Starch

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Background: It has been reported that the use of photo-optical fibrinogen activity methods on plasmas diluted with hydroxyethyl starch (HES) can lead to clinically relevant overestimation of fibrinogen concentrations.

Aims: The aim of this study was to investigate the effect of HES on Clauss fibrinogen measurements with samples diluted with HES on a photo-optical instrument.

Methods: Two citrated plasma pools (Pool 1: a pool of 48 donors; Pool 2: cryoprecipitated to achieve a low fibrinogen level) were diluted in a 1:1 ratio with 0.9% NaCl and HESPAN (6% hetastarch in 0.9% NaCl injection, B. Braun, Bethlehem, PA, USA), respectively, generating two levels of HESPAN (0% and 50%) for each specimen. The two dilutions were used to prepare different HESPAN levels (0, 12.5, 25, 37.5 and 50%). Fibrinogen activity was tested on an Instrumentation Laboratory (IL) photo-optical based ACL TOP 700 analyzer using IL´s Q.F.A. Thrombin kit, and the average results were compared to the respective baseline mean.

Results: The two citrated plasma pools diluted with 50% NaCl had corresponding baseline fibrinogen levels at 166 mg/dL and 79 mg/dL, respectively.

Pool 1 had fibrinogen activity within the Q.F.A. test range. The Q.F.A. test reported no more than a 2% increase (within 3SD of the baseline) in the fibrinogen level when the sample was diluted with HESPAN.

HESPAN, %	0.0	12.5	25.0	37.5	50.0
Fibrinogen, mg/dL	166	169	160	170	170
SD	2.2	7.2	2.8	4.1	3.9
%CV	1.3	4.2	1.8	2.4	2.3
%Baseline	100	101	96	102	102

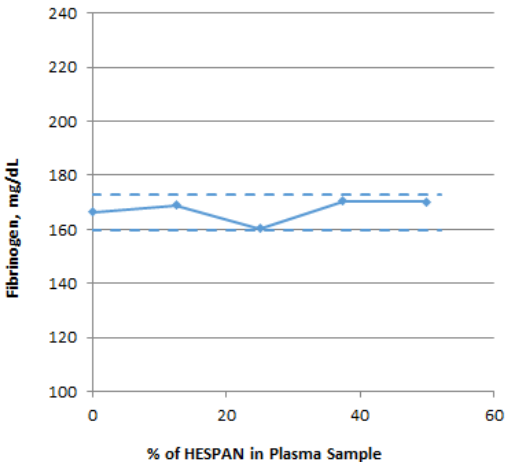

FIGURE 1 Fibrinogen measurement of HES diluted samples on ACL TOP 700: results from the Q.F.A. test with dashed lines representing the 3SD limits

HESPAN, %	0.0	12.5	25.0	37.5	50.0
Fibrinogen, mg/dL	79	78	82	82	86
SD	6.1	2.4	1.6	4.3	1.8
%CV	7.8	3.1	1.9	5.3	2.1
%Baseline	100	98	103	104	109

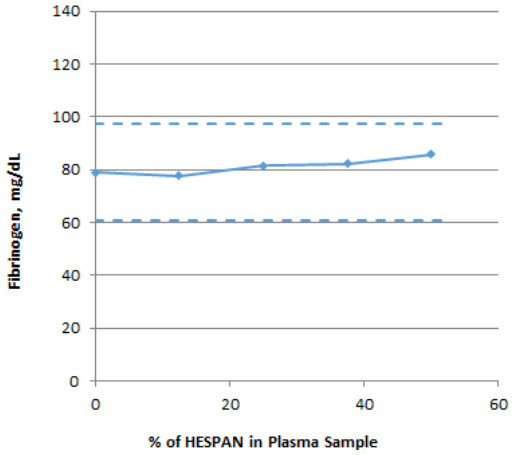

FIGURE 2 Fibrinogen measurement of HES diluted samples on ACL TOP 700: results from the Q.F.A. Low test with dashed lines representing the 3SD limits

Pool 2 had fibrinogen activity within the Q.F.A. Low test range. The Q.F.A. Low test reported no more than a 9% increase (within 3SD of the baseline) in the fibrinogen level when diluted with HESPAN.

Conclusions: The studies demonstrate that when fibrinogen measurements are performed on IL´s photo-optical ACL TOP Family analyzer using the Q.F.A. Thrombin kit, no statistically or clinically significant differences are observed in fibrinogen activity on plasma diluted with HES.

Hemorrhagic Disorders, Hemophilia

HEMORRHAGIC DISORDERS, HEMOPHILIA

PB 199 | Evaluation of the Ankle Brachial Index in Persons with Hemophilia: Unexpected Findings

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Background: The prevalence of cardiovascular disease (CVD) is rising in persons with hemophilia (PWH). Previous studies of atherosclerosis in PWH have been limited to carotid and coronary arteries.

Aims: We analyzed the prevalence of subclinical peripheral artery occlusive disease (PAOD) in PWH by measuring the ankle brachial index (ABI), an established method to diagnose PAOD.

Methods: In consecutive PWH visiting our hemophilia treatment center, systolic blood pressure (SBP) was measured at the brachial and ankle arteries (dorsalis pedis and tibialis posterior) in supine position after a few minutes rest, using Doppler ultrasound. The ABI was calculated as the ratio of the highest SBP at the ankle to the highest SBP at the arm. We evaluated three groups: low (< 0.9), normal (0.9-1.3) and high ABI (≥1.3). Creatinine and HbA1c were measured; medical history was reviewed.

Results: 65 PWH (median age 52 years [IQR 42-64], 39% severe hemophilia, A:B 48:17) were enrolled. Nine (14%) patients had a history of CVD, 7 (11%) diabetes and 15 (23%) hypertension. Three (5%) patients had an estimated glomerular filtration rate < 60 ml/min. The median ABI was 1.26 (IQR 1.19-1.33). Only one patient had an ABI< 0.9, indicating PAOD. Notably, 32 (49%) patients had an high ABI in at least one leg. No significant differences were found between patients with an ABI ≥1.3 and patients with an ABI< 1.3 in age, HbA1c, renal function, hypertension or severity of hemophilia.

Conclusions: We found a very low prevalence of subclinical PAOD in PWH. However, we found a high prevalence of a high ABI, suggesting calcification of the medial layer of the peripheral arteries rather than thickening of the intimal layer. Medial arterial calcification (MAC) can lead to arterial stiffness and thereby cause hypertension. Indeed, literature reports an increased prevalence of hypertension in PWH. The hypothesis that persons with hemophilia have increased MAC needs further investigation.

PB 200 | A Single Center Cross-sectional Study of the Age Related Effects of Intensive Prophylactic Therapy on Annual Bleed Rate and Joint Arthropathy in 338 Patients with Severe Haemophilia A

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Background: In severe Haemophilia A current prophylactic regimens, although very effective, do not completely prevent joint disease in a long-term perspective. Absence or presence and degree of joint arthropathy can be regarded as a cumulative measure of the qualitiy of prophylactic treatment during life-time.

Aims: The aim of this study is to present age dependent data on FVIII concentrate consumption, total and joint annual bleed rate (ABR) and degree of joint arthropathy.

Methods: A cross-sectional study was performed comprising 330 patients with severe Haemophilia A that were treated in 2010. Patients were analysed in 6 groups according to age in -6 (G1, n=35), 7-12 (G2, n=34),13-18 (G3, n=35), 19-40 (G4, n=108), 41-60 (G5, n=108) and > 60 (G6, n=18) years of age. Data were obtained on concentrate consumption (U/kg/year), total and joint ABR, and joint status (Gilbert and Ptterson scores). Joint bleeds were verified by a phone interview of the physician.

Results: Median annual concentrate consumption was 4898 iIU/kg BW/year and within the age groups G1-G6 10909, 8260, 4228, 3818, 4978 and 4978 IU/kg B/year, respectively.Total ABRs was 2.8 and within the age groups G1-G6 5.4, 2.9, 2.8, 2.0, 2.7, 3.5 and 2.8. Joint ABR increased continuously from 0.2 in the youngest age group to 0.5 in the oldest age group. A Non-pathologic Petterson scores in G1-G6 were found in 100%, 91%, 65%, 15%, 2% and 0% and

non-pathological Gilbert scores in 82%, 90%,72%, 32%, 1% and 0% of the patients, respectively.

Conclusions: Most patients up to an age of 40 years (G1-G4) have received a life-long intensive prophylactic regimen. This treatment results in 1 joint bleed every 2-5 years depending on the patient´s age. Despite this intensive treatment the majority of the patients developed joint artropathy when reaching an age of 19-40 years. Thus even intensive prophylactic regimens do not prevent joint disease in a life time perspective.

PB 201 | Patient, Regional and Center-level Variation in Utilization of Arthroplasty and Arthrodesis Interventions in Persons with Hemophilia: An Analysis of the American Thrombosis and Hemostasis Network (ATHN) Dataset

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Background: Loss of joint function and pain due to arthropathy are the most prevalent long-term morbidities associated with hemophilia A and B (HA and HB). Severe, end-stage arthropathy can be treated with interventions such as total joint arthroplasty and arthrodesis. Data describing utilization of these procedures in US hemophilia treatment centers (HTCs) are lacking.

Aims: To determine the impact of patient-level factors, HTC size and geographic region on utilization of arthroplasty and arthrodesis in individuals with hemophilia A or B.

Methods: We included male enrollees with HA or HB in the ATHN Dataset as of 6/30/2016. We developed a negative binomial multivariable regression model to estimate the relative contributions of independent variables to the lifetime prevalence of arthroplasty or arthrodesis.

Results: Data from 12,324 persons with hemophilia (PWH) were included, of whom 532 (4.3%) had a history of arthroplasty and/or arthrodesis. Older age, increasing hemophilia severity, HIV, and HCV increased the odds of having had either procedure (Table 1). Surgery was more common in persons with HA compared to HB, as well as in those currently using clotting factor prophylaxis. Race/ethnicity and history of inhibitor did not impact the outcome. The likelihood of having arthroplasty or arthrodesis varied significantly among the HTC regions, and being at a large (>150 patients) or medium (51-150 patients) HTC increased the odds of surgery compared to small (≤50 patients) HTCs (Table 2).

TABLE 1 Impact of patient-level variables on the likelihood of subjects having a history of at least one arthroplasty or arthrodesis procedure

Variable	Odds Ratio	95% Confidence Interval	P-Value
Age (each additional 10-year increment)	2.16	2.00-2.33	<0.001
Hemophilia A vs. B	1.46	1.12-1.90	0.005
Hemophilia Severity			
Severe vs. Mild	7.10	4.97-10.13	<0.001
Moderate vs. Mild	2.23	1.55-3.20	<0.001
HIV	1.44	1.11-1.88	0.007
HCV	1.82	1.46-2.27	<0.001
Prophylaxis- current use	1.77	1.34-2.33	<0.001

TABLE 2 Impact of geographic region and HTC size on the likelihood of subjects having at least one arthroplasty or arthrodesis procedure

Variable	Odds Ratio	95% Confidence Interval	P-Value
Region (compared to Western Region)			
New England	2.58	1.56-4.26	<0.001
Southeast	3.22	1.98-5.25	<0.001
Great Lakes	3.42	2.12-5.52	<0.001
Northern	2.98	1.77-4.99	<0.001
Mountain	3.98	2.36-6.71	<0.001
HTC Size			
Large vs. Small	1.77	1.09-2.87	0.020
Medium vs. Small	1.86	1.16-2.98	0.009

Conclusions: Use of orthopedic interventions for end-stage arthropathy is driven in part by patient and disease characteristics, but also by HTC size and geographic region. These findings suggest that factors beyond patients' joint status affect the decision of whether to pursue these orthopedic surgical procedures to treat hemophilic arthropathy. Further investigation of the availability of orthopedic surgeons, access to hospitals capable of surgical management of PWH, and differences in HTC regional practice patterns is warranted.

PB 202 | New Tools for the Optimization of the Primary and Secondary Prophylaxis in Pediatric Patients with Severe Hemophilia: Ultrasound Thromboelastography (UT) and Joint Echotomography

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Background: Hemophilia A and hemophilia B are hereditary hemorrhagic diseases, characterized by a partial or total deficiency of factor VIII or IX. Patients with severe haemophilia are at risk of spontaneous bleeding. Joint damage is the major complication of hemophilia, needing replacement therapy to prevent morbidity and mortality.

Aims: Addressing critical hemorrhages implies a correct interpretation of the bleeding and its consequent therapeutic approach. Therefore carrying out a dynamic coagulation monitoring aiming at an overall assessment within a short running time is crucial to assess, the risk of bleeding and provide information on clot stability. The purpose of our study is to provide a complementary tool to the laboratory tests, meanwhile demonstrating the usefulness of prophylaxis optimized by means of ultrasound thromboelastography (UT), muscle-joint ultrasonography and thrombin generation assessment.

Methods: 10 patients with severe and moderate haemophilia A receiving FVIII concentrates in the secondary prophylaxis regimen were evaluated. Factor VIII PK, UT, joint ultrasound, and monitoring of clinical response as a function of the number of monthly bleeding were analysed. Factor VIII kinetic was performed before infusion, at time 0, after 2 h and 24h. UT was performed in three stages, in line with the kinetics. Joint ultrasound was performed in supine and prone position, transverse and longitudinal sections.

Results: 40% of patients receiving prophylaxis with 1000/1500 IU of rFVIII showed sonographic signs of joint damage and more than half of patients had alterations of the parameters analysed. Table 1 shows the results.

TABLE 1

	T0	T2h	T24h
FVIII	2.71 +/- 1.86	44.26 +/- 15.6	8.61 +/- 15.6
R	40.03 +/- 29.7	18.04 +/- 18.25	33.66 +/- 10.6
K	10.16 +/- 4.7	4.62 +/- 3.1	7.24 +/- 3.5
MA	51.97 +/- 6.3	57.62 +/- 9.3	52.23 +/- 7.9
Alpha-angle	36.27 +/- 16	56.8 +/- 31.9	48.8 +/- 6

Conclusions: Customization of therapy and its management based on UT represents an innovative way to deal with patients with severe and moderate hemophilia A. This tool has to be considered complementary to routine laboratory tests and allows a good representation of in vivo clotting mechanisms.

PB 203 | Responsiveness and Construct Validity of Goal Attainment Scaling for Hemophilia (GAS-Hēm): A Novel, Personalised, Patient-reported Outcome for Haemophilia

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Background: People with haemophilia have complex medical issues that require a multidimensional assessment, including the development of patient-centred treatment goals. GAS-Hēm is a novel, personalised outcome measure developed to establish and measure progress towards individualised goals.

Aims: To establish the construct validity and responsiveness (sensitivity to change) of the GAS-Hēm in adult and paediatric patients with moderate or severe haemophilia in a real-world clinical setting.

Methods: In a 12-week study of patients aged 5-65 years with haemophilia A or B on prophylaxis recruited from four North American centres, we evaluated the construct validity of GAS-Hēm by assessing the correlation at baseline and study end (12 weeks) of GAS-Hēm scores (range 0-100) with widely used measures: the SF-36 Physical Health Component Score (SF-36 PCS), SF-36 Mental Health Component Score (SF-36 MCS), PedsQL and annualised bleed rate (ABR). Responsiveness of GAS-Hēm was calculated using the standardised response mean (SRM; >0.2 corresponds to small, >0.5 to moderate, and >0.8 to large effect).

Results: The 42 patients (5-12 years, n=9; 13-18 years, n=9; and 19-65 years, n=24) set 63 goals. Mean subject- and clinician-scored GAS-Hēm scores at baseline (n=42), 6 weeks (n=40), and 12 weeks (n=41) are shown (Table). Correlations of total subject-scored GAS-Hēm scores with ABR, SF-36 PCS, SF-36 MCS, and PedsQL, respectively, were: 0.07, 0.19, 0.07 and -0.06 at baseline; and -0.06, 0.22, 0.00 and -0.16 at 12 weeks. The SRMs at 12 weeks for adults were: GAS-Hēm 1.25, SF-36 PCS 0.16, SF-36 MCS 0.18; and for paediatric and adolescents combined were: GAS-Hēm 1.21 and PedsQL 0.58.

TABLE 1 Mean GAS-Hēm Scores Stratified by Age Group

GAS-Hēm Version	Age Stratum	Baseline	Interim Follow-up (6 weeks)	End Point (12 weeks)
Subject-scored	Paediatric	39.2	43.9	52.0
	Adolescent	39.5	44.4	53.6
	Adult	38.4	49.1	53.0
	TOTAL	38.8	47.2	52.9
Clinician-scored	Paediatric	39.2	46.3	53.4
	Adolescent	39.5	46.7	55.8
	Adult	38.4	50.3	54.8
	TOTAL	38.8	48.8	54.8

Conclusions: GAS-Hēm was highly responsive in adult and paediatric populations. However, correlations between GAS-Hēm and standard outcome measures were small. Taken together, these results suggest that GAS-Hēm is tapping constructs not captured by ABR or QoL tools. The individualised goal setting facilitated by GAS-Hēm may account for these findings.

PB 204 | Compartmental High Resolution 3D Imaging by XtremeCT Reveals Low Bone Mineral Density in Hemophilia Patients is Attributed to Loss of Trabecular Bone

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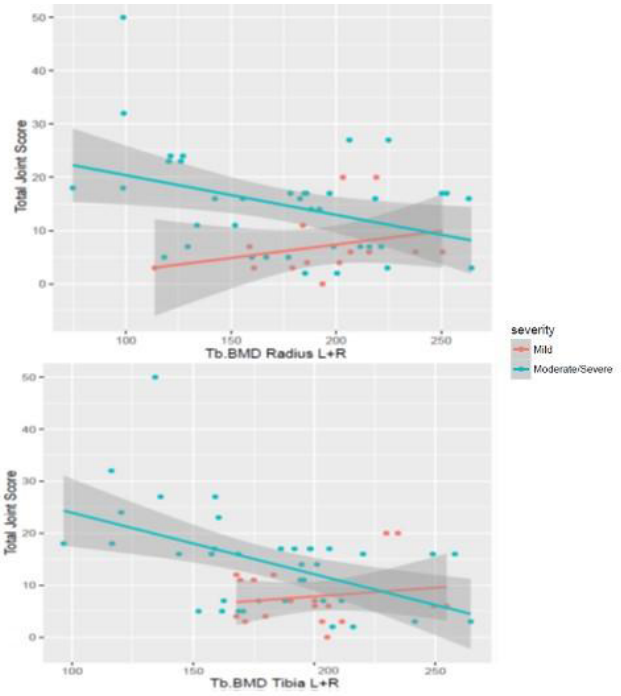
Background: Low bone mineral density (BMD) in persons with hemophilia (PWH) is a recognized concern. The contribution of arthropathy and hemophilia severity to localized and systemic BMD loss is not known.

Aims: To determine the compartmental (cortical and trabecular bone) contribution to low BMD in severe/moderate (S/M) vs. mild (Mi) PWH.

Methods: Cortical and trabecular BMD at bilateral radius (rad) and tibia (tib), was measured in S/M and Mi PWH by XtremeCT (high resolution 3D bone imaging) and compared to normative population data. BMD by DXA, bone turnover markers (BTMs) and hemophilia joint scores were measured in the PWH cohorts.

Results: Comparison of baseline characteristics and BMD results are in table 1. Total joint score was significantly higher in S/M group. XtremeCT data was available on 25 S/M and 18 Mi PWH. Trabecular BMD (Tb.BMD) at both rad and tib were significantly lower in S/M vs. Mi PWH. 64.4% of S/M (vs. 50% Mi) had total BMD (Tt.BMD) < 50th percentile of normative controls with 82.6% of S/M (vs. 60.8% Mi) having Tb.BMD < 50th percentile. Lower Tb.BMD at both rad and tib significantly correlated with worse (higher) total joint scores (Figure 1) in S/M, but there was no correlation of limb-specific joint scores with respective limb-specific BMD measures. More S/M PWH had high BTMs (CTX or NTX) compared to Mi (35 vs 20%) and NTX was significantly higher in S/M PWH (94.5 vs. 70.8, p-value=0.03).

Conclusions: S/M vs. Mi PWH have significantly lower Tb.BMD and correlates with higher total joint scores. Proximity/severity of arthropathy of a particular limb, does not seem to contribute to limb-specific BMD loss. A large proportion of S/M PWH have abnormally low Tt.BMD and Tb.BMD percentiles compared to normative, but low Tb.BMD percentiles in Mi PWH suggests adverse BMD changes are still present. Along with greater proportion of S/M PWH with abnormal BTMs, this suggests that BMD loss is due to a systemic rather than localized process with majority of loss occurring in the trabecular compartment.



Tb.BMD = trabecular BMD, measured in mgHA/cm3. L+R = left and right. Total joint score using measured by World Federation of Hemophilia joint score. Pearson correlation total joint score to Tb.BMD tibia (r= -0.487, p = 0.02). Pearson correlation total joint score to Tb.BMD radius (r= -0.377, p=0.04).

FIGURE 1 Total joint score vs. trabecular BMD, tibia and radius

TABLE 1 Baseline characteristics and BMD results of severe/moderate vs. mild patients

Clinical characteristic	Severe/moderate (n=57)	Mild (n=48)	p-value
Age (years), mean (range)	41.3 (19-76)	48.6 (19-83)	0.03
Hemophilia A/B, n (%)	43(75.5)/14(24.5)	39(81.3)/9(18.7)	n/a
Total joint score, mean (range)	16.7 (2-50)	5.0 (0-20)	<0.001
DXA BMD L-spine T/Z-score, median (IQR)	-1.1 (-1.8, -0.4)	-0.6 (-0.6, 0.2)	0.14
DXA BMD Femoral neck T/Z-score, median (IQR)	-0.8 (-1.6, -0.2)	-0.3 (-1.1, 2.6)	0.05
XtremeCT BMD results (mgHA/cm3)	Severe/moderate (n=25)	Mild (n=18)	p-value
Radius Tb.BMD, median (IQR)	184.9 (139.2, 214.7)	203.2 (184.7, 219.4	0.016
Tibia Tb.BMD, median (IQR)	177.0 (159.2, 202.1)	200.9 (178.6, 206.0)	0.020

PB 205 | No Association between HFE and HMOX1 Polymorphisms, Important in Iron and Heme Handling, and the Severity of Haemophilic Arthropathy

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Background: Marked heterogeneity in joint damage exists among haemophilia patients, and potentially genetic differences affecting iron handling impact blood-induced joint damage. Iron levels are regulated by the HFE gene and heme degradation is influenced by polymorphisms in the $(GT)_n$-repeat length in the HMOX1 gene.

Aims: Our objective is to evaluate whether the severity of haemophilic arthropathy is associated with genetic differences in iron handling.

Methods: Patients with severe (n=211) or moderate (n=41) haemophilia A or B were included. The presence of an HFE gene mutation (C282Y and H63D) and the $(GT)_n$-repeat length in the HMOX1 promoter region were analysed via PCR. In serum samples the iron status was assessed. Joint damage was quantified on X-rays using the Pettersson score. By linear regression analysis the association of the HFE mutation (present/absent) and HMOX1 polymorphism (long (≥25) repeat/short repeat) with the Pettersson score was assessed. Analyses were adjusted for haemophilia severity, presence of inhibitors, annual joint bleeding rate (AJBR), age at evaluation, age at entrance of the Van Creveldkliniek, and birth cohort.

Results: 252 patients were evaluated at a median age of 44y (range 18-79y). In severe haemophilia the median AJBR and Pettersson score were higher than in moderate haemophilia (AJBR 2.3 (IQR 1.0-4.6) vs 0.5 (IQR 0.1-2.2); Pettersson 22 (IQR 5-44) vs 4 (IQR 1-13)). In 95 patients an HFE mutation was detected and their levels of iron and the transferrin saturation were significantly increased (both $p < 0.05$). Patients with heterozygosity for a long/short $(GT)_n$-repeat length more frequently suffered form haemophilia A compared to homozygous patients. Neither presence of an HFE mutation, nor a long $(GT)_n$-repeat length was associated with an increase in the Pettersson score.

Conclusions: This study rejects the hypothesis that carriership of an HFE mutation or a long $(GT)_n$-repeat is associated with an increase in haemophilic arthropathy.

TABLE 1 Linear regression analysis for determinants of Pettersson score

Predictor	Beta	95% CI	P-value
Age at evaluation	0.43	0.20 - 0.65	0.000
Clotting factor activity	-18.2	-23.3 - -13.0	0.000
Presence of inhibitors	7.8	2.9 - 12.7	0.002
Annualized joint bleeding rate	1.3	0.8 - 1.8	0.000
Birth cohort	-8.4	-12.1 - -4.5	0.000
Age at entrance of the Van Creveldkliniek	7.6	3.5 - 11.8	0.000
HFE mutation	1.4	-2.3 - 5.2	0.446
HMOX1 polymorphism	-0.9	-3.8 - 2.0	0.536

PB 206 | Factors Associated with Perception of Functional Abilities in US Adults with Hemophilia Beyond Joint Status: Analysis of the Pain, Functional Impairment, and Quality of Life (P-FiQ) Study

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Background: P-FiQ enrolled US adults with hemophilia and a history of joint bleeding or pain. Participants completed patient-reported outcome instruments to assess quality of life. Functional impairment, as measured by Hemophilia Activities List (HAL), was associated with lack of college education, unemployment, older age, history of joint procedures, viral disease, comorbidities, and severe hemophilia.

Aims: This post hoc analysis assessed association between demographic and clinical characteristics independent of joint status (Hemophilia Joint Health Score [HJHS]) and HAL.

Methods: The association of HAL with HJHS and other covariates was examined using simple regression models and by a multiple regression model where HJHS overall score, age, hemophilia severity, and treatment were included with other covariates having bivariate correlations with HAL scores ($\alpha < 0.05$) using forward selection.

Results: Of 381 adults, physiotherapist-completed HJHS was available for 240 respondents (median age 32 years). Poor HJHS overall score was found to have strong association with poor HAL overall score, in both simple and multiple regression models. The overall R-squared value of 0.70 for the model with multiple predictors was indicative of high combined predictive power of HJHS and several covariates including older age, more severe hemophilia (with inhibitor patients as most severe), non-employed status, higher BPI pain severity, and higher EQ-5D-5L pain/discomfort score **(Table 1)**. EQ-5D-5L anxiety/depression and use of anxiolytics, antidepressants, and opiates were significant in bivariate but not multiple regression.

TABLE 1 Multiple Regression Models - HAL Overall Score

	Hemophilia Activities List (n=177; R2=0.700)			
Characteristic	ß Estimate*	Lower 95% CI	Upper 95% CI	P Value
HJHS overall score (10-point change)	-2.64	-3.82	-1.46	<0.0001
Age (5-year change)	-1.31	-2.12	-0.51	0.0016
Hemophilia severity				<0.0001
Mild (reference)				
Moderate	-8.35	-15.40	-1.29	0.0207
Severe	-8.28	-14.59	-1.98	0.0104
Inhibitor	-22.39	-31.00	-13.79	<0.0001

TABLE 1 Multiple Regression Models—HAL Overall Score (continued)

Characteristic	ß Estimate*	Lower 95% CI	Upper 95% CI	P Value
Employed	7.18	2.22	12.13	0.0048
BPI pain severity	-2.75	-4.14	-1.36	0.0001
EQ-5D-5L pain/ discomfort				0.0102
No problem (reference)				
Slight problem	-5.30	-10.15	-0.46	0.0322
Moderate problem	-9.06	-15.89	-2.24	0.0096
Severe problem	-16.80	-26.59	-7.00	0.0009
Extreme problem	-24.97	-46.80	-3.14	0.0252

BPI, Brief Pain Inventory v2 Short Form; HAL, Hemophilia Activities List; HJHS, Hemophilia Joint Health Score. * ß estimate measures change in outcome per change in covariate.

Conclusions: Beyond joint status, demographic factors such as older age and non-employment and clinical factors including inhibitor status and pain severity were associated with functional abilities. Variation in functional capacity was explained well (70%) using a combination of observed measures (HJHS joint status) along with patient-reported pain.

PB 207 | Long-term Treatment with Human-cl rhFVIII in Previously Treated Children with Severe Haemophilia A

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Background: Human-cl rhFVIII is a 4th generation recombinant FVIII produced in a human cell line without chemical modification or protein fusion. It is approved in Europe, USA and elsewhere for treatment and prophylaxis of bleeding in patients with haemophilia A.

Aims: The aim of the study was to investigate the safety and efficacy of long-term prophylactic treatment with Human-cl rhFVIII in previously treated children with severe haemophilia A.

Methods: This study (GENA-13) enrolled children who had completed the 6-month predecessor study GENA-03. Patients continued prophylaxis with injections every other day or 3 times per week. Inhibitor tests (Bethesda assay, Nijmegen modification) were performed at study start, then every 3 months and at study completion. Adverse events were recorded throughout the study. The study was approved by the local ethics committees and all patients/legal guardian(s) gave written informed consent.

Results: The study enrolled 49 patients from 10 centers across 6 European countries. Their median (range) age at study start was 6.0 (3.0-13.0) years. They received a total of 27.5 million International Units (IU) and 20,518 injections of Human-cl rhFVIII over a median (range) period of 30.1 (9.6-53.2) months. The mean (± SD) dose per prophylactic injection was 38.6 ± 6.7 IU/kg. The spontaneous annualized bleeding rate (ABR) was lower than in the same patients in GENA-03 (Table 1).

TABLE 1 ABRs* estimated by negative binomial regression (95% CIs) in GENA-13 and GENA-03 (n=49)

ABR	GENA-03	GENA-13
Total ABR	3.54 (2.42-5.17)	2.88 (1.86-4.46)
Traumatic ABR	1.88 (1.16-3.06)	1.76 (1.16-2.67)
Spontaneous ABR	1.6 (0.74-2.50)	0.67 (0.44-1.02)
Joint ABR	1.00 (0.54-1.83)	0.84 (0.53-1.2)

Conclusions: The data suggest that long-term prophylaxis with Human-cl rhFVIII has a favorable safety profile and reduces even further the already low spontaneous bleeding rates observed in the predecessor prophylaxis study.

PB 208 | Realworld Health Care Utilization and Costs of Extended and Standard Half-life Recombinant Factor IX Products in Hemophilia B Patients

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Background: Hemophilia B management requires intravenous factor IX (FIX) infusions to replenish the missing coagulation factor. Extended half-life (EHL) FIX products were recently introduced that require fewer infusions compared to standard half-life (SHL) FIX products.

Aims: We analyzed USA real-world data to determine usage and costs associated with EHLs vs SHLs. We examined how switching from SHL to EHL impacts healthcare costs.

Methods: The Truven MarketScan claims database was queried for factor replacement costs over one year in EHL patients were compared with the patients in the SHL cohort (overall analysis), starting June 2014 (first EHL claim). Separately, we analysed FIX utilization and costs in patients who switched from SHL to EHL

TABLE 1 Hemophilia B related utilizations and costs of FIX replacement - All patients on SHL or EHL

	1-3		4-6		7-9		10-12		Overall for all available quarters
Characteristic	EHL	SHL	EHL	SHL	EHL	SHL	EHL	SHL	EHL/SHL
N	21	71	17	43	13	28	12	23	21/71
FIX Utilization - Metric Ius									
Mean/Std	95,116	66,043	58,081	66,637	61,240	71,351	69,977	64,128	75,881/61,606
Median	68,870	40,530	56,142	41,800	55,278	73,005	56,796	62,300	70,059/44,978
FIX Utiliztion Costs									
Mean/Std	$223,375	$89,194	$166,712	$82,819	$180,267	$100,339	$207,414	$91,000	$207,206/$81,664
Median	$178,650	$60,753	$152,990	$67,632	$149,436	$102,850	$162,929	$82,530	$167,909/$58,616

product during 2 years pre and post switch (switch analysis). Medians for cost and IUs were used to accommodate for date distribution skewness.

Results: In the overall analysis, quarterly costs and IUs dispensed were analyzed for 92 patients over 12 months (71 SHL; 21 EHL). The median quarterly study period factor cost was $109,292 higher (2.8 times) in the EHL cohort ($167,909) compared with the SHL cohort ($58,616). Median quarterly study period IU usage was also higher in the EHL cohort (70,059 IU) vs SHL users (44,978 IU).

In the switch analysis, 14 patients with hemophilia B switched to EHL product and had at least 3 months data pre and post switch. Median quarterly factor costs analyzed over 2 years pre/post-switch was $73,795 in the period prior to switch, and $189,083 after switch. Median quarterly metric IUs utilization in the period prior to switch was 58,535 and 65,700 IUs in the post-switch period.

TABLE 2 Costs and Utilization of FIX pre- and post-switch (SHL to EHL)

	Overall for all available quarters (24 months pre- and post-switch)	
Characteristics	Pre-Switch	Post-Switch
N	14	14
FIX Utilization - Metric IUs		
Mean/Std	77,557	79,264
Median	58,535	65,701
FIX Utilization Costs		
Mean/Std	$94,520	$235,322
Median	$73,795	$189,083

Conclusions: These analyses using real-world data suggest that total FIX costs and IU utilization are higher with EHL vs SHL use in the US. Additional real world data analyses with a larger sample size should be pursued to confirm these findings.

PB 209 | Three-year Bleeding Frequency in >1000 Haemophilia A Patients on Prophylaxis: A Reliable Effectiveness Benchmark

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Background: New products are becoming available leading to an increased need to compare their effectiveness in prophylaxis to standard products.

Aims: Establish an effectiveness benchmark.

Methods: To establish an effectiveness benchmark, bleeding frequency was assessed in the AHEAD studies (an International [INT] and a German [GER] arm). These non-interventional, prospective long-term cohort studies include severe and moderate (FVIII < 1-5%) haemophilia A (HA) patients treated with octocog alfa (ADVATE).

Results: Overall 1,117 patients (715 INT and 402 GER) were enrolled so far. Preliminary data of 869 patients from 22 countries were analyzed. Of these, 687 patients completed year 1, 536 year 2 and 385 year 3. Mean age at screening was 23.4 years, 67% had severe HA and 79% were on prophylaxis.

Over the first 3 years of observation, patients on prophylaxis had a median annual bleeding rate (ABR) of about 2 bleeds: median ABR 1.7 and 2.5 in year 1; 1.6 and 2.2 in year 2; 2.2 and 1.9 in year 3 in the INT and GER arm, respectively. Median annual joint bleeding rates (AJBRs) were ≤1 in the 3 years: a median of 0.9 in both arms in year 1, 1.0 INT and 0 GER in year 2, and 1.0 INT and 0.8 GER in year 3.

In the first 3 years a median of 36.3% of patients on prophylaxis (min-max: 35.1%-36.9%) had an ABR < 1. An additional 13.1% (min-max: 12.5%-15.6%) had an ABR of 1 to < 2 and further 8.8% (min-max:7.6%-9.8%) had an ABR of 2 to < 3. A median of 54.6% (min-max: 53.3%-56.4%) of patients on prophylaxis had an AJBR < 1 during the first 3 years. An additional 14.1% (min-max: 12.7-14.4) had an AJBR of 1 to < 2, and further 6.7% (min-max: 6.5-10.0) an AJBR < 3%.

Conclusions: These data show that ~30% patients on prophylaxis had an ABR < 1 and about 50% had an AJBR < 1 in routine clinical practice. About 50% of patients had an ABR < 2 and about 70% an AJBR < 2. The AHEAD studies jointly represent one of the largest long-term prospective HA cohort, providing a reliable benchmark for new products or therapeutic approaches.

PB 210 | Bleeding Phenotype In Non-severe Hemophilia A Has a Better Association with the One-stage Than the Chromogenic Assay

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Background: Measuring factor VIII activity (FVIII) is of importance to diagnose and monitor hemophilia A (HA) patients. Two types of assays are generally used: the one-stage (OSA) and chromogenic assay (CSA). FVIII assay discrepancies have been reported in non-severe HA patients. However, it is still uncertain which assay corresponds best with bleeding phenotype.

Aims: To analyze the association between FVIII measured with the OSA and CSA and bleeding phenotype.

Methods: Non-severe HA patients (according to OSA at diagnosis) seen in the outpatient clinic of the Erasmus University Medical Center were included. FVIII was measured using the OSA and CSA during a routine visit. Severity of hemophilia was determined with the results of both assays and classified according to the SSC/ISTH criteria. Clinical agreement between assays was calculated. All documented bleedings between 2011 and 2015 were collected from patient files. Annual bleeding rate (ABR) was calculated by dividing the total number of bleeds by years of follow-up. The relationship between ABR and severity of HA was determined with Mann-Whitney U tests.

Results: In total, 140 non-severe HA patients were included (median age: 47 years [range 9-91]). Median FVIII with the OSA was 0.10 IU/mL [IQR: 0.05-0.19]. Median FVIII with the CSA was 0.09 IU/mL [IQR 0.06-0.18]. According to the OSA, 2 patients (1%) were diagnosed with severe HA, 36 (26%) with moderate and 102 (73%) with mild HA. According to the CSA, these numbers were 8 (6%), 23 (16%) and 109 (78%), respectively. The clinical agreement between assays regarding the severity of HA was 77%. Overall, median ABR was 0.40 [range 0-7.60]. Moderate HA patients had a significantly higher ABR (0.80) than mild HA patients (0.33) when analyzed with the OSA ($p< 0.01$), but not when analyzed with the CSA ($p=0.54$): median ABR was 0.40 in both moderate and mild HA patients.

Conclusions: In non-severe HA, bleeding phenotype, defined as ABR, is best associated with FVIII measured with the OSA in comparison with the CSA.

PB 211 | Inhibitor Development and Efficacy in Previously Untreated Patients with Severe Haemophilia A Treated with Human-cl rhFVIII, a 4th Generation Recombinant FVIII of Human Origin

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Background: Human-cl rhFVIII (simoctocog alfa) is a 4th generation recombinant FVIII produced in human cells without chemical modification or protein fusion. The immunogenicity, efficacy and safety of Human-cl rhFVIII in previously untreated patients (PUPs) with severe haemophilia A are being assessed in the ongoing multinational NuProtect study. One hundred evaluable (110 enrolled) male PUPs of all ages and ethnicities will be studied for up to 100 exposure days (EDs) or a maximum of 5 years.

Aims: To assess the immunogenicity, efficacy and safety of Human-cl rhFVIII in PUPs with severe haemophilia A.

Methods: Inhibitor activity was defined as ≥0.6 BU as measured by the Nijmegen modified Bethesda assay at a central laboratory (low-titre inhibitors ≥0.6 to < 5 BU; high-titre inhibitors ≥5 BU). Efficacy outcomes were assessed during inhibitor-free periods.

Results: Data for 66 PUPs with ≥20 EDs (by when most inhibitors arise) from a pre-planned interim analysis were analysed. The median age at first treatment was 13 months (range 3-135). The cumulative incidence was 12.8% for high-titre (95% CI 4.49-21.15), 8.4% for low-titre (95% CI: 1.28-15.59) and 20.8% for all inhibitors (95% CI 10.68-30.95). Twelve of the 13 inhibitor patients had identifiable *F8* gene mutations, all were null, and all except one were high-risk. The annualised bleeding rate for spontaneous breakthrough bleeds (Poisson model) was 0.96 (95% CI: 0.65-1.36). Efficacy in treatment of bleeds was excellent or good for 324/353 (91.8%) of rated bleeds. The overall efficacy of surgical prophylaxis for 9 procedures with available ratings was excellent or good for 8 (89%; 7 [78%] excellent, 1 [11%] good) procedures and moderate for 1 (11%) procedure.

Conclusions: These interim data support the low rate of inhibitor development in true PUPs with severe haemophilia A treated with Human-cl rhFVIII and demonstrate haemostatic efficacy during inhibitor-free periods. Final data are expected in 2019.

PB 212 | Long-term Impact of rFVIIIFc Prophylaxis in Paediatric, Adolescent, and Adult Subjects with Target Joints and Severe Haemophilia A

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Background: Long-term safety and efficacy of rFVIIIFc are being evaluated in the ongoing ASPIRE extension study of subjects with severe haemophilia A who completed A-LONG or Kids A-LONG.

Aims: To present data on re-bleeds and target joint resolution from subjects with target joints at entry into the parent study through the third ASPIRE interim data cut.

Methods: Subjects with ≥1 target joint (major joint with ≥3 bleeding episodes in a 6-mo period) at parent study entry with available pre-parent study data and on-study data were evaluated. ASPIRE treatment groups (≥12 y) were: individualised prophylaxis (IP), weekly prophylaxis (WP), modified prophylaxis (MP) or episodic treatment (ET); or (< 12 y) IP or MP. Outcomes were analysed over the duration of the parent study through the third ASPIRE interim data cut (11 Jan 2016).

Results: 113 A-LONG subjects had target joints at baseline; 111 with evaluable data had 287 target joints at baseline and a cumulative median (IQR) 4.0 (2.8, 4.1) y on rFVIIIFc. 13 Kids A-LONG subjects had 15 target joints at baseline and 3.0 (0.5, 3.1) y on rFVIIIFc. Target joint annualised bleeding rates were low for subjects on rFVIIIFc prophylaxis (**Table 1**). 43.9% of IP, 42.3% of WP and 6.3% of MP A-LONG subjects and 53.8% of Kids A-LONG subjects (all IP) had no target joint bleeding episodes. Among prophylaxis subjects with target joints at baseline and 12 mo follow-up, 100% of A-LONG and Kids A-LONG subjects had ≥1 target joint resolved (≤2 spontaneous bleeding episodes in 12 consecutive mo); 99.6% and 100% of evaluable target joints in A-LONG and Kids A-LONG subjects were resolved, respectively. **Table 2** shows prophylactic dose and dosing intervals. In adults/adolescents, 96.4% of target joint bleeding episodes were controlled with ≤2 rFVIIIFc injections; patients rated 82.0% of injections to control a bleeding episode as excellent or good.

Conclusions: Low target joint ABRs and effective target joint resolution occurred in children, adolescents, and adults on long-term rFVIIIFc prophylaxis.

TABLE 1 Summary of on-study target joint ABRs among subjects with target joints at baseline and an efficacy period

	Adults/adolescents				Paediatric subjects (<6 years)[a]	Paediatric subjects (6 to <12 years)
Median (IQR) on-study target joint ABR	IP (n = 82)	WP (n = 26)	MP (n = 16)	ET (n = 18)	IP (n = 6)	IP (n = 7)
Overall	0.4 (0.0, 1.7)	0.4 (0.0, 2.0)	1.6 (0.5, 6.4)	14.6 (6.1, 22.2)	0.7 (0.0, 2.0)	0.0 (0.0, 2.0)
Spontaneous	0.0 (0.0, 0.8)	0.1 (0.0, 0.8)	0.7 (0.3, 3.3)	8.6 (1.4, 16.3)	0.7 (0.0, 2.0)	0.0 (0.0, 2.0)
Traumatic	0.0 (0.0, 0.5)	0.0 (0.0, 0.5)	0.3 (0.0, 2.0)	0.5 (0.0, 6.9)	0.0 (0.0, 0.0)	0.0 (0.0, 0.3)

ABR, annualised bleeding rate; IQR, interquartile range; IP, individualised prophylaxis; WP, weekly prophylaxis; MP, modified prophylaxis; ET, episodic therapy.

[a]For 1 subject <6 years of age on MP on-study, overall target joint ABR was 1.0, spontaneous target joint ABR was 1.0, and traumatic target joint ABR was 0.

TABLE 2 Summary of dosing characteristics for subjects with target joints at baseline[a]

	Adults/adolescents			Paediatric subjects (<6 years)[b]	Paediatric subjects (6 to <12 years)[c]
Median (IQR)	IP (n = 82)	WP (n = 26)	MP (n = 16)	IP (n = 6)	IP (n = 7)
Average weekly dose (IU/kg)	78.1 (73.9, 97.6)	65.4 (62.7, 66.8)	70.1 (57.3, 81.2)	89.6 (75.3, 97.5)	82.2 (79.4, 113.2)
Dosing interval (days)	3.5 (3.2, 4.5)	7.0 (6.9, 7.0)	5.6 (4.0, 7.0)	3.5 (3.5, 3.5)	3.5 (3.0, 3.6)

IQR, interquartile range; IP, individualised prophylaxis; WP, weekly prophylaxis; MP, modified prophylaxis.

[a]Subjects who changed regimens during the study were included in summary analyses of each treatment regimen for the time period they were in that regimen and, thus, may be included in >1 regimen.

[b]For 1 subject <6 years of age on MP on-study, average weekly dose was 118.7 IU/kg and dosing interval was 2.3 days.

[c]There were no subjects 6 to <12 years of age on MP on-study.

PB 213 | Administration of Recombinant Factor VIII by Continuous Infusion (CI) versus Intermittent Bolus Infusion (BI) in the Intra- and Post-operative Setting

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Background: Factor VIII (FVIII) replacement therapy by continuous infusion (CI) in patients with hemophilia A undergoing surgery may offer a safe and efficacious alternative to bolus infusion (BI).

Aims: To compare the peri- and post-operative (post-op) hemostatic efficacy and safety of Advate [antihemophilic factor (recombinant), plasma/albumin-free method] administered via CI or BI in patients undergoing major orthopedic surgery requiring drain placement.

Methods: In this randomized multicenter phase 3/4 study, previously treated patients with severe or moderately severe hemophilia A (baseline FVIII level ≤ 2% of normal) aged 18-59 years undergoing elective unilateral orthopedic surgery were randomized to treatment by CI or BI of Advate through post-op Day 7 according to their PK profile. The primary endpoint was the cumulative packed red blood cell (PRBC) volume in the drainage fluid during the first 24 hours post-op.

Results: A total of 63 patients were included; 32were randomized to receive Advate by CI and 31 to BI during unilateral knee replacement (n=48), hip surgery (n=4) or shoulder/elbow/ankle/knee surgery (n=8). The ratio of cumulative PRBC volume in the 24-hours drainage fluid was 0.92 with a 95% confidence interval of 0.82 to 1.05. Higher Advate doses were used in BI compared to CI intraoperatively, on post-op Day 0. and from post-op Day 15 to end of study; higher doses were used for CI on post-op Days 1-14. Transfusions were required in 18/32 and 13/31 patients on CI and BI, respectively. Of 4 reported bleeding episodes, 3 occurred in subjects on BI. Related adverse events (n=14) were reported in 5 subjects treated by CI (n=8) and 5 treated by BI (n=6).

Conclusions: CI administration of Advate is a viable alternative to intermittent BI in the peri- and postoperative hemostatic management of patients with hemophilia A undergoing major orthopedic surgery by providing comparable efficacy and safety.

PB 214 | Assessment of Clot Kinetics of Recombinant Human Factor VIII (rhFVIII), Recombinant Porcine Factor VIII (rpFVIII) Recombinant Factor VIIa (rFVIIa) and Activated Prothrombin Complex Concentrate (APCC) Utilizing Thromboelastography in Patients with Severe Hemophilia A and Inhibitors

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Background: Approximately 30% of FVIII deficient patients will develop inhibitors (antibodies which neutralize the activity of factor replacement therapy) and therefore rely on bypassing agents (BPA) to manage bleeding.

Aims: The purpose of this study was to utilize TEG to compare clot kinetics of rhFVIII (Advate) and the 3 BPA: rpFVIII (Obizur), rFVIIa (Novosven) and APCC (FEIBA) in patients with FVIII deficiency and high titer inhibitors.

Methods: Subjects who signed informed consent to participate in an IRB-approved institutionally-funded study were enrolled. After appropriate washout, blood samples were drawn into citrated tubes from all subjects for measurement of FVIII activity, hFVIII inhibitor, pFVIII inhibitor, and baseline kaolin-activated TEG. In addition, 3 sets of kaolin-activated TEG experiments were performed:

1) *in vitro* spiking of rhFVIII and BPA at clinically-relevant concentrations;

2) *in vitro* spiking of rhFVIII and BPA at clinically-relevant concentrations after incubation of the blood at 37°C for 90 minutes; and

3) *in vivo* assays before and 30 minutes after infusion of rhFVIII 100 IU/kg in patients on immune tolerance.

Results: Two major observations were noted: 1) All 3 BPA significantly improved clot kinetics with shorter R and higher MA values in a concentration-dependent manner with a more potent effect noted for rpFVIII and FEIBA (table 1), and 2) the neutralizing effect of the inhibitor was not observed in the *in vitro* spiking experiments (even after incubation) but was observed on the *in vivo* samples (table 2).

TABLE 1 Study summary of group 1 in vitro experiments with all TEG parameters expressed as means (n=8)

	Baseline	rhFVIII 50 IU/mL	rhFVIII 200 IU/mL	rpFVIII 50 IU/mL	rpFVIII 200 IU/mL	FEIBA 50 IU/mL	FEIBA 100 IU/mL	rFVIIa 1.5 mcg/mL	rFVIIa 4.5 mcg/mL
R	124.4	4.3	4.7	4.6	3.6	5.4	3.8	20.3	10.7
K	34	1.5	1.8	1.6	1.5	3.9	1.5	3.9	2.6
angle	3.2	68.8	65.2	68	68.9	45.1	66.5	48.3	57.8
MA	15.2	65.2	59.6	64	60.2	48.3	63.5	65.9	65.3
G	1	9.9	7.5	9.7	7.8	4.9	9.1	10.3	9.8

TABLE 2 Study summary response to rhFVIII in in vitro and in vivo experiments on group 2 (n=3). TEG parameter results expressed as means.

	Baseline	rhFVIII 100 IU/mL in vitro spiking	rhFVIII 100 IU/mL in vivo
R	127.3	2	29.9
K	41.9	5.9	12.6
Angle	11.6	46.8	62
MA	33	43	60.4
G	3.3	4	5.8

Conclusions: TEG can demonstrate the *in vitro* effect of BPA at relevant concentrations and thus can be used to guide individualized therapy. However, the results of the rhFVIII *in vitro* spiking experiments did not demonstrate the neutralizing effect of the inhibitor, calling into question utilizing this method to assess the impact of FVIII therapy for bleed management in patients with high titer inhibitor patients.

PB 215 | Obesity in Patients with Hemophilia: Prevalence by Age, Clinical Correlates and Impact on Joint Bleeding

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Background: The prevalence of obesity in patients with hemophilia (PWH) quite varies among different countries. The reports on the prevalence of obesity and impact of obesity in PWH from Asian countries are very limited.

Aims: We investigated the prevalence and clinical correlates of obesity in PWH in Taiwan, and explored the impact of body mass index (BMI) on hemophilic arthropathy (HA) and annual joint bleeding rate (AJBR).

Methods: We retrospectively collected 140 severe/ 40 moderate PWH from two hemophilia centers from 2006 to 2014. The patients' median age was 31.5 years old, ranged from 6-73 years Their BMI, AJBR and other clinical information were analyzed. HA was assessed by roentgenograms of 6 index joints including elbow, knee and ankle joints, and their Pettersson scores were calculated.

Results: The prevalence of overweight and obesity by age group were 7.1% and 0% in PWH aged 6-10 years, 6.9% and 27.6% in PWH aged 11-18 years, 35.6% and 11.1% in PWH aged 18-29 years, 23.5% and 38.2% in PWH aged 30-39 years, 24.2% and 36.4% in PWH aged 40-49 years, and 36% and 12% in PWH aged >50 years, respectively. Age, HCV infection, knee, elbow joints and total 6 index joints scores were found to have positive correlation with BMI, but severity of hemophilia, ankle scores, HBV and HIV infection were not found to have correlation with BMI. Additionally, BMI, age and HCV infection were found to have positive correlation with AJBR in both adult and pediatric PWH.

Conclusions: There were remarkably higher prevalence of overweight and obesity in both adult and pediatric PWH than those in general male population in Taiwan. Age, HCV infection, higher index joints except ankle scores were associated with higher BMI. Higher BMI or obesity had a significant correlation with higher AJBR in PWH, although the direct effect of BMI on HA could not be shown by total 6 index joints score.

PB 216 | Influence of Genetic Polymorphisms of Biological Mediators on the Phenotypic Heterogeneity in Hemophilic Arthropathy

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Background: Heterogeneity in hemophilic arthropathy indicates the difference of bleeding frequency and severity of hemophilic arthropathy. Some studies have shown that some polymorphisms or mutation of hemostatic factors, cytokines and genes involved in inflammation and angiogenesis in hemophilic arthropathy.

Aims: The aim of this study was to explore whether there is a correlation of

polymorphisms of the 8 biological mediators encoding genes with the heterogeneity of hemophilic arthropathy.

Methods: There were 74 consecutive severe type hemophilia A (HA) patients (10 with inhibitor) in this study. Their median age was 42 years old, ranged from 14-71 years. Their DNA of white blood cells were extracted. Genotyping of gene polymorphism of 8 biological mediators including Thrombomodulin/Ala25Thr, TFPI/Val264Met, TNF-α/(-308G>A), IL 6/(-174 G>C), IL 10/(-1082G>A), VEGF/(936 C>T), MDM2/(309T>G) and HFE/ C28Y&H63D were sequenced. Each patient's X ray score of 6 index joints including elbow, knee and ankle joints was calculated by Pettersson score. Unpaired Student's *t* test was used to evaluate the comparison of the eight genotype of polymorphisms.

Results: There were high frequency of variants of MDM2 and VEGF genes in our cohort. The MDM2/(309T>G) was the most frequent variant with frequency of 78.8%, followed by VEGF (936C>T) with frequency of 41.9%. The total 6 index joints score in patients with

heterozygous polymorphism of VEGF (C/T) was 35.86± 3.85 which was significant lower than the 44.67 ± 2.89 in patients with wild type VEGF (C/C) (p= 0.0336).

Conclusions: Our study demonstrates that there were high frequency of MDM2 and VEGF gene polymorphisms in severe type HA patients and patients with heterozygous polymorphism of VEGF gene (C/T) had less severity of HA of 6 index joints evaluated by X ray Pettersson score than patients without polymorphism. The mechanism for this remarkable finding warrants further investigation.

PB 217 | Joint Status and Other Factors Associated with Pain Severity and Interference in US Adults with Hemophilia Beyond Joint Status: Analysis of the Pain, Functional Impairment, and Quality of Life (P-FiQ) Study

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Background: P-FiQ enrolled US adults with hemophilia and included administration of patient-reported outcome instruments to assess pain, functional impairment, and quality of life. Brief Pain Inventory (BPI) severity was associated with lack of college education, unemployment, older age, history of joint procedures, viral disease, and severe hemophilia.

Aims: This post hoc analysis assessed association between demographic and clinical characteristics independent of joint status (Hemophilia Joint Health Score [HJHS]) and BPI pain severity and interference scores.

Methods: The association of BPI scores with HJHS and other covariates was examined using simple regression models and by a multiple regression model where HJHS overall score, age, hemophilia severity, and treatment were included with other covariates having bivariate correlations with HAL scores (α< 0.05) using forward selection.

Results: The study enrolled 381 patients in total. Physiotherapist-completed HJHS was available for 240 respondents, with a median age of 32 years; most were employed (65%), college educated (65%), had severe hemophilia (63%) or inhibitors (9%), experienced pain (84%), and had prior joint procedures (54%). Worse joint status (higher HJHS score) was associated with higher pain scores in the multiple regression models, which had R-squared values of 0.422 for pain severity and 0.386 for pain interference. In addition to HJHS score, other predictors of worse pain outcomes included non-employed status, opiate use, anxiety, and use of anxiolytics **(Table 1)**.

Conclusions: Joint health status was an independent predictor of pain severity and interference, and opiate/anxiolytic use, anxiety, and work status were also associated with pain outcomes. Only 42%/39% of pain severity/interference was explained by the model. Continued attention to psychosocial issues, including assessing and managing pain, is essential to clinical care and future research in the hemophilia population.

PB 218 | Ranges of Risk Associated with Sports/Recreational Activities in People with Hemophilia: Results of the Activity-Intensity-Risk (AIR) Consensus Survey of US Physical Therapists

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Background: Limited evidence supports assessment of activity-associated risk for people with hemophilia (PWH) and other bleeding disorders. Existing consumer resources provide a single risk score for each activity based on opinions of a few physical therapist (PT) authors.

TABLE 1 Multiple Regression Models—BPI Pain Severity or Interference

	BPI Pain Severity (n=177; R2=0.422)				BPI Pain Interference (n=181; R2=0.386)			
Characteristic	ß Estimate*	Lower 95% CI	Upper 95% CI	P Value	ß Estimate*	Lower 95% CI	Upper 95% CI	P Value
HJHS overall score (10-point change)	0.30	0.14	0.47	0.0003	0.32	0.13	0.52	0.0012
Employed	-0.67	-1.38	0.03	0.0617	-0.94	-1.77	-0.12	0.0244
Patient-reported use of opiates	1.27	0.71	1.83	<0.0001	1.41	0.73	2.09	0.0001
Site-reported anxiety	1.33	0.49	2.18	0.0022	2.26	1.34	3.18	<0.0001
Site-reported use of anxiolytics	1.68	0.05	3.30	0.0429				

BPI, Brief Pain Inventory v2 Short Form; HJHS, Hemophilia Joint Health Score. *ß estimate measures change in outcome per change in covariate.

TABLE 1 Physical Therapist Ratings of Risk

Ball Sports	Low/Min Risk Median (IQR)	High/Max Risk Median (IQR)	Water Sports	Low/Min Risk Median (IQR)	High/Max Risk Median (IQR)	Winter Sports	Low/Min Risk Median (IQR)	High/Max Risk Median (IQR)
Baseball - Pitcher	3 (2, 3)	4 (4, 5)	Jet Skiing	2 (1, 3)	4 (3, 4)	Ice Hockey	4 (3, 4)	5 (5, 5)
Baseball - Catcher	3 (3, 3)	4 (4, 4)	Kayaking	1 (1, 2)	3 (2, 4)	Skating - Recreation	2 (1, 2)	3 (2, 4)
Baseball - Other	2 (2, 3)	3 (3, 3.3)	Sailing	1 (1, 2)	3 (2, 3.5)	Skating - Figure	2 (2, 3)	4 (3, 5)
Basketball	2 (2, 2)	4 (4, 4)	Diving Recreational	2 (1.8, 2.3)	3 (2, 3)	Ski - Cross Country	1 (1, 2)	2 (1.8, 3)
Football- Flag	2 (2, 3)	4 (3, 4)	Paddle Boarding	1 (1, 1)	2 (1.8, 3)	Ski - Down Hill	2 (2, 3)	4 (4, 5)
Football - Tackle	5 (5, 5)	5 (5, 5)	Surfing	2 (2, 3)	4 (3, 5)	Snowboarding	3 (2, 3)	5 (4, 5)
Rugby	5 (4.5, 5)	5 (5, 5)	Swimming	1 (1, 1)	2 (2, 3)	Other Activities		
Soccer - Goalie	2 (2, 3)	4 (4, 5)	Water Polo	2 (2, 3)	4 (3.8, 4)	Horseback Riding	2 (1, 3)	4 (3, 4.3)
Soccer - Other	2 (2, 2.3)	4 (3.8, 4)	Water Skiing	2.5 (2, 4)	4 (3, 5)	Trampoline	3 (2, 4)	4 (4, 5)

Aims: To assess ranges of risk for PWH associated with different sport/recreational activities, based on consensus of US PT experts.
Methods: Peer-nominated PTs in the US hemophilia treatment center (HTC) network were invited to participate in a survey of 100 sports/recreational activities. For each activity, respondents provided a low/minimum and high/maximum risk assessment on a 5 point scale (low=1, high=5). Position-specific assessments were made for some team sports (eg, baseball pitcher, catcher, and field positions). Sports with distinctly different participation levels were evaluated separately (eg, flag vs tackle football, boys vs girls lacrosse, Frisbee vs ultimate).
Results: Of 32 invited PTs, 17 responded with median (mean) 26.5 (22.4) years as a PT and 15.5 (16.8) years at an HTC. Most treated adults (94%) and children (88%), and worked in the HTC full-time (29%) or nearly full-time (41%); 94% worked in a comprehensive care clinic. Median (Q1, Q3) PT ratings are shown for selected activities **(Table 1)** and ball sports **(Fig 1)**. Overall, few activities had low/minimum and high/maximum risk assessments at the lower (1) or upper (5) end of the response range. For example, while swimming is typically associated with low risk, maximum risk includes potential year-round competitive swimming. In contrast, rugby and American football (tackle) had consistently high scores.

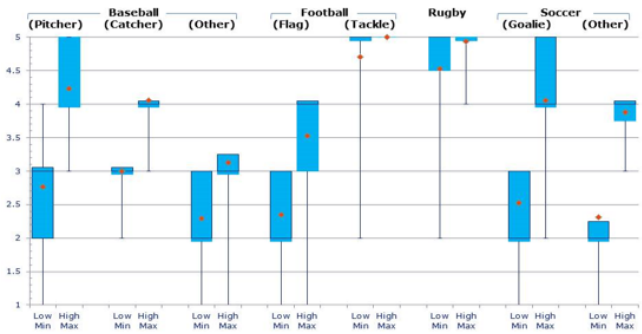

FIGURE 1 Physical Therapist Rated Range of Risk for Selected Ball Sports

Conclusions: Consensus of experienced PTs demonstrates most sports/recreational activities can be best described by a range of risk. This finding highlights the importance of individualized activity-specific risk assessment over time (eg, from recreational to competitive) as part of regular HTC discussions with patients/families.

PB 219 | The Impact of Switching Recombinant Factor VIII Product Concentrates on Inhibitor Development among Haemophilia A Patients in Australia

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Background: The pathogenesis of the development of inhibitors in patients with haemophilia A (HA) is complex, involving the immune system, genetics and environmental factors. It is controversial if switching recombinant factor VIII (FVIII) concentrates is a risk factor for inhibitor development.
Aims: This retrospective study explores if switching between recombinant FVIII concentrates increases the risk of inhibitor development in HA in Australia.
Methods: Inhibitor and Product Usage data was obtained from the Australian Bleeding Disorder Registry on HA patients who had to switch between full-length & B-deleted FVIII products in 2012 and 2014 as part of a national tendering process. With Ethics approval, data was extracted on patient diagnosis, severity, age, date of product switch and inhibitor test results pre and post product switch including titres. Positive inhibitor is ≥0.5 BU/mL; negative < 0.5BU/mL. Post product switch inhibitor test results were recorded as transient

(1 positive followed by 1 negative) or persistent (>1 positive inhibitor result within 12 months). Multivariate regression analysis was performed to determine if product switching contributed to inhibitor development in patients with HA.

Results: 712 patients switched their FVIII product. Pre and post product switch inhibitor tests were performed on 609 patients. Post switch, 47 (7.7%) had a transient and 23 (3.8%) a persistent inhibitor. 10 of the persistent inhibitors were de novo (7/336 [2.1%] severe; 3/273 [1.1%] non-severe); and 13 occurring in patients with a prior history of inhibitor (8/336 [2.4%] severe; 5/273 [1.8%] non-severe) (table 1). Multivariate analysis showed that product switching did not impact inhibitor development, but severity of HA did (p=0.000) (figure 1).

TABLE 1 Inhibitor development post FVIII product switch by haemophilia A severity and presence or absence of past inhibitor

	Past inhibitor prior to product switch	
Haemophilia A severity	Yes	No
Non-severe	5	3
Severe	8	7

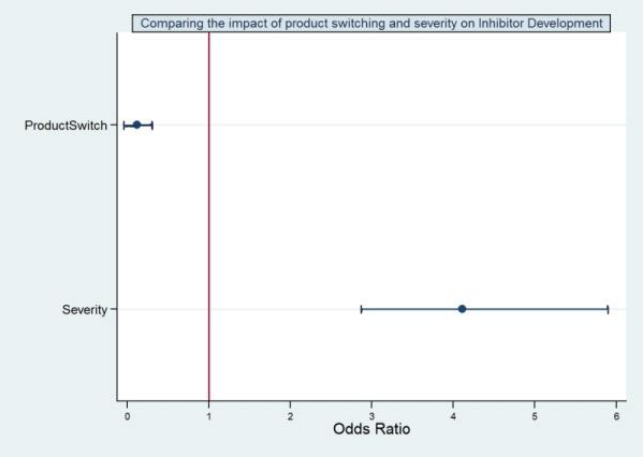

FIGURE 1 Comparing the impact of FVIII product switching and severity of HA on inhibitor development

Conclusions: Switching of FVIII concentrate in HA patients does not appear to increase the risk of inhibitor development.

PB 220 | Sociodemographic, Clinical and Psychosocial Characteristics of People with Haemophilia (PWH) in Portugal: Preliminary Results of the First National Survey

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Background: Understanding PWH clinical characteristics and psychosocial needs is paramount for improvement of integrated health care. However, despite recent surveys conducted among PWH, no demographic, clinical and psychosocial data is available in Portugal.

Aims: To collect information on sociodemographic and clinical data, pain, quality of life (QoL) and functionality in Portuguese PWH, ultimately contributing for the improvement of health policies and care.

Methods: Recruitment was made through a national patient organization (Portuguese Haemophilia Association) and 477 surveys were sent by post (Table 1). Data was obtained from 71 adults [age range=18-74; M(SD)=44.18(14.02)] and 28 children/teens [age range=1-17; M(SD)=9.64(4.73)]. The survey was approved by ethical committee and informed consent was signed by all respondents or legal guardians.

TABLE 1 Survey variables and measures

Variables	Measure
Sociodemographic: age, education, employment, marital status, income and household.	Sociodemographic Questionnaire
Clinical: age of diagnosis, Haemophilia type and severity, inhibitor status, appointments with Haemophilia specialist, emergency hospital admissions, work absences, frequency and location of bleeds, Haemophilia treatments, damaged and target joints, orthopedic surgery, HIV/ Hepatitis C status, medical comorbidities and chronic medication.	Clinical Questionnaire
Pain: duration, frequency, location, triggering factors, impact, intensity, interference, relief strategies, pain specialists and satisfaction with pain treatment.	Pain Questionnaire
Pain catastrophizing	CSQ-R - Catastrophizing Subscale (Riley & Robinson, 1997)
Functional Ability (Adults / Children and Teens)	HAL (van Genderen et al., 2006) / PedHAL (Groen et al., 2010)
Quality of Life (Adults / Children and Teens)	A36HemofiliaQoL (Remor et al., 2005) / CHO-KLAT (Young et al., 2004)

Results: Haemophilia A was reported by 89.9% of participants, the presence inhibitors by 17.4%, and all disease severities were considered (mild=12.1%; moderate=31.3%; severe=56.6%). The mean of annual bleeds was 10.92 (SD=15.06) for adults and 4.00 (SD=3.66) for children/teens. In this latter group, 75% were on prophylaxis (vs.

26.8% adults). A target joint was reported by 69.0% of adults, most frequently the knees (52.1%) and by 34.6% of children/teens, most often the elbows (44.4%) and ankles (44.4%). Chronic pain was reported by 59.2% of adults and 32.1% of children/teens. Adults reported greater functional limitations on ‚Functions of the Legs' [M(SD)=55.29(29.27)] and ‚Lying/Sitting/Kneeling/Standing' [M(SD)=58.22(28.77)] and the mean normative score for QoL was 53.98(26.21). Children/teens revealed greater impairment on ‚Leisure Activities and Sports' [M(SD)=88.65(15.75)] and QoL mean score was 76.52 (SD=12.62).

Conclusions: These findings are expected to contribute to deepen the knowledge about Portuguese PWH, in order to implement multidisciplinary approaches that improve disease management, promote well-being and quality of life.

PB 221 | Understanding Burden of Illness of People with Hemophilia A with or without a Current Inhibitor

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Background: The CHOICE Project was conducted in partnership between the CDC and Hemophilia Federation of America to survey persons with bleeding disorders (PWBD) in the US, including those who do not receive care at federally-funded hemophilia treatment centers (non-HTC PWBD).

Aims: To retrospectively characterize clinical utilization and bleeding among people with hemophilia A with or without a current inhibitor (PWH) to better understand burdens of illness and how to address them.

Methods: Demographic and clinical data were collected through CHOICE from 04/2013-07/2015 on PWBD. PWBD were recruited to take a 20-minute survey in English or Spanish, online or on paper. Non-HTC PWBD were solicited specifically but others were not excluded from participation. Participants' status as non-HTC PWBD was determined by algorithm. Descriptive statistical analysis of PWH was performed based on the chi-square test (*P* values).

Results: Of 439 PWH, 13% are non-HTC PWH and 11% have a current inhibitor (WI). In the last 12 months, due to their bleeding disorder, 18/48% (PWH without an inhibitor (WO)/WI) were admitted to a hospital (p=0.001); 31/58% visited an ER (p=0.0004); average ER visits for WO 2.8 v. WI 3.7 (p=.004); 22/13% delayed care **(Figure 1)**; 75/70% had a joint bleed, 41/58% a muscle bleed, 65/78% another type of bleed with an average of number of bleeds of 5.3/7.6 **(Figure 2)**; 26/19% always had joint problems and these problems always limited activity for 16/27%, with 35/56% taking OTC and 13/13% prescription pain medication on a few days to treat pain.

Conclusions: Burden of illness for WI is reflected in hospital admittance and emergency care along with bleed types/number, care delay, and joint problems. Additional analysis is needed to investigate the disease burden associated with different treatment regimen for PWH. This sample does not necessarily represent all PWH, as targeted outreach in some regions may have led to over-representation of some participant characteristics.

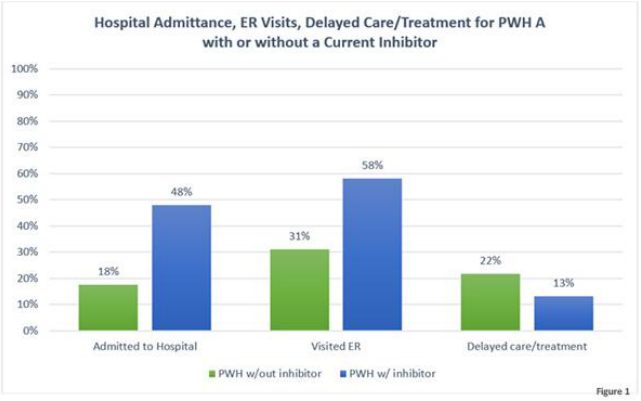

FIGURE 1 Hospital Admittance, ER Visits, Delayed Care/Treatment for PWH A with or without a Current Inhibitor

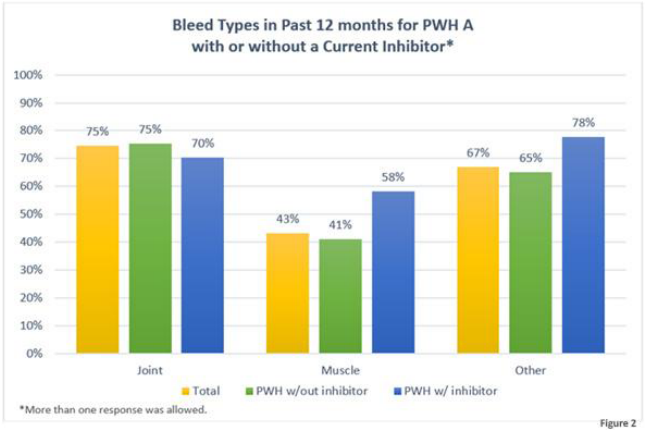

FIGURE 2 Bleed Types in Past 12 months for PWH A with or without a Current Inhibitor

PB 222 | The Continued Use of Cryoprecipitate and Plasma to Treat Bleeding Disorders

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Background: Plasma contains all the coagulation factors and can be used to treat bleeding disorders (BD). Cryoprecipitate (cryo) is preferable to plasma for the treatment of hemophilia A (HA) and von Willebrand disease. Although it is possible to apply virucidal treatment to plasma and cryo, this is not common practice. Despite the risk of transfusion-transmitted infection, these products are still used in countries unable to afford sufficient supplies of clotting factor concentrates (CFC).

Aims: To evaluate the use of cryo and plasma globally to treat BD.

Methods: The World Federation of Hemophilia Annual Global Survey (AGS) collects basic data on demographics and access to care and treatment products. The AGS asks specifically about availability and use of cryo and plasma for treatment of BD. Using data reported by 115 countries (~90% of world pop.) from 2013 to 2105, we analyzed the use of plasma and cryo around the world.

Results: 115 countries answered the AGS questions on the use of plasma and cryo. All countries reported plasma and cryo being available, but 69 (60%) reported never using them to treat BD. 40 countries (34.8%) reported using plasma (Table). Of this group, 31/40 (78%) reported receiving humanitarian aid donations of CFC and in 20/40 (50%) virtually all CFC treatments were with donated product. 83,903 patients with BD have been identified in countries reporting use of plasma. High GNI countries specified it is used for rare BD, not hemophilia. Cryo was used to treat patients in 36 countries (31.3%). Many of these countries reported some access to CFC but use of cryo when CFC were not available. Median FVIII/cap in countries using cryo was 0.07 IU. 63,848 patients with HA have been identified in countries reporting cryo use.

TABLE 1 Countries reporting use of plasma and cryo by Gross National Income (GNI)

GNI per capita US$ (World Bank data)	Countries using plasma n = 40	Countries using cryo n = 36
high income, ≥$12,475	4 (10%)	1 (3%)
upper middle income, $4,036 - $12,475	11 (28%)	11 (31%)
lower middle income, $1,026 - $4,035	19 (48%)	20 (56%)
lower income, $0-$1,025	6 (15%)	4 (11%)

Conclusions: Despite the risks, plasma and cryo are still used to treat people with BD, underscoring the need for improved safety of these lifesaving treatments and increased access to affordable and sustainable supplies of CFC.

PB 223 | Anti-CD20 and Sirolimus for Immune Tolerance in Congenital Hemophilia A with Inhibitors

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Background: The development of factor VIII (FVIII) inhibitors in 30% of patients with severe hemophilia A (HA) results in significant morbidity. Immune tolerance induction (ITI) is only successful in 2/3 of patients and others relapse after ITI. Use of anti-CD20 (rituximab) demonstrated inconclusive benefit as a solo therapy (Thromb Haem 2014). A combined B and T cell approach aimed to increase Tregs has potential to improve refractory inhibitor patient outcomes.

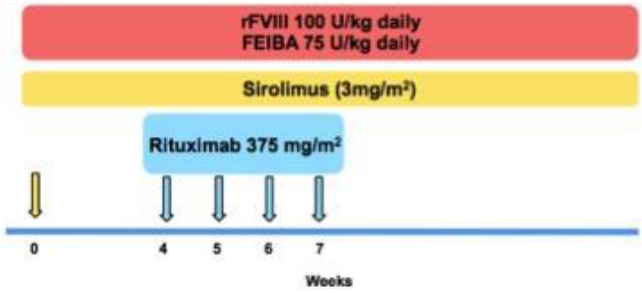

FIGURE 1 Experimental Protocol for Dual Therapy

Aims: Here we analyzed response to dual T and B cell therapy with rituximab (anti-CD20) and sirolimus in 2 severe HA patients with refractory inhibitors.

Methods: Patients consented to an IRB approved protocol. They were treated with sirolimus, rituximab and FVIII per Figure 1. Bypass agents were stopped upon high titer inhibitor resolution. Patient plasma samples were analyzed to determine Nijmegen modified Bethesda titer and FVIII-specific IgG1 and IgG4 by ELISA to evaluate for FVIII non-neutralizing and neutralizing antibodies, respectively, and compared to HA without inhibitor control.

Results: Patient 1 failed prior ITI and rituximab therapies. At enrollment his inhibitor was 23 BU. He had a rapid response to therapy with an undetectable Bethesda titer within 2 months of sirolimus initiation and 1 month after rituximab. His FVIII-IgG1 and IgG4 were undetectable at 7 and 19 months, respectively. Patient 2 failed multiple ITI attempts. His inhibitor was 384 BU with corresponding greater FVIII-IgG1 and IgG4 at enrollment (Table 1). He had a slower therapeutic response with a continued decrease in Bethesda titer, FVIII-IgG1 and IgG4, but remains on bypass therapy. Both patients had clinical improvement with marked reduction in bleeding.

TABLE 1 Bethesda Assay, FVIII-specific IgG1 and IgG4 over time

Patient	Time from rituximab (months)	Inhibitor Titer (BU)	IgG1 (mcg/ml)	IgG4 (mcg/ml)
1	-2	23	1.22	1.98
	1	0	0.17	0.11
	7	0	0	0.08
	19	0	0	0
2	-1	384	52.78	10.25
	1	166	7.99	4.86
	11	48	2.18	1.35
	17	7	2.54	0.26
HA Control	--	0	0	0

Conclusions: To our knowledge, this is the first study of sirolimus and rituximab co-administration for ITI in HA inhibitor patients. One patient tolerized rapidly to FVIII while the other has promising results at 17 months. Dual sirolimus and rituximab therapy may be a viable alternative in refractory inhibitor patients.

PB 224 | A Prospective Surveillance Study of Inhibitor Formation in Quebec Hemophilia A Patients Following a Population Switch to a Third-generation B-Domain-deleted Recombinant Factor VIII

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Background: For administrative reasons, most subjects with hemophilia A (HA) were switched from their current treatment to a third-generation B-domain-deleted recombinant factor VIII (FVIII). Despite a low risk of inhibitor development in previously treated subjects, we considered it important to monitor our population for this serious outcome.

Aims: To evaluate FVIII recovery and the risk of inhibitor formation in subjects switching to this product.

Methods: All subjects with severe and moderate HA were eligible unless they had less than 150 exposures days (ED) to any FVIII or detectable inhibitor at enrollment. Plasma samples were collected prior to the first infusion (ED1), at 15min post ED1, then at 6 and 12 months after switching, where feasible. All samples were analyzed for FVIII levels and inhibitors at a central laboratory. FVIII recovery (FVIIIrec) was expressed as the observed FVIII increase per unit of FVIII infused per kg ($IU.dL^{-1}/IU.kg^{-1}$). The study was approved by the REB of participating institutions and all subjects provided written informed consent.

Results: 125 male subjects were recruited (mean age 27.83±13.93). 96% had severe HA, 8% had a family history of inhibitors, and 9.6% had a personal history of inhibitors. Pre and post infusion samples were collected from 97.6 % of subjects at ED1 (mean FVIIIrec 2.45±0.61); from 68.8% of subjects at 6.58±1.60 months (mean FVIIIrec 2.43±0.73) and 84% of subjects at 12.85±2.04 months (mean FVIIIrec 2.49±0.64). No recurrence of inhibitors was observed in any of the subjects with a personal history of inhibitors. Two subjects (1.6%) developed an inhibitor: one (age 19) developed a transient inhibitor (peak titer 2.6BU) after 38ED to the new product; another (age 59) developed an inhibitor (peak titer 56.3BU) after 58ED.

Conclusions: Our results suggest that switching to this product was not associated with an increased risk of inhibitor formation as reported in similar studies.

PB 225 | Evaluation of Reagent Substitutions in the Centers for Disease Control and Prevention Modified Nijmegen-Bethesda Assay (CDC-NBA) for Factor VIII (FVIII) Inhibitors in Hemophilia A

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Background: The Nijmegen modification of the Bethesda assay introduced 2 changes: buffering of normal pooled plasma (NPP) in test and control mixtures with imidazole (IB-NPP) and substitution of FVIII-deficient plasma (FVIIIDP) for imidazole buffer (IB) as diluent in control mixtures and specimen dilutions for stabilization during incubation. Other reagents have been proposed to reduce cost.

Aims: To investigate the efficacy of reagent substitution in the CDC-NBA.

Methods: NPP, IB-NPP, and HEPES-buffered NPP were tested for FVIII and pH before and after 2 hours at 37°C. IB-NPP mixtures with other diluents substituted for FVIIIDP were tested for FVIII during incubation. Subsets of 1,565 specimens submitted to the Registry for Bleeding Disorders Surveillance conducted by CDC, the American Thrombosis and Hemostasis Network, and the US Hemophilia Treatment Centers Network were tested using the CDC-NBA, which includes preanalytical heat inactivation, and in parallel with reagent substitutions. IgG_4 anti-FVIII antibodies were measured by fluorescence immunoassay.

Results: IB-NPP showed greater FVIII and pH stability during incubation. As diluent, 4% bovine serum albumin (BSA), imidazole-buffered BSA (IB-BSA), and IB were not significantly different; all produced greater FVIII stability than native FVIIIDP. BSA, IB-BSA, and IB were comparable to FVIIIDP on 15 positive (≥0.5 Nijmegen-Bethesda units (NBU)) specimens with slightly higher NBU on 311 negative specimens; BSA with a cut-off of ≥0.6 NBU showed the best performance relative to FVIIIDP. Among 1206 negative and 33 positive specimens, BSA at a cut-off of ≥0.6 had sensitivity 0.97, specificity 0.99, positive predictive value 0.70, and negative predictive value >0.99 relative to the original assay. Anti-FVIII IgG_4 increased at ≥0.5 NBU with FVIIIDP and ≥0.7 NBU with BSA.

Conclusions: Substitution of BSA for FVIIIDP in the CDC-NBA produces similar results to the original assay if the cut-off for positivity is adjusted to ≥0.6 NBU. Imidazole buffering is preferred for NPP.

PB 226 | Evaluation of an Optimized Thrombin Generation Assay for Measurement of rFVIIa in Haemophilia A Patient Plasma

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Background: NovoSeven® (rFVIIa) shows high efficacy in the treatment of bleedings in haemophilia patients with inhibitors. The standard commercial thrombin generation assay CAT (Calibrated Automated Thrombogram) is currently being evaluated for monitoring efficacy of haemostatic therapy of rFVIIa in haemophilia patients. However, CAT standard condition for haemophilia plasma samples is not sensitive to therapeutic relevant rFVIIa levels [Turecek 2003].

Aims: To evaluate an optimized CAT for *ex vivo* measurements of rFVIIa in therapeutic relevant levels in haemophilia A plasma.

Methods: Haemophilia A (HA) plasma samples (n=6 patients) were spiked with various concentrations of rFVIIa (0-75 nM, 75 nM corresponds to ≈ 270 ug/kg), Novo Nordisk A/S) and thrombin generation was measured with an optimized CAT using human soluble tissue factor (sTF, 2 nM final concentration (f.c.), Novo Nordisk A/S) + MP phospholipid reagent (4 mM phospholipids f.c., Thrombinoscope). CAT was also determined in a different set of HA plasma samples (n=11) spiked with comparable rFVIIa concentrations but using the standard CAT reagent PPP Reagent Low (Thrombinoscope) containing f.c. of 1 pM TF and 4 μM phospholipids. Thrombin generation was initiated by addition of a fluorogenic thrombin substrate containing $CaCl_2$ (Fluca, Thrombinoscope).

Results: Optimized CAT showed a clear dose-response of rFVIIa. Relevant pharmacological rFVIIa levels (1-25 nM) resulted in peak thrombin concentrations ranging from approximately 50-250 nM. The optimized assay was able to detect rFVIIa concentrations down to 0.1 nM. As expected, the standard CAT condition (PPP Reagent Low) resulted in a peak thrombin increase from 25 nM to approximately 35 nM for comparable rFVIIa plasma concentrations.

Conclusions: With the optimized CAT it is possible to detect and distinguish relevant pharmacological rFVIIa levels in Haemophilia A patient plasma. Clinical data are needed for evaluation of the optimized assay as an efficacy assay.

NB is supported by FIS-Fondos Feder CP14/00024

PB 227 | Efficacy and Safety of Human-cl rhFVIII in Patients with Severe Hemophilia A Undergoing Surgical Procedures

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Background: Hemophilia A patients are at a high risk of excess bleeding during surgery. The aim of hemostatic therapy during the perioperative period is to prevent bleeding for as long as the bleeding risk persists and until wound healing is complete.

Aims: To examine the efficacy and safety of Human-cl rhFVIII (simoctocog alfa), a 4th generation recombinant FVIII produced in a human cell line without chemical modification or protein fusion, for surgical prophylaxis in patients with severe hemophilia A.

Methods: This analysis assessed the efficacy of Human-cl rhFVIII during surgical procedures and in the postoperative period in 7 clinical studies of previously treated patients (PTPs) with severe hemophilia A.

Results: Thirty-six patients (17 adults and 19 children), aged 3 to 55 years, received surgical prophylaxis with Human-cl rhFVIII for 60 surgeries. Of these, 28 were major and 32 were minor. The success rate (efficacy assessed as ‚excellent' or ‚good') of Human-cl rhFVIII treatment was 98.1% (52 of 53 evaluated surgeries); hemostatic efficacy was assessed as ‚excellent' or ‚good' in all but one major surgery (assessed as ‚moderate'). Treatment was assessed as ‚excellent' in all 24 (15 major and 7 minor) evaluated procedures in children. The number of infusions ranged from 1 to 19 for minor surgeries and from 3 to 76 for major surgeries. The median (range) daily doses were 42.0 (28.2-100.9) IU/kg for minor surgeries and 69.3 (43.3-135.6) IU/kg for major surgeries. Actual blood loss was no higher than the maximum expected blood loss for any procedure except for one minor dental extraction (actual blood loss 3 mL, maximum expected 0 mL). There were no serious treatment-related adverse events, and none of the patients developed FVIII inhibitors.

Conclusions: The results of this pooled analysis show that Human-cl rhFVIII was efficacious in maintaining hemostasis during and after major and minor surgical procedures in PTPs with severe hemophilia A.

TABLE Pooled efficacy ratings for surgical prophylaxis

Efficacy rating	Major surgeries (n = 26)	Minor surgeries (n = 27)	Total (n = 53)*
Excellent, n (%)	21 (80.8)	27 (100)	48 (90.6)
Good, n (%)	4 (15.4)	0	4 (7.5)
Moderate, n (%)	1† (3.8)	0	1 (1.9)
None, n (%)	0	0	0

*Efficacy ratings were not available for 7 of 60 surgical procedures (2 major and 5 minor) †For this patient, intraoperative efficacy had been rated as ‚good'; however, during the postoperative period the patient experienced several minor nose bleeds.

PB 228 | Comparison of Biomarkers and Immunological Parameters between Hemophilia Patients, Rheumatoid Arthritis Patients, and Control Subjects

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Background: Patients with haemophilia or rheumatoid arthritis (RA) may develop severe joint damage caused by recurrent joint bleeds in haemophilia and by chronic inflammation in RA, respectively.

Aims: Biomarkers are useful diagnostic tools to assess joint damage in RA. However, to date only limited data are available for biomarkers in haemophilia arthropathy.

Methods: A panel of biomarkers was assessed in 129 men older than 30 years (40 haemophilia patients without arthropathy, 23 haemophilia patients with arthrophathy, 23 patients with RA and 43 control subjects). During follow-up examination 61 different biomarkers were analyzed including immunological, inflammation, coagulation, angiogenesis-related parameters and cytokines. Arthropathy was characterized by painfull swelling, loss of function, typical radiology images

and surgical treatment of joints. The RA patients were classified according to ACR/EULAR criteria.

Results: We identified 24 parameters of angiogenesis and cytokines with significant differences between hemophilia patients, RA patients, and healthy individuals. Most of them (20) were reduced (e.g. VEGFR1 or TNF-alpha) whereas only EGFR, osteopontin, IL6-RA and IL-7 were elevated. In a second statistical analysis, we identified cytokine MIP-1b, VEGFR- 2 and HGF significantly declined and IP-10 elevated in hemophilia patients with arthropathy and rheumatoid arthritis patient compared to healthy individuals.

Conclusions: Twenty-four out of 61 immunological parameters and biomarkers were different in hemophilia patients. In addition, significant differences could be demonstrated between hemophiliacs (with and without arthropathy) and RA patients compared to controls. Therefore, we could show a specific immunological profile for hemophilia as well as a common biomarker profile for the arthropathy in haemophilia and RA. Further research should be performed to evaluate the potential of established and new biomarkers to follow-up joint damage and chronic arthrophathy in hemophilia.

PB 229 | Frozen Autologous Platelet Gel and Fibrin Glue in Hemophilia A, B and von Willebrand Surgical Patients

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Background: Haemostatic, germicidal and regenerative properties of platelet-derived growth factors justify their application in in patients with congenital hemorrhagic disorders for hard-to-heal wounds or improvement of hemostasis in clean surgical wounds.

Aims: To estimate the safety and effectiveness of frozen autologous platelet gel (FAPG) and fibrin glue (FG) applied to clean postoperative or chronically infected wounds in patients with congenital hemorrhagic disorders.

Methods: 17 surgical procedures were performed in 13 patients, aged 23 - 57years (11 male, 2 female), 9 - hemophilia A, 1 - hemophilia B , 3 - von Willebrand disease. The performed surgical procedures were: strumectomy (4), fasciotomy of forearm compartment syndrome (1), necrotic skin excision after arthroplasty (3), foot and hand trauma (2), removal of hemophilic pseudotumor (3 patients, 7 procedures). FAPG was obtained by thrombopheresis using Cobe-Spectra; FG by cryoprecipitation of autologous plasma. Sealants were divided into portions, immediately frozen and thawed before application. Both were activated by thrombin solution and applied on either clean or infected wounds. Activated sealants were applied to infected wounds on Bactigras dressing or directly on clean wounds. In the perioperative period all patients were supplemented with deficient clotting factors.

Results: Clean wounds healed by primary adhesion with no bleeding or inflammatory reactions. Infected wounds presented rapid granulation tissue formation and reduction of ulcerationarea. For hemophilic pseudo tumors a gradual decrease in cavity size was observed.

Conclusions: In patients with congenital hemorrhagic disorders application of platelet gel and fibrin glue to either clean or infected wounds presented accelerated healing and shorter hospital stay.

PB 230 | Safety and Efficacy Of Moroctocog Alfa (AF-CC) in Patients with Hemophilia A: Results of a Post-authorization Study in Usual Care Settings in China

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Background: Acquisition of real-world data is important to understand the efficacy and safety profiles of marketed medicines. Moroctocog alfa (AF-CC) is approved in China for control and prevention of bleeding events and for surgical prophylaxis in patients (pts) with hemophilia A.

Aims: Assess safety and efficacy of moroctocog alfa (AF-CC) in a real-world setting in Chinese pts with hemophilia A.

Methods: Ethics committees approved this open-label, multicenter, prospective study conducted at hemophilia treatment centers in China. Pts provided informed consent and received treatment on-demand (OD) for 6 mo or 50 exposure days, or for surgical prophylaxis, dosed according to the label. Key assessments included factor VIII (FVIII) inhibitor development; adverse events (AEs); response to treatment, no. of infusions needed to treat new bleeding events, and less-than-expected therapeutic effect (LETE) in the OD setting; and hemostatic efficacy, blood loss, and transfusion requirements in the surgical prophylaxis setting.

Results: In all, 85 pts received treatment (mean age, 9.5y; severe hemophilia, 58%). Seven pts tested positive for FVIII inhibitors (Table). Common AEs (>20%) included joint swelling, arthralgia, nasopharyngitis, and pain in extremity. In pts receiving OD treatment (n=73), 63% of bleeding events resolved with 1 infusion. Most OD infusions (87%) were rated as "excellent" or "good." One bleeding event met LETE criteria. Results were similar with subgroup analysis based on age, disease severity, and prior factor exposure. All surgical prophylaxis pts (n=14) had efficacy responses of "excellent" or "good" on day of surgery and in postoperative period. All blood loss was assessed as normal; transfusions were required in 2 pts.

TABLE FVIII inhibitor development in patients treated with moroctocog alfa (AF-CC)

Parameter	On-demand (n=73)[a]	Surgical prophylaxis (n=14)	All patients (n=85)
Patients with inhibitors, n	6	1	7
Inhibitor rate, % (95% CI)	8.2 (3.1, 17.0)	7.1 (0.2, 33.9)	8.2 (3.4, 16.2)
High-titer[b] inhibitors, n (rate [%])	1 (1.4)	0 (0)	1 (1.2)
Low-titer[c] inhibitors, n (rate [%])	5 (6.9)	1 (7.1)	6 (7.1)

[a]Two pts rolled over from the surgical prophylaxis group to the on-demand group and are counted once in the "all patients" column. [b]≥5 BU/mL. [c]<5 BU/mL.

Conclusions: The efficacy and safety profiles of moroctocog alfa (AF-CC) in the real-world setting when used for OD treatment or surgical prophylaxis in Chinese pts are similar to those previously reported in clinical interventional studies.

PB 231 | Post-authorization Study to Evaluate Pharmacokinetics of FVIII after Moroctocog Alfa (AF-CC), as Well as Safety and Efficacy in Previously Treated Pediatric Patients with Severe Hemophilia A

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Background: Moroctocog alfa (AF-CC), a recombinant B-domain deleted factor VIII (FVIII) protein, is indicated for the treatment and prophylaxis of bleeding events in patients (pts) with hemophilia A. There is limited pharmacokinetic (PK) information available for pts aged < 12 y.

Aims: The primary objective of this open-label, interventional study was to assess the PK of FVIII after administration of moroctocog alfa (AF-CC) in pediatric hemophilia A pts. Assessment of safety and efficacy were secondary objectives.

Methods: This study, which was approved by ethics committees and conducted in 11 EU countries, enrolled previously treated males with severe hemophilia A into 2 cohorts: 1, pts aged < 6 y (n=18); 2, pts aged 6 to < 12 y (n=19). Parents for all pts provided informed consent. Serial plasma samples to measure FVIII were collected up to 48 h after infusion of a 50-IU/kg dose in cohort 2, while cohort 1 had only recovery assessment. Recovery assessments were repeated in all pts through the final visit. After the PK or recovery assessment, pts continued treatment according to approved labels until they reached a minimum of 50 exposures days (EDs; max: 100 EDs).

Results: PK data are shown in Table 1. Recovery was consistent throughout the study. ABRs and incidence of LETE are summarized in Table 2. Among 804 first infusions, 713 (89%) were rated as having an "Excellent" response, and 761 (95%) of 804 bleeding events resolved after 1 infusion. Low-titer, transient FVIII inhibitor development was observed in 4 pts.

TABLE 1 Summary of PK Assessments

Parameter	N	Summary Statistic[a]
Recovery, IU/dL/IU/kg		
Aged <6 y	17	1.7 ± 0.4
Aged 6 to <12 y	19	2.1 ± 0.8
Cmax, IU/mL[b]	19	0.9 (45)
AUCinf, IU·h/mL[b]	14	9.9 (41)
t½, h[b]	14	9.1 ± 1.9
CL, mL/h/kg[b]	14	4.4 (30)
Vss, mL/kg[b]	14	56.4 (15)

[a]Geometric mean (geometric CV%) for all, except for arithmetic mean ±SD for incremental recovery and $t^{½}$. [b]Patients aged 6 to <12-years only. C^{max}, maximum observed plasma concentration; CV, coefficient of variation; AUC^{inf}, area under the plasma concentration-time profile from time zero extrapolated to infinite time; $t^{½}$, terminal half-life; CL, clearance; V^{ss}, steady-state volume of distribution.

TABLE 2 Summary of Selected Efficacy Assessments

Parameter	N	On-demand	N	Prophylaxis
ABR, mean (SD)	14[a]	27.5 (20.4)	22[b]	4.2 (3.8)
LETE	804[c]	0	2457[d]	2
Incidence (95% CI)		0 (0.00, 0.46) [c]		0.08% (0.01, 0.29)

ABR, annualized bleed rate; CI, confidence interval; LETE, less-than-expected therapeutic effect; SD, standard deviation. [a]Number of pts at baseline following on-demand regimen (pts not required to maintain regimen throughout study). [b]Number of pts at baseline following prophylaxis regimen (pts not required to maintain regimen throughout study). [c]Number of bleeding events. [d]Number of prophylaxis infusions.

Conclusions: The PK and recovery of FVIII activity after moroctocog alfa (AF-CC) administration in this study were consistent with the limited available PK data in this age group. Moroctocog alfa (AF-CC) is efficacious for on-demand and prophylactic treatment of hemophilia A in pediatric pts aged < 12 y. The inhibitor rate was 11%, but all were noted to be low-titer, transient, and without clinical significance. Otherwise, no new safety risks were observed.

PB 232 | Understanding Care Utilization and Perceptions of People with Hemophilia A with or without a Current Inhibitor

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Background: The CHOICE Project was conducted in partnership between the CDC and Hemophilia Federation of America to survey persons with bleeding disorders (PWBD) in the US, including those who do not receive care at federally-funded hemophilia treatment centers (non-HTC PWBD).

Aims: To retrospectively explore and characterize demographics, disease severity, and care perceptions among people with hemophilia A (PWH) with or without a current inhibitor (WI or WO) to better understand burdens of illness and how to address those burdens.

Methods: Demographic and clinical data were collected through CHOICE from 04/2013-07/2015 on PWBD. PWBD were recruited to take a 20-minute survey in English or Spanish, online or on paper. Non-HTC PWBD were solicited specifically but others were not excluded from participation. Participants' status as non-HTC PWBD was determined by algorithm. Descriptive statistical analysis was performed based on the chi-square test.

Results: Of 439 PWH, 13% are non-HTC PWH; 17% are female; 11% have a current inhibitor; 2% of WI are mild severity v. 19% of WO (p= 0.0001) **(Figure 1)**. For their bleeding disorder, 96/89% (WI/WO) visited a HCP regularly; 96/84% saw an HCP at least once/year; 94/87% usually see* a hematologist, 14/25% a general practitioner/internist, 35/33% a social worker. Usual places of care* include: HTC 74/62% (WI/WO) and doctor's office 15/15%. In the last 12 months, WI reported a slightly higher satisfaction with care than WO, 3.02 v. 2.95 **(Figure 2)** (*More than one response allowed).

Conclusions: Despite differences in disease severity levels, care utilization, and where and who they usually see for care of their bleeding disorder, WI or WO care satisfaction was nearly equal. Additional analysis is needed to investigate care utilization by age and severity to support care improvements. This sample does not necessarily represent all PWH, as targeted outreach in some regions may have led to over-representation of some participant characteristics.

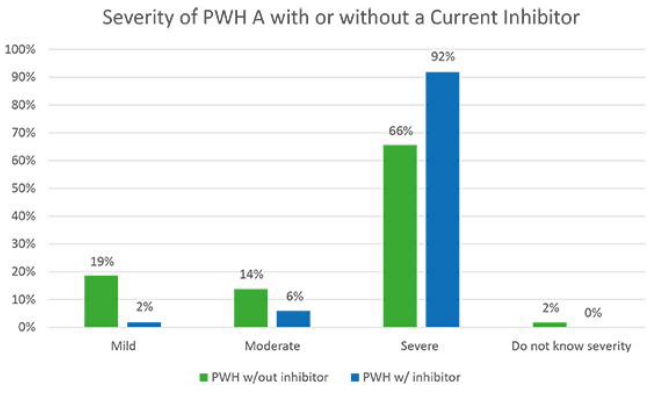

FIGURE 1 Severity of PWH A with or without a Current Inhibitor

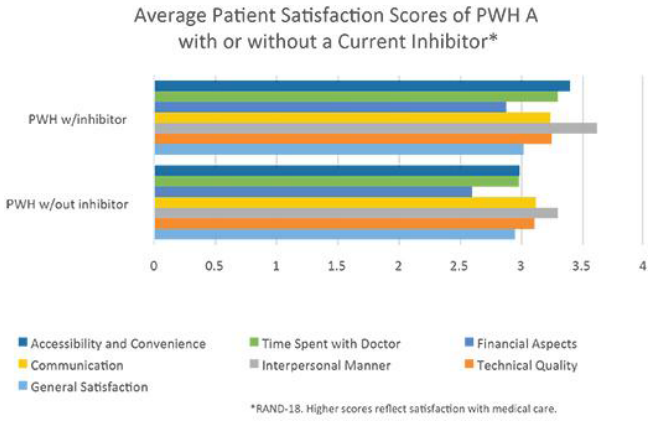

FIGURE 2 Average Patient Satisfaction Scores of a PWH A with or without a Current Inhibitor*

PB 233 | Utilisation of Smartphone Technology in Managing Prophylaxis for Patients with Haemophilia

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Background: Treatment outcomes in Haemophilia are significantly influenced by effectiveness of FVIII/FIX replacement. Adherence is critical, but often difficult to verify. We introduced HomeScan smartphone app (Crimson Tide, Ireland) to facilitate clotting factor concentrate (CFC) scanning, traceability, adherence for patients with Haemophilia taking prophylaxis in Ireland.

Aims: This study aimed to correlate factor replacement prescribed, delivered and infused over a 12 month period and to identify factors associated with use of the HomeScan app.

Methods: The records of patients with Haemophilia using HomeScan from 1 January to 31 December 2016 were reviewed. Demographic data (age, weight, severity of Haemophilia) and data on CFC infusions (dose and frequency prescribed, units of factor delivered and confirmed infusions) were collected and analysed.

Results: 128 adult patients (>16 years) with severe Haemophilia (93 FVIII, 35 FIX deficient) are registered at the National Coagulation Centre, Dublin. Of these, 52 adults use HomeScan to record prophylaxis (34 FVIII, 17 FIX deficient; median age 34, range 17-74, median HJHS 17, range 0-52).

Prophylaxis was prescribed at a median 30.8 IU/kg of FVIII/FIX per dose (range 15.5-78). HomeScan recording of prophylaxis was higher than with paper diaries, with 63.5% patients scanning >50% of prescribed doses and 52% scanning >75% doses. Age was not a

barrier to app usage with no significant difference in percentage prescribed doses scanned in patients >40yo (n=19) or < 40yo (n=33) (median 85.6% vs 51%;p=0.07). A significant relationship was seen between prescribed, dispensed and scanned doses (r^2=0.23, p< 0.005) demonstrating adherence to prophylaxis in those using HomeScan.

Conclusions: Use of the HomeScan App for Haemophilia prophylaxis assures the safety and traceability of CFC and provides real-time, reliable data on concentrate usage. Given the benefits of using this App, maximising uptake by all patients on prophylaxis is a priority.

PB 234 | Retrospective Analysis of Hemostatic Efficacy by Continuous Infusion of Recombinant Factor VIIa: A Single Institution Study

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Background: Recombinant factor VIIa(rFVIIa) is administered to control bleeding of congenital hemophilia with inhibitors and acquired hemophilia patients by frequent every 2-3 hours injections. Some reports show continuous infusions (CI) of rFVIIa can be managed safely and may be useful.

Aims: To estimate efficacy of CI of rFVIIa by global coagulation assays.

Methods: rFVIIa was administered using CI for 19 cases including spontaneous bleeding and surgical procedures of six congenital and two acquired hemophilic patients. Hemostatic efficacy was judged in the first 24 hours after rFVIIa administration was started. Coagulation study including PT, APTT, Fibrinogen, FDP, D-dimer and FVII:C were checked during rFVIIa treatment. Global coagulation assays, ROTEM and TGA were also performed to evaluate the effectiveness of rFVIIa.

Results: There were no adverse effects including thrombosis and thrombophlebitis. According to clinical hemostatic efficacy, effective was 12/19 (63%), partially effective was 6/19 (32%), and ineffective was 1/19(5%). rFVIIa was bolus infused followed by CI in 16 out of 19 cases. The median dose of bolus infusion(BI) of rFVIIa was 110μg kg^{-1} (range: 67-273μg kg^{-1}), the mean dose of BI of rFVIIa was 143μg kg^{-1} followed by CI whose median rate was 42μg kg^{-1} h^{-1}(range: 17-50μg kg^{-1} h^{-1}), mean rate was 36μg kg^{-1} h^{-1}, the median duration of treatment was 7 days(range: 2-16 days), and the median total dose was 5273 μg kg^{-1}(range: 1000-12583μg kg^{-1}). As for global coagulation assays, not TGA with platelet poor plasma (PPP) but ROTEM assay, which showed compatible results with clinical hemostatic efficacy.

Conclusions: CIs of rFVIIa were safe and effective. ROTEM might be better to monitor comprehensive coagulation activity than TGA with PPP during FVIIa treatment.

PB 235 | An Evaluation of the Pharmacokinetics of Turoctocog Alfa in Relation to Body Mass Index (BMI) in Patients with Haemophilia A (HA)

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Background: Management of overweight/obese patients is a challenge in haemophilia treatment. Obese patients have a lower volume of plasma per kilogram of body weight compared with non-obese individuals, resulting in higher post-dose factor FVIII (FVIII) activity levels and higher incremental recovery of injected FVIII as FVIII products are dosed by body weight. The long-term safety and efficacy of turoctocog alfa (NovoEight®), a B-domain truncated recombinant FVIII (rFVIII) product developed by Novo Nordisk for the treatment and prophylaxis of bleeding in HA patients, has been assessed in an international open-label extension trial, guardian™2. The rationale for the pharmacokinetic (PK) subtrial of guardian™2 was to collect PK data as a basis for further investigations of FVIII dosing in HA patients with a BMI ≥30 kg/m^2.

Aims: To investigate single-dose PK following turoctocog alfa administration in patients with a BMI ≥30 kg/m^2.

Methods: Previously treated male patients with severe HA and no history of FVIII inhibitors with a BMI ≥30 kg/m^2 were selected for PK analysis. Patients received a single dose of turoctocog alfa 50 IU/kg after a washout of ≥96 hours. Blood samples taken pre-dose and at time points up to 72 hours post-dose were used for PK analysis. PK results from a patient cohort with BMI < 30 kg/m^2 with turoctocog alfa were used for comparison.

Results: The PK population comprised six patients who received a mean turoctocog alfa dose of 52.7 IU/kg. Mean PK parameters, based on a chromogenic assay, are shown in the Table.

Conclusions: The trend for higher incremental recovery and area under the plasma concentration-time curve in this high BMI group suggests that obese patients should be dosed based on individual PK and clinical response. Further investigation of turoctocog alfa in patients with a BMI ≥30 kg/m^2 is warranted.

TABLE 1

Parameter	PK trial Mean (SD) [Median] Range N=20	guardian™2 PK subtrial Mean (SD) [Median] Range N=6
BMI, kg/m^2	23.4 (3.1) [23.1] 18.1–29.2*	33.35 (2.37) [33.05] 30.5–37.2
AUC, IU*h/ml	18.70 (5.08) [17.72] 10.28–31.06	31.02 (9.78) [33.13] 12.87–41.85
CL, ml/h/kg	2.87 (0.80) [2.82] 1.61–4.87	1.94 (0.95) [1.70] 1.17–3.81
Incremental recovery, (IU/dl)/(IU/kg)	2.8 (0.6) [2.8] 1.5–4.1	3.5 (0.96) [3.4] 2.4–5.0
$t_{½}$ (h)	11.96 (9.28) [9.82] 6.06–48.50	12.40 (3.16) [13.21] 7.23–15.19

*BMI at baseline for patients in the full analysis set (n=23; three patients did not have any chromogenic assay results).

AUC, area under the plasma concentration–time curve; BMI, body mass index; CL, clearance; PK, pharmacokinetics; SD, standard deviation; $t_{½}$, half-life.

PB 236 | Pharmacokinetic Studies of FVIII in Chinese Boys with Severe Hemophilia A

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Background: Although much attention has been paid to the pharmacokinetics (PK) of FVIII products, these have not been well characterized in Chinese boys with severe hemophilia A.

Aims: To assess the PK and the parameters influencing PK of plasma-derived FVIII (pd-FVIII) and recombinant FVIII (rFVIII) products in Chinese severe HA boys.

Methods: A total of 36 boys (pd-FVIII, n=15; rFVIII, n=21) were enrolled in this study, including 6 boys who underwent both rFVIII and pd-FVIII PK studies when they switched from rFVIII to pd-FVIII. Pharmacokinetic characteristics of two different FVIII products were studied according to a reduced 4-point design(1hour, 9hours, 24hours, and 48 hours after the end of infusion). At baseline, all patients' blood groups and levels of VWF:Ag and VWF:C were determined.

Results: The FVIII half-life time ($t_{1/2}$) varied widely from 5.5 to 20.0 hours, with a median of 10.8 hours.The PK of the two FVIII products given separately to the same 6 boys showed that $t_{1/2}$,adjusted in vivo recovery(IVR), area under the plasma concentration curve(AUC), and maximal plasma concentration(C_{max}) of pd-FVIII were slightly higher than those of rFVIII($t_{1/2}$:11.56±3.87hoursvs. 10.21±3.48hours; IVR:2.01±0.42 vs.1.76±0.48; AUC:1364±515.8 vs.1097±367.3;C_{max}(1hour):100.60±21.70vs.86.38±23.68) (*P*=0.2374, *P* = 0.0713,*P* = 0.0124, *P*=0.0018). The clearance (CL), however, was slightly longer for rFVIII(3.93±1.60 mL·Kg^{-1}·h^{-1}vs.4.74±1.72 mL·Kg^{-1}·h^{-1}; *P*=0.0018).Young patients have a shorter FVIII half-life than older patients. Patients with blood group O had a shorter FVIII half-life than patients with non-O blood group. Age and VWF antigen and activity levels were closely associated with FVIII half-life and IVR.

Conclusions: Chinese boys with HA had similar PK values to other ethnic groups and large differences in FVIII PK between individual patients. Age, blood group, and VWF levels were important determining factors for FVIII. The pd-FVIII product had a higher AUC and C_{max} and lower CL than rFVIII product.

PB 237 | Preliminary Results of a Personalized One-year Program to Individualize the Prophylactic Treatment with Recombinant Factor VIII in Severe or Moderate Hemophilia A Patients

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Background: Knowledge of the individual's pharmacokinetics (PK) using Bayesian analysis with 2-3 samples could help to individualize prophylactic therapy with recombinant factor VIII (rFVIII) in severe or moderate hemophilia A patients (HA) and minimize the risk of bleeding.

Aims: Analyze if PK tailored dosing can influence in bleeding control in a single centre cohort.

Methods: Retrospective study in HA with rFVIII (Advate®) prophylaxis from January 2014 to December 2016. A previously available Bayesian model (myPKFit® 2.0) was employed to get individual PK profile using FVIII trough levels retrospective data. PK parameters analyzed were: clearance (Cl); steady state volume (Vss); plasma half-life ($t_{1/2}$); trough level at 48 or/and 72 h (TL); and time to reach rFVIII levels < 1% (T1%). Kruskal-Wallis test (R® 3.1.2) was used to compare

TABLE 1 Clinical variables and PK parameters in severe or moderate HA treated with rFVIII prophylaxis (Part 1)

Patient #	Age (years)	Dose (IU/kg/week)	Gilbert score	AJBR in 2016	Cl (mL/h/kg)	Vss (mL/kg)	t1/2 (h)	TL at 48h/72h (IU/dL)	T1% (h)
1	26	54.5	8	1	4.0	50	10.2	0.8/ND	57
2	45	69.0	22	0	2.8	50	14.4	5.5/1.7	83
3	37	71.4	17	0	2.7	50	14.8	6.2/2.0	86
4	30	64.1	17	0	1.8	50	21.8	13.0/5.9	128
5	17	65.0	3	0	3.0	50	13.2	2.6/1.7	66
6	30	79.4	5	1	2.6	50	15.7	6.3/2.5	83
7	15	75.0	0	1	3.9	60	11.5	3.0/ND	66
8	38	36.5	42	0	2.4	40	14.6	4.1/1.3	77
9	47	72.9	31	1	3.8	50	11.0	1.7/ND	56

TABLE 2 Clinical variables and PK parameters in severe or moderate HA treated with rFVIII prophylaxis (Part B)

Patient #	Age (years)	Dose (IU/kg/week)	Gilbert score	AJBR in 2016	Cl (mL/h/kg)	Vss (mL/kg)	t1/2 (h)	TL at 48h/72h (IU/dL)	T1% (h)
10	29	50.0	5	0	3.3	50	12.6	2.3/ND	63
11	69	50.0	28	3	2.1	50	18.6	11.5/4.7	113
12	32	60.4	14	0	3.3	40	11.0	1.5/0.9	54
13	37	57.2	23	2	2.2	50	16.5	9.4/3.4	101
14	36	51.4	5	0	3.0	50	13.2	8.4/5.9	71
15	19	79.4	8	0	3.2	50	12.8	3.6/1.3	71
16	42	63.4	19	0	3.1	50	13.2	3.1/ND	66
17	50	80.0	36	1	2.5	50	15.9	6.7/2.3	91
18	46	43.9	23	0	2.9	50	14.5	2.9/1.6	70

PK parameters and clinical variables: age, dose/kg, annualized joint bleeding rate (AJBR), Gilbert score.

Results: Eighteen patients were analyzed. Mean age was 35.8 years (SD: 13.2; range 15-69) and 115 PK monitoring (6.4 per patient) were made. Mean PK values (SD) were: Cl 2.9 (0.6) mL/h/kg; Vss 49.4 (0.04) ml/kg; $t_{1/2}$ 14.2 (2.9) h; TL at 48h 5.1 (3.5) IU/dL, TL at 72h 2.7 (1.7) IU/dL and T1% 77.9 (20.1) h. Table 1 and 2 summarizes individual clinical and PK variables.

Along 2017, 7 patients (38.9%) reported joint bleeds (JB). No association between clinical variables and PK parameters were noted, although in 3 patients (#1, #7, #9) JB were related to low $t_{1/2}$. Several JB were reported in 3 patients with high Gilbert score and severe arthropathy (#11, #13, #17), whereas their estimated TL were appropriated.

Conclusions: Bayesian estimate with myPKFit® allows to know the individual PK profile and could be a useful tool to individualize prophylaxis, along with physical activity and bleeding pattern. We are running a personalized one-year program to identify and treat the specific causes of poor bleeding control in HA on prophylactic therapy.

Study supported by Baxalta/Shire grant.

PB 238 | International Clinical Study Investigating the Incidence of Inhibitors in Previously Untreated Patients with Severe Haemophilia A Treated with a High Purity FVIII/VWF Concentrate in a 1:1 Ratio

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Background: The development of neutralising antibodies against FVIII (inhibitors) is the biggest complication of haemophilia A treatment. The SIPPET study shows that up to 37% of previously untreated patients (PUPs) develop inhibitors, mostly within the first 50 exposure days (EDs)[1]. wilate® is a high-purity FVIII/VWF complex concentrate with a physiological 1:1 ratio.

Aims: The primary objective was the assessment of the immunogenicity of this 1:1 FVIII/VWF concentrate in PUPs with severe haemophilia A.

Methods: This prospective, open-label, multi-centre study included 28 subjects with severe haemophilia A and no previous exposure to blood products. Patients were screened for FVIII inhibitors by Bethesda assay every 3 to 4 EDs until ED20 and every 10th ED or at least every 3 months thereafter. Gene analysis was performed in 28 subjects.

Results: 3 of 28 patients (10.7%) developed high titre inhibitors and 4 (14.3%) showed transient low titre inhibitors, not requiring change of treatment regimen or FVIII/VWF concentrate. One patient (3.6%) had a single positive test for a low-titre inhibitor, but died in an accident, so no follow up is available.

The most common gene mutation was an intron 22 inversion in 16/28 patients (57.1%) - higher than the overall prevalence for this mutation in severe haemophilia A patients (30%).[2,3] Despite this high-risk population only 8/28 (28.6%) patients developed inhibitors. All 3 high-titre inhibitors and 3/5 low-titre inhibitors developed in patients with intron 22 mutations.

Conclusions: The SIPPET study reported overall inhibitor rates of 23.2% (29/125) and 37.3% (47/126) for PUPs treated with pd FVIII/VWF and rFVIII from hamster cell lines, respectively.[1] The overall inhibitor incidence (28.6%; 8/28) during this study with a 1:1 pdFVIII/VWF concentrate was in line with these results. Moreover, the incidence of high titre inhibitors in this study (10.7%) compares favourably to that in the pdFVIII/VWF arm of the SIPPET study (16%, 20 of 125).

PB 239 | Magnitude of Dosing Adjustment of AFSTYLA in Clinical Trials

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Background: AFSTYLA (rVIII-SingleChain) is indicated in adults, adolescents and children with hemophilia A for control and prevention of bleeding episodes, routine prophylaxis to prevent or reduce the frequency of bleeding episodes, and for perioperative management. In one adult/adolescent (12-65 years) trial and one pediatric trial (<

12 years), dosing was according to investigator's discretion in order to reflect practice in the clinical setting. Adjustment of dose or frequency was allowed at any time during the studies.

Aims: To estimate the difference in factor use of AFSTYLA between the final and the initial dosing regimens (DELTA), in two pivotal clinical trials.

Methods: Factor use per week for prophylaxis was calculated based on frequency of injection and dose per injection, for the initial and final dosing regimens. DELTA was then taken (in IU/kg per week, for prophylaxis). Patients were grouped by initial dosing frequency and DELTA was summarized for each group (in which the same patients were followed).

Results: In the adult/adolescent trial, 47 and 79 subjects initially received two and three injections per week, respectively. DELTA < =0 occurred in 78.72% of subjects receiving 2 injections per week and 77.22% of subjects receiving 3 injections per week. In the pediatric trial, 43 subjects initially received 2 injections per week with DELTA < =0 in 69.77% of the subjects; 24 subjects initially received 3 injections per week with a DELTA < =0 in 75.00% of the subjects.

Conclusions: The majority of subjects had the same or decreased factor use between the initial and final dosing regimens. Although real world factor consumption of AFSTYLA is yet to be measured, these results indicate that it is likely to be predictable and stable during the course of treatment.

PB 240 | Validity of Angiography and Therapeutic Embolization in the Treatment of Acute Bleeding in Hemophilia Patients

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Background: Clotting factor replacement is often inadequate for bleeding control in hemophilia patients, and surgery may impose a real challenge to clinicians. Arterial angiographic intervention has been utilized for the control of bleeding in the local blood vessels. Furthermore, arterial angiography is considered for hemophilia patients with various complications, especially severe bleeding at the site of a puncture.

Aims: This study inquired into the clinical courses and prognosis in hemophilia patients with bleeding, who had undergone angiography, and also evaluated the validity of diagnostic angiography and therapeutic embolization in these patients.

Methods: In this study, angiography was carried out in five hemophilia patients. These patients had uncontrolled bleeding even after clotting factor replacement.

Results: Three of all five severe hemophilia patients had antibodies to factor VIII. Two patients had postoperative hemorrhage after surgery, those were small bowel segmental resection for hemorrhagic infarction and total knee arthroplasty. Two patients had spontaneous bleeding—one with retroperitoneal hematoma and the other with hemoptysis after pneumonia. One patient had hemothorax caused by trauma. The common femoral artery puncture was performed under ultrasonography. Of these 5 patients, four were confirmed as having active bleeding and underwent angiographic embolization. Clotting factor concentrates were administered to reach the 100% factor level for hemophilia patients without inhibitors, while adequate doses of bypassing agents were administered for hemophilia patients with inhibitors. However, one patient developed uncontrolled bleeding at the puncture site, and eventually expired.

Conclusions: The consideration is that angiography and therapeutic embolization may be the preferential procedures in the treatment of hemorrhagic complications, refractory to clotting factor concentrates. Further comprehensive, skilled multidisciplinary team studies are necessary to reduce hemorrhagic complications.

PB 241 | Postraumatic Anterior and Posterior Thigh Pseudotumor in Young and Adolescent with Hemophilia

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Background: The management of the patient with a haemophilic pseudotumour is complex and carries a high rate of potential complications. There are a number of therapeutic alternatives for this dangerous condition: embolization, radiation, percutaneous management, surgical removal and exeresis, and filling of the dead cavity.

Aims: To describe 2 cases of posttraumatic pseudotumors successfully treated by well planning of elective surgery.

Methods: We describe two patients, one of 21 years of age with moderate Hemophilia A on demand treatment and recent history of mass of the posterior part of the left thigh after traumatic injury. The mass slowly progressed asymptomatically with a large mass on the posterior part of the thigh, magnetic resonance and thigh angiography were obtained showing pseudotumor of the posterior muscular compartment of the thigh. Second patient is a 13-year-old patient with a right upper quadrant mass of the thigh after traumatic injury 4 months evolution. The patient consulted due to a mass with progressive increase associated with paresthesias of the anterior thigh. Rx images, soft tissue ultrasound and magnetic resonance revealed a mass with approximately 300 mls of blood in different stages of coagulation with formation of a pseudo capsule without direct contact with the anterior cortical of the right femoral shaft.

Results: Therapeutic intervention: Due to the large size and location of the mass, it was decided to perform a surgical treatment. A large mass was found during surgery that could be completely resected and sealed with the use of fibrin sealants with a satisfactory postoperative evolution. Second patient underwent surgery for drainage and application of fibrin sealants with a remarkable postoperative outcome.

Conclusions: Untreated, proximal pseudotumours will ultimately destroy soft tissues, erode bone and may produce neurovascular complications. Surgical excision is the treatment of choice but should only be carried out in major haemophilia centres by a multidisciplinary team.

PB 242 | Vitamin D Deficiency in Patients with Haemophilia: Initial Workup and Follow-up of Supplementation

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Background: Patients with haemophilia are at increased risk for developing bone loss. Predisposing factors include overweight, decreased physical activity, and prolonged periods of immobility. Although it is well established that vitamin D deficiency contributes to bone loss, only few studies have evaluated vitamin D status in haemophilia patients.

Aims: The aim of the present study was to evaluate the vitamin D status in haemophilia patients.

Methods: From thirty adult patients with haemophilia A (n=24, aged 19 to 73 ys) or haemophilia B (n=6, aged 15 to 74 ys) treated at the Regional Haemophilia Centre Rostock, twenty six were investigated for vitamin D deficiency. Levels of 25-hydroxyvitamin D were measured by immunoassay (DiaSorin).

Results: At baseline, vitamin D concentrations of only two patients were in the normal range (> 75 nmol/L). In contrast, 22 of 26 patients (85 %) were considered deficient in vitamin D (< 50 nmol/L) including six patients with concentrations below 20 nmol/L. A supplementation regimen consisting of 40,000 units per week was accepted by 22 patients and monitored by subsequent vitamin D analyses at six months intervals. Sixteen of twenty two patients (73 %) reached improved values within six months and did not return to vitamin D deficiency. Improved vitamin D concentrations were accompanied by normal PTH levels in all but two patients. Furthermore, follow-up analyses of TRAP5b as indicator of osteoclastic activity demonstrated decreasing bone resorption with vitamin D reaching normal levels. Compliance issues were the most likely explanation for the failure of the remaining six patients to obtain benefit.

Conclusions: The present study suggests that measuring vitamin D may form part of assessing the risk of bone loss in haemophilia patients. Vitamin D supplementation is an inexpensive and safe measure to correct vitamin D deficiency and may contribute to the prevention of bone loss as has been demonstrated by improved bone metabolism markers in the present study.

PB 243 | Severe Haemophilia A Patients Born between 2000- 2015 at a Single Haemophilia Centre: Characteristics and Inhibitor Development

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Background: Haemophilia is a rare disease and detailed documentation and evaluation of treatment and outcomes is essential.

Aims: The aim of this review was to determine the characteristics and outcomes of the severe haemophilia A (HA) patients at our centre.

Methods: This was a single-centre retrospective observational study. All severe HA (FVIII < 1%) patients born 2000-2015 and diagnosed at or early referred to our centre were included. Date of birth, mode of delivery, family history of haemophilia, F8 mutation, neonatal head

TABLE 1 Characteristics of the 7/40 patients that developed clinically relevant inhibitor

Year of birth	F8 mutation	Family history/ Mode of delivery	Neonatal head bleed/ Treatment	Age at haemophilia diagnosis (years)	Age at first treat (years)	Age at inh diagnosis (years)	ED at inh diagnosis	Type of treatment at inh diagnosis	Max inh titre (BU)
2010	Intron 22 Inv	Sporadic/ Vaginal	No	0.8	0.9	1.0	8	Prophylaxis 1 / week	134
2009	Nonsense subst	Sporadic/ Vaginal	Cephalohematoma/ Not treated	0.8	0.8	1.2	7	On-demand	51
2006	Small insert	Sporadic/ Vaginal	No	0.8	0.8	1.2	8	On-demand	24
2005	Intron 22 Inv	Sporadic/ Vaginal	No	1.0	1.3	1.7	15	Prophylaxis 1 / week	45
2004	Nonsense subst	Sporadic/ Forceps assisted	Subgaleal haematoma/ FVIII	0.0	0.0	1.2	21	On-demand (intensive treatment)	43
2004	Small insert	Sporadic/ Forceps assited	Subgaleal haematoma+ cephalohematoma/ FVIII	0.0	0.0	2.3	50	Prophylaxis 3/ week	54
2000	Small insert	Sporadic/ Forceps assited	Subdural haematoma +cephalohematoma/ FVIII	0.0	0.0	0.6	38	Prophylaxis 3/ week	4

bleeding, age at diagnosis and age at first treatment and occurrence of clinically relevant inhibitor were collected from medical files.

Results: Forty severe HA children were evaluated (median age at evaluation 7.4 years; range 1.2-16.6 years). All patients were receiving prophylactic treatment (except 1 on-demand) via peripheral vein infusion and 39/40 received recombinant FVIII products. FVIII gene mutation was known in all cases (92% null-mutations; 53% Intron22 Inversions). Twelve (30%) patients had a family history of haemophilia and 28 (70%) were sporadic cases. Type of delivery was predominantly vaginal (80%) in spontaneous cases and caesarean (58%) in familial. Diagnosis was done at a median age of 0.5 years (range 0-1.2 years) and first FVIII infusion at 0.8 years (0-1.9 years). There were 7 delivery-related head bleeds (3 minor cephalohematomas and 4 subgaleal and/or subdural haematomas), all in spontaneous cases. Clinically relevant inhibitor developed in 7 (17.5%) patients at a median age of 14 months (range 7-28 months) and after 15 ED (7-50 ED) (Table 1). In the period 2010-15 only 1/18 children developed inhibitor, compared to 6/22 in 2000-09 (5.5 % versus 27.2%). All inhibitors were eradicated with immune tolerance treatment with FVIII.

Conclusions: A high ratio of sporadic versus familiar cases has been found in our cohort. The cumulative incidence of inhibitor in the last 15-year has been low (17.5%) and we detected a tendency of decreasing over the studied period.

PB 244 | The Effectiveness of PK-Tailored Tertiary Prophylaxis for Severe Hemophilia A Children: A Pilot Study from BCH

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Background: Hemophilia A (HA) is an X-linked inherited coagulation disorder, regular prophylaxis is the standard treatment method of severe HA. The pharmacokinetics (PK) driven prophylaxis is a feasible but also expensive in developing countries, like China. PK-tailored prophylaxis using more infusion frequency and allowing the 30h per week duration for FVIII level < 1% in PK driven prophylaxis might be more cost-effective method.

Aims: To explore the efficacy of PK-tailored tertiary prophylaxis in severe HA children.

Methods: Since January 2015 to May 2016, we have launched a pilot study on 15 cases from 5 to 16 year-old at Beijing Children´s Hospital (BCH). The study period was divided in two phases:

Phase I: using the original prophylaxis for 6 months (86.7% in low-dose and 13.3% in moderate prophylaxis regimens);

Phase II: separate the study cohort in two groups:

(1) PK-tailored group (modified the prophylaxis regimen according to PK data for the next 6 months),

(2) Maintain group (continued with their original regimen for the next 6 months). Comparing the bleeding rate, infusion frequency and factor consumption between two groups.

Results: In the PK-tailored group, Annual Bleeding Rate (ABR) of 8 cases was reduced from 14.2 to 4 times with annual factor consumption increased from 1619 IU· kg^{-1} to 2401.9 IU· kg^{-1} and the infusion frequency increased from 104 times y-1 in Phase I to 156 times y^{-1} in Phase II ($p< 0.05$), but without significant different in AJBR(Joint) ($P>0.05$). Between two groups, there were higher ABR in PK-tailored group in Phase I, but without difference in Phase II.

Conclusions: In conclusion, the pilot study showed that PK-tailored prophylaxis maybe the more cost-effective individual prophylaxis in China, but need further study.

PB 245 | Korea Hemophilia Foundation Registry Trends 1991-2015: Patient Registry, Demographics, Health Services Utilization

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Background: The Korea Hemophilia Foundation (KHF) founded in 1991 has registered Korean hemophiliacs, and updated the cohort by establishing the network of medical information between affiliated clinics.

Aims: The KHF data of over 20 years' experience was analyzed, with inhibitors change over 10 years since 2004.

Methods: Among the 2,482 patients registered in the KHF, 2,303 were alive at the end of 2015 because 179 patients had died; there were 1,654 patients (71.8%) with hemophilia A and 409 (17.8%) with hemophilia B and 116 (5.0%) with von Willebrand disease (VWD).

Results: From 1991 to 2015, while the general South Korean population grew by 15%, the KHF registration patients grew about 200% (n=2,303). Registered hemophilia patients was approximately 1:20,000 males at 1995, which gradually increased over years, and up to 0.9:10,000 males at 2015, with 80% having factor VIII deficiency. Two third of registered patients had ´severe´ disorder, 20% had moderate hemophilia and 11% was classified with mild deficiency. The registered patients with VWD grew by 116; they are now roughly one-eighteenth of the population of hemophiliacs. In patients with hemophilia A, the overall prevalence of inhibitors has a little decreased over 10 years. At least the prevalence of inhibitors in hemophilia A has not increased in spite of the increased use of recombinant factor VIII (rFVIII) agents. The prevalence of inhibitors in hemophilia B has not changed in spite of the increased use of recombinant factor IX agents.

Conclusions: Although the KHF patient base has grown much faster than the general Korea population, some patients have still been left out, especially those with VWD. Therefore, a more efficient and nationwide registry system should be fixed for the provision of comprehensive management for this disorder. Decreased or non-changed prevalence of inhibitors in hemophilia A may warrant the use of rFVIII use.

PB 246 | Safety and Efficacy of Nonacog Alfa in Patients with Hemophilia B: Results of a Post-authorization Study in Usual Care Settings in China

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Background: Acquisition of real-world data is key to understanding the efficacy and safety of marketed medicines. Nonacog alfa is a recombinant coagulation factor IX (FIX) approved in China for the control and prevention of bleeding events and for perioperative management in hemophilia B patients (pts).

Aims: Evaluate the safety and efficacy of nonacog alfa in a real-world setting in Chinese pts with hemophilia B.

Methods: A prospective, open-label, multicenter study, approved by ethics committees, was conducted in hemophilia treatment centers in China. All pts provided informed consent and received nonacog alfa (on-demand [OD] or as prophylaxis) according to approved labeling for 6 mo or 50 exposure days. Key assessments in both the OD and prophylaxis settings included incidence of FIX inhibitor development, allergic reactions, and thrombotic events, serious adverse events (SAEs), annualized bleeding rates (ABRs), and incidence of less-than-expected therapeutic effect (LETE). The number of infusions needed to treat each new bleeding event and treatment response were also assessed in the OD setting.

Results: Of 70 enrolled pts (mean ±SD age, 7.8 ±7.2y; severe hemophilia [FIX < 1%], 51%; previously untreated, 16%), 66 (94%) pts completed the study. One pt (aged < 6y) developed a transient low-titer FIX inhibitor (0.71 BU/mL) during prophylaxis, which was considered an SAE. Another pt had an SAE of oral mucosa hematoma. No pt had an allergic reaction or a thrombotic event. The table summarizes ABR and LETE results. The mean number of infusions to treat each new bleeding event was 1.5 ±1.7; 79% resolved with 1 infusion. Most OD infusions (88%) were rated "excellent" or "good." Results were similar across subgroups defined by age, disease severity, and prior factor exposure.

TABLE Efficacy Results

Parameter	Prophylaxis Patients (n=57)	On-demand Patients (n=18)
ABR, mean (SD)	6.5 (9.1)	26.3 (23.1)
LETE, n	2	0
Incidence (95% CI)	0.1% (0.0%, 0.4%)	0

ABR, annualized bleeding rate; CI, confidence interval; LETE, less-than-expected therapeutic effect; SD, standard deviation.

Conclusions: The efficacy and safety profiles of nonacog alfa in the real-world setting in Chinese pts are similar to those previously reported in clinical interventional studies.

PB 247 | Spinal & Cranial Neurosurgical Procedures in Patients with Hemophilia

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Background: Optimal hemostatic management of patients with hemophilia (PwH) undergoing surgery is challenging. Current surgical management in PwH is based on expert opinion and experience as guidelines generated from prospective trials are lacking; infrequent procedures have less supporting data to guide therapeutic decision making.

Aims: To describe the Indiana Hemophilia and Thrombosis Center (IHTC) experience with neurosurgical procedures (NP) in PwH.

Methods: The IHTC surgical database (SD) contains 1962 surgical plans with outcomes. The SD was queried for spinal/cranial NP and cases analyzed; procedure related medical records were reviewed. Both hemophilia A (HA) and B (HB) and 2 female carriers (levels of 20%) were included. IRB approval was obtained.

Results: Twenty NP were performed between 1998-2016 in 18 PwH including 16 spinal (9 fusions, 3 discectomies, 4 other) and 4 craniotomies (2 tumor resections, lobectomy, subdural hematoma). Thirteen HA underwent 14 procedures; 5 HB underwent 6 procedures. Median age was 41.5 years (range 1.5-71). Two PwH had remote history of transient or low titer inhibitors.

Target preoperative factor levels were ≥80%. Continuous infusion factor concentrate was used for severe/moderate, and 50% of mild PwH. Median therapy duration for severe/moderate was 19 days (range 8-30). Antifibrinolytics were not used in spinal procedures. Hemostatic complications occurred in 2 of 20 NP, both spinal surgery; one with intraoperative hemorrhagic shock, the 2nd with delayed surgical site hematoma after factor replacement stopped. No postoperative inhibitors or thrombotic complications occurred. Table 1 summarizes NP based on factor deficiency, age, length of therapy, and hemostatic complication.

Conclusions: Two of 20 PwH undergoing NP experienced bleeding, both in spinal surgery (2/16); no antifibrinolytics were used. Case details will be presented with recommendations; hemostatic control in specific NP may benefit from antifibrinolytics while others may require more prolonged therapy.

TABLE 1

Surgery Type	Spinal Surgery						Craniotomy	
Severity§	Mild HA	Mild HB	Moderate HA	Moderate HB	Severe HA	Severe HB	Severe HA	Severe HB
# Patients N=18	7±	2	1	1	2	1	3	1
# Procedures N=20	8	3	1	1	2	1	3	1
Age (years)† 51; 46 (20-59) 32, 59, 63			71	45	12, 34	38	14, 16, 1.5	43
Baseline Factor Level (%)†	18; 15 (6-20)±	17, 26	1	2	<1	<1	<1	<1
Days Hospitalized†	3; 4.4 (2-12)	2, 2, 10	5	2	4, 15	5	11, 14	18
Total Days Treated†	4; 9 (3-19)	7, 8	16	8	11, 29	15	22, 30	29
Bleeding Complications	1*	0	0	0	1**	0	0	0

§ Mild HA >5-<40%; Moderate 1-5%; Severe <1% ± Two low level (20%) female carriers † For N >3: Median; Mean (Range), For N≤3 actual values reported * 57 year old male with mild (20%) HA underwent cervical discectomy and fusion, bleeding from incision site POD #20 after completing 2 weeks of therapy requiring additional factor therapy and hematoma evacuation ** 12 year old with severe HA underwent scoliosis repair complicated by profound blood loss, hemorrhagic shock and coagulopathy

PB 248 | Pseudotumors in Hemophilia: Effective Surgery with Application of Frozen Autologous Platelet Gel

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Background: Hemophiliac pseudotumor (HP) is an encapsulated, chronic, slowly growing hematoma . Frozen autologous platelet gel (FAPG) contains a number of growth factors with beneficial effect on wound healing and post surgery tissue reconstruction.

Aims: Was to present our experience in management of hemophilic pseudotumors-

surgical procedures followed by application of FAPG.

Methods: Four haemophiliac patients aged 38- 57 were subjected to surgical treatment within the last 9 years.

Results:

Case 1: Hemophilia A (38yrs) patient with HP of left femur tumor (21x12x12 cm) pressed to popliteal fossa and limited knee extension. Surgery consisted in tumor evacuation with excision of its pseudocapsule and drainage. No tumor recurrence was reported.

Case 2: A 55-year old hemophilia A patient admitted to IHTM for recurrent HP in left retroperitoneal region 33x21x20 cm tumor pressing on left pelvic bone and hip joint. The front wall of the tumor was removed, the cavity drained and filled with FAPG. Replacement therapy with factor VIII and 18 portions of FAPG were applied into the HP cavity.

Case 3: A 41-year-old Hemophilia A patient (41yrs) with diagnosed factor VIII inhibitor (0.7 BU) and HP of the left iliopsoas muscle infiltrating iliac bone. HP mass was surgically removed and tumor cavity was drained. Replacement factor VIII therapy and 23 portions of FAPG were applied into the HP cavity. A significant reduction of HP cavity size was achieved and the wound healed.

Case 4: A 57-year Hemophilia B (57yrs) patient with bleeding HP (mass 2.0kg), damaging the left iliac bone, sacral bone and one lumbar vertebra. HP mass was removed. Infusion drainage, antibiotics 24 portions of FAPG and factor IX concentrate were applied.

Conclusions: One-stage surgery complete removal of HP from the abdominal cavity is often difficult. Further surgical procedures are required to remove residual parts of the tumor and these combined with simultaneous FAPG application significantly improve the quality of life of hemophilia patients.

PB 249 | Rationale and Study Design for a Prospective, Non-interventional Phase 4 Study Evaluating the Effectiveness of Recombinant Factor VIII Fc Compared with Conventional Factor VIII Concentrates in the Prophylactic Treatment of Haemophilia A: The A-SURE Study

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Background: The efficacy and safety of recombinant factor VIII Fc (rFVIIIFc) have been established in phase 3 studies and extension

studies are ongoing, but there is still a need to provide routine clinical practice data to physicians, payers and patients, assessing the possibility of improved protection and reduced treatment burden.

Aims: To describe the rationale and design of the ongoing A-SURE study evaluating the effectiveness of rFVIIIFc compared with conventional FVIII in the prophylactic treatment of haemophilia A.

Methods: A-SURE is a prospective non-interventional study (NCT02976753) including males with haemophilia A from approximately 45 centres across Europe. Eligible are patients without inhibitors who currently receive and have received FVIII prophylaxis ≥12 months pre-enrolment. Personal history concerning bleeding and prescription over the pre-study period is collected. Patients in other clinical studies or those who received other extended half-life products than rFVIIIFc one year prior to enrolment will be excluded. The protocol will not dictate any treatments and all visits will be according to local practice. Each patient on rFVIIIFc will be matched for age and FVIII concentrate consumption with a patient on conventional FVIII. Safety reporting will follow European Union regulation guidelines. Statistical analyses will be adjusted for propensity scores based on baseline characteristics.

Results: The investigators plan to enrol 350 patients. The annualised bleeding rate (overall, joint and target-joint), injection frequency and concentrate consumption are measures of the primary study outcome evaluating the effectiveness of rFVIIIFc compared with conventional FVIII during 24 months. Secondary endpoints will assess patient reported outcomes and health economic parameters.

Conclusions: A-SURE, a prospective and comparative haemophilia A study, will provide evidence on the effectiveness of prophylaxis with an extended half-life FVIII compared with conventional FVIII in routine clinical practice.

PB 250 | Outcomes in Acquired Hemophilia A Based on the Choice of Hemostatic Agent: A Single Institution Retrospective Cohort Study

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Background: Acquired Hemophilia A (AHA) is a rare disease affecting factor VIII. Only 2 products are FDA approved for bleed management in AHA - recombinant activated factor VII (rFVIIa) and recombinant porcine factor VIII (rpFVIII) - but it is not yet known how these drugs compare in terms of efficacy and overall cost.

Aims: Retrospectively review patients admitted with AHA to compare outcomes and costs based on the hemostatic agent used.

Methods: We identified patients with AHA admitted at to our facility from Dec 2014-Dec 2016, extracting relevant data including bleed severity, transfusions use, type and amount of hemostatic agent used, and survival. We calculated the estimated cost of each hemostatic therapy, using average wholesale costs.

Results: Nine patients met inclusion (mean 71.3 yo, mean inhibitor titer 63.5 BU). All presented with bleeding: 2 with Grade 4, 6 with Grade 3, and 1 with Grade 2 bleeding per WHO criteria. 8 of 9 required transfusion (mean 4.3 units/pt). 2 required surgery: internal iliac artery embolization, and spinal decompression. Of the 7 receiving hemostatic agents, 6 received rFVIIa alone and 1 received rFVIIa and rpFVIII. The average maximum rpFVIIa cost per day was $103,618 USD, with a mean total cost of $329,896/admission based on these real clinical scenarios. For the patient treated with rpFVIII, the maximum daily cost was $123,676, with a total cost of $920,923/admission. All patients achieved adequate hemostasis and all survived.

Conclusions: While our study is too small to draw strong conclusions, we found that both agents achieved adequate hemostasis in AHA patients, and all patients survived to hospital discharge. rpFVIII was more expensive per day than rFVIIa in our clinical experience. Because there are no head-to-head trials of rpFVIII vs rFVIIa, and no compelling data to suggest greater hemostasis with one versus the other, clinicians may consider reserving rpFVIII for cases of severe bleeding.

PB 251 | The Use of Multiple Drugs in a Sample of Adult Patients with Hemophilia: Considerations and Perspectives on Aging

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Background: Owing to their much improved management with coagulation factor replacement therapy, patients with hemophilia (PWH) have currently reached a life expectancy similar to that of males in the general population and thus attain older ages much more often than before. However, their aging is associated with an array of hemophilia-related comorbidities (arthropathy, viral infections, liver and renal disease) that begun when treatments were inadequate but are becoming superimposed on the multimorbidities typical of aging. Thus, more and more older PWH need, beside replacement therapy, other drugs that may interfere with their bleeding disorder, increase its severity and cause additional morbidities.

Aims: To tackle these novel and so far poorly investigated issues, this study had the goal to retrospectively describe the number of comorbidities and multimorbidities and the associated polypharmacy in a sample of adult PWH.

Methods: A cross-sectional study analyzed the therapeutic agents employed by patients with severe hemophilia (A or B). The study was focused on adults aged 40 or older living in Italy.

Results: Of 135 patients included with a mean age of 47.7(SD= ±12.3) we found that older patients (50 years of age or more) took more drugs than younger patients (mean 3.4; SD ± 2.0; vs = 2.6; ± 1.8, p=0.0393). Only older hemophiliacs took anti-hypertension drugs (16.2%) and angiotensin-converting-enzyme (ACE) inhibitor drugs (40.5%) but they

did use less frequently anti-inflammatory drugs in comparison with younger PWH (18.9% vs 21.4%).

Conclusions: These findings present a favorable picture pertaining to multiple drug intake in adults and old people with hemophilia. Even if this study is an explorative description, it is specifically oriented to increase the currently scarce knowledge on the health status of older PWH, including their comorbidities, multimorbidities, disease clusters and polypharmacy features.

PB 252 | Sudden Decrease of High-titer Anti-FVIII Antibodies with Anti-inhibitor Coagulant Complex in a Patient with Severe Hemophilia A and Poor Response to Immune Tolerance Induction

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Background: This 7-year-old patient was diagnosed with severe hemophilia A (HA) at 1 month of age. The hemophilia carrier status of the mother was previously unknown, and he was born full term in Jan-2010 by instrumental delivery with suction cup. Neurologic symptoms and cephalohematoma were observed after a few hours. Computed tomography revealed subgaleal and subdural hematoma in the right cerebral hemisphere. Anemia (Hb 5.7 g/dl) was determined and the patient received blood transfusions before and after emergency craniotomy. The subsequent clinical history and laboratory data are summarized in Table 1. The patient developed high-titer inhibitors early after exposure to replacement factor. Viral infection serology is negative. **Aims:** To describe the experience of inhibitor management in this patient.

Methods: At 3 years and 10 months, rescue immune tolerance induction (ITI) was initiated due to poor progress (Table 1). Response to treatment in Jan2016 (6 years old) was poor. Hemorrhagic episodes had increased and frequent infusion of high doses of NovoSeven were needed. NovoSeven was suspended and treatment with anti-inhibitor coagulant complex Feiba (1000 U/8 h) was initiated due to left knee hemarthrosis. After resolution, prophylaxis regimen was set at 1000 U/day.

Results: FVIII inhibitors reduced dramatically until almost undetectable (Figure 1), and clinical manifestations have greatly improved, with an important favorable impact in the QoL of the patient and his family. At present, the patient is receiving Fandhi 4000 IU and Feiba 1000 U every 48 h.

Conclusions: In this patient with poor response to ITI strategies, Feiba achieved negative inhibitor titer and elicited successful bleeding prophylaxis.

Note: *Labeled use of Feiba is 50-100 U/kg, ≤200 U/kg a day. Feiba is the only agent with an approved indication for bleeding prophylaxis in HA patients with anti-FVIII antibodies. Labeled use of NovoSeven® is 90 µg/kg initial dose in intervals adjusted according to clinical situation.*

TABLE 1 Patient's hemophilia history

Age	Clinical Event	Clinical Management
Birth	Full term delivery with hemorrhagic complications (described in text)	Blood transfusions (Hb 5.7 g/dl) and emergency craniectomy
13 days	Hematoma in right hand related to fluid infusion site and ecchymosis in other puncture sites	Hematology consultation in view of perinatal history.
15 days	Patient diagnosed with severe HA. Intron 22 inversion FVIII:C: <1; FVIII:Cr undetectable; Inhibitor: 0 BU	Replacement therapy with octocog-alpha (Advate® 200 IU/24 h) initiated. (Patient's weight approx. 3-3.5 kg)
45 days	After 23 exposures to replacement factor, patient developed low-response inhibitor (1.07 BU). FVII:C 10.6%	ITI with 500 IU/24 h of activated eptacog alfa (Novoseven®) initiated
3 years 10 months	Poor evolution of FVIII inhibitors with increasing titer in the previous year. FVIII:C 0.8%; FVIII:Cr undetectable Inhibitor: 6.8 BU	FVIII administration suspended. When inhibitor reached 6.8 BU, initiation of rescue ITI with plasma-derived FVIII concentrate rich in vWF (Fandhi®) 2000 IU/24 h (137 IU/kg) and prophylaxis with eptacog alfa 2 mg/48 h
6 years	Poor response to anti-inhibitor treatment (See methods)	Treatment with anti-inhibitor coagulant complex Feiba® (See methods)

Hb, hemoglobin; HA, hemophilia A; BU, Bethesda Units; ITI, immune tolerance induction; vWF, von Willebrand factor

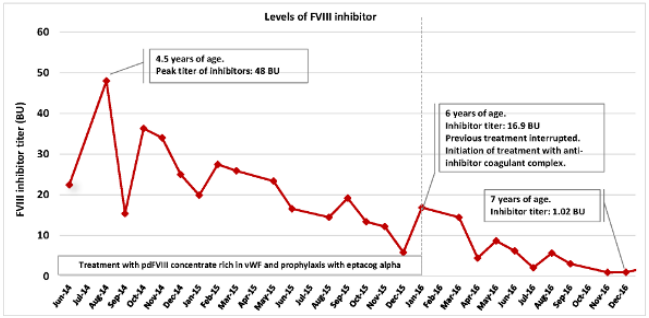

FIGURE 1 Evolution of inhibitor titer and clinical events in the last 2.5 years (since the present laboratory data monitoring system for frequent sa

PB 253 | Projecting Product Consumption of AFSTYLA vs. ADVATE in the United States

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Background: AFSTYLA (rVIII-SingleChain) is indicated in adults, adolescents and children with hemophilia A for control and prevention of bleeding episodes, routine prophylaxis to prevent or reduce the frequency of bleeding episodes, and for perioperative management.

Aims: To estimate product consumption of AFSTYLA from the perspective of a US health plan and to compare against another recombinant factor VIII product, ADVATE.

Methods: Average consumption of product per patient (IU/kg) was calculated and compared between AFSTYLA and ADVATE, based on a previously-developed budget impact model. The patient population consisted of those with hemophilia A, stratified based on age (< 6 years, 6 to < 12 years, 12 to < 18 years, and ≥18 years) and proportion on prophylaxis versus on-demand. Dosing, median annualized bleeding rates, and average number of infusions needed to treat bleeding were based on product prescribing information and other published data. Dosing frequency was 2 to 3 times weekly for AFSTYLA and 3 to 4 times weekly for ADVATE.

Results: In the total prophylaxis population, the average monthly consumption of AFSTYLA (including treatment for breakthrough bleeding) was lower than ADVATE by 6.4% among children < 6 years and 6 to < 12 years of age, and by 16.7% among adolescents 12 to < 18 and adults ≥18 years of age. When excluding treatment of breakthrough bleeds, the average monthly consumption of AFSTYLA was lower than ADVATE by 4.8% among children, and by 16.7% among adolescents and adults. In the on-demand population, consumption of AFSTYLA per bleeding episode was lower than ADVATE by 41.3% among children, and by 27.2% among adolescents and adults.

Conclusions: Without compromising the efficacy, the introduction of AFSTYLA to a US health plan may result in lower average product consumption, compared to ADVATE, in both the prophylaxis and on-demand settings.

PB 254 | Correlation between Pharmacokinetic of Factor VIII and Bleeding Frequency of Children withsevere Hemophilia A in China - A Single Center Data Analysis

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Background: The pharmacokinetic (PK) of factor VIII(FVIII)is individualized in hemophilia A (HA) children, the result of PK will be the indicatorof patients' bleeding phenotype and the instruction for their personalized replacement regimen.

Aims: The aim of this study was toinvestigate the relationship of PKof FVIII and time< 1 IU dL^{-1}per weekwith bleedingfrequencyof severe HA children in China.

Methods: Total24 cases enrolled in our center during Feb. 2015 to Oct. 2015, all of them were given 50IU/kg of FVIII concentrates after 72h washout period. Five points' samples FVIII:C activities were tested: baseline, 1 h, 9h, 24h, and 48h, usingWinNonlin(Pharsight Corp., Phoenix, AZ, USA) soft for PK testing, then calculated the individual half-life($t_{1/2}$) and the time (h) of FVIII concentrations < 1IU dL^{-1} within a week during prophylaxis. Date of baseline and rate of bleedings(joint bleeds and ,other bleeds') was recorded.

Results: The median age was 8.95 (range 5 to 15.98) years old, and the median annual bleeds rate / joint bleeding (ABR/AJBR) were 20 (range 5 to 30)times/12 (range 2.2 to 30)times. The mean half-life of FVIII was 10.20±2.72hand the mean hours of FVIII< 1IU dL^{-1} in one week was 50.6 h (-90.56~102.33h). Significant relationship betweenthe $t_{1/2}$ of FVIII and ABR/AJBR was found (r=0.62 and 0.75, respectively). ABR/AJBR had positive correlation with the time (h) of the hours of FVIII< 1IU dL^{-1}in one week (r=0.46 and 0.51, P< 0.05).

Conclusions: There indeed existed the significantvariation in the FVIII $t_{1/2}$ in Chinese severe HA children and had the relationship with-ABR/AJBR and time< 1 IU dLper weekduring prophylaxis.It showed that $t_{1/2}$was the important indicator to prevent bleeding for HA and shorten the time< 1 IU dL according tot$_{1/2}$ wouldreduce the bleeding of severe HA during prophylaxisin China.

PB 255 | Assessment of the Quality of Life in Korean Hemophiliacs: Impact of Disease-related Factors, Social Status and Treatment Factors on the Quality of Life of Korean Hemophiliacs

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Background: The identification of disease-related factors, treatment factors and the social status of hemophiliac patients which may have an impact on their QoL in each country, is vital in establishing potential intervention strategies for these patients.

Aims: We evaluated the impact of these factors on the QoL of Korean hemophiliac patients.

Methods: QoL was evaluated using the SF36 standardized questionnaires. Disease-related factors were obtained from the patients' medical records. Socio-demographic data, such as marital status, occupational status, education years, as well as a treatment data, self-injection ability were obtained using questionnaires.

Correlation analysis and multiple regression analysis were conducted to elucidate the impact of the observed data on the QoL of these patients.

Results: The disease-related factors were correlated with physical health of the patients. Marital status was the only social status shown to have a correlation with the QoL of the hemophiliac patients; marital status was shown to only affect patients' physical health. However, self-injection ability, the only treatment factor surveyed in this study, was significantly associated with both the physical and mental health of the patients. Mental health was revealed to be poorer in Korean hemophiliac patients when compared with that of patients residing in countries with well-organized hemophilia treatment systems.

Conclusions: One social factor (marital status) and the disease-related factors correlated with the physical health of the Korean hemophiliac patients. Self-injection ability was associated with both the physical and mental health of the patients. The differences in treatment factors across different countries may contribute to the difference in the physical and mental health of hemophiliac patients.

PB 323 | Extracellular Nucleosome Levels in the Etiopathogenesis of Sepsis-associated Coagulopathy

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Background: Nucleosomes (DNA-histone complexes) can be expelled into the blood as part of the host defense process. While this contributes bacterial clearance, nucleosomes (NUCs) can play a role in disease pathology by leading to inflammation, endothelial damage, and pathological thrombosis. NUCs may contribute to the link between infection, host response, and systemic coagulation in sepsis-associated disseminated intravascular coagulation (DIC).

Aims: The purpose of this study was to demonstrate the relationship between NUCs with other mediators of inflammation in sepsis associated coagulopathy.

Methods: Plasma was collected from patients with sepsis and suspected DIC on ICU days 0, 4, and 8 under an IRB approved protocol. DIC score was evaluated using the ISTH scoring algorithm. Plasma from healthy individuals was purchased from George King Biomedical. Plasma NUCs were measured using the Cell Death Detection ELISA (Roche Diagnostics).

Results: NUCs were significantly elevated in patients with sepsis and suspected DIC compared to healthy individuals on ICU days 0 ($p = 0.028$), 4 ($p < 0.0001$), and 8 ($p = 0.013$). When patients were categorized according to ISTH DIC score, a non-significant trend towards increasing NUCs with increasing DIC score was observed. NUCs were significantly elevated in patients with overt DIC compared to normal on day 0 ($p = 0.02$). On day 4, nucleosomes were significantly elevated in patients with overt and non-overt DIC compared to controls ($p < 0.01$). NUCs correlated with D-Dimer, F1.2, PCT, IL-8, and IL-10.

Conclusions: Plasma NUCs were elevated in patients with sepsis and DIC, with a trend towards increasing NUCs with increasing DIC score, suggesting that NUCs contribute to the pathophysiology of DIC. The correlation of nucleosomes with PCT and inflammatory markers suggests that the presence of NUCs in the plasma of patients may be due to infection-related processes. The correlation of NUCs with markers of coagulation suggests that NUCs play a role in coagulation activation in DIC patients.

PB 324 | Mediators of Inflammation and Infection in Sepsis Associated Disseminated Intravascular Coagulation and Their Prognostic Role

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Background: Sepsis is a severe systemic inflammatory response to infection that manifests with widespread inflammation as well as endothelial and hemostatic dysfunction. Both nucleosomes (NUC) and procalcitonin (PCT) are associated with the inflammatory and infectious processes that play a key role in the pathogenesis of this syndrome.

Aims: The purpose of this study is to investigate the role of various mediators of inflammation including nucleosomes and their relevance with each other in determining the severity of DIC.

Methods: De-identified citrated plasma samples from patients diagnosed with SAC (n=137) were collected from patients in the ICU upon admission and on days 4 and 8. In addition, plasma samples from healthy volunteers (n=50) were purchased from George King Biomedical (Overland, KS). Plasma samples were analyzed for procalcitonin (PCT) (Abcam) and extracellular NUC (Roche) using commercially available ELISA methods. Markers of inflammation including interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10) and tumor necrosis factor α (TNF α) were measure using the Biochip Array from Randox (Antrim, UK).

Results: The levels of PCT were elevated in all three groups compared to normal ($p< 0.05$). In addition, the patients with overt DIC had a higher level of PCT on days 0 and 4, compared to patients with non-overt DIC or sepsis alone. On day 8, the overt and non-overt DIC patients had similar levels of PCT. Similarly, markers of inflammation, including IL-6, IL-8, IL-10 and TNF α were higher in the overt DIC group compared to the other groups on day 0 and day 4. The PCT levels correlated with NUC, IL-6, IL-8, IL-10 and TNF α levels ($p< 0.05$, Spearman $r>0.20$).

Conclusions: This study demonstrates the diagnostic and prognostic value of profiling several biomarkers of inflammation and infection in patients with SAC and DIC to assess the severity of illness. This study provides an initial framework in developing a multiparametric profile of biomarkers in DIC for diagnostic and prognostic purposes.

PB 325 | Crotoxin, a phospholipase A2 from Crotalus durissus terrificus rattlesnake venom, attenuates coagulation and inflammatory parameters in endotoxin-induced DIC

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Background: Crotoxin (CTX) is the major component present in the venom of the rattlesnake *Crotalus durissus terrificus*, and has been widely investigated due to its immunomodulatory and anti-inflammatory properties.

Aims: Therefore, we aimed to evaluate the capacity of CTX to modulate the systemic activation of coagulation upon inflammatory stimulus, using the disseminated intravascular coagulation (DIC) model in mice.

Methods: The DIC was induced by injection of 30mg/Kg lipopolysaccharide (LPS via i.p.) in swiss mice, and blood collected by cardiac puncture after 24 hours. Mice were pretreatment with CTX (30µg/Kg via s.c.) 6 hours before LPS administration. Control mice were given saline, CTX or LPS only. Prothrombin time (PT), activated partial thromboplastin time (aPTT), platelet counting and pro-inflammatory citokine IL-6 were analyzed.

Results: CTX was capable to reduce both plasma PT and aPTT compared to animals challenged only by LPS, which presented both parameters prolonged. Moreover, mice pretreated with CTX partially recovered platelet counting (607.5±86.76x10^6platelets/mL) compared to LPS challenged animals (582.5±19.59x10^6platelets/mL). As concerning to cytokine IL-6, the pretreatment with CTX before LPS stimulation reduced in 38% the plasma concentration of the pro-inflammatory mediator.

Conclusions: The preliminary results shows that CTX is capable to reduce both inflammatory and coagulation parameters triggered by LPS. Therefore, in order to obtain more information on CTX effects we intend to continue the research on CTX. One effort consists in the measurement of an important biomarker associated with the inflammation-coagulation axis: tissue factor. Moreover, a second approach consist in using pharmacological antagonists in order to elucidate CTX anti-inflammatory mechanism possibly involved. The results obtained assign CTX as a novel approach in DIC resolution, and further steps open perspectives for different targets in DIC therapeutics.

PB 326 | Evaluation of the Diagnostic Utility of Individual Parameters in the Disseminated Intravascular Coagulation (DIC) Panel for Use in Under-resourced Settings

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Background: Disseminated intravascular coagulation (DIC) is a clinicopathological condition caused by a number of pro-thrombotic inflammatory stimuli which results in dysregulation of normal coagulation homeostasis. Systemic fibrin clots are generated which result in consumption of both platelets and coagulation factors, critical organ ischaemia and ultimately in a bleeding diathesis. DIC is a strong predictor of mortality. The DIC testing panel at our centre consists of the following parameters: Prothrombin Index (PI), activated Partial Thromboplastin Time (aPTT), Platelet count, Antithrombin, Thrombin Time, Fibrinogen and D-dimers.

Aims: To assess the predictive value of the individual parameters included in the DIC screen panel at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa.

Methods: A retrospective review of all DIC screens performed at CMJAH over a period of 1 year was conducted. The ISTH DIC score was applied to each screen. A score of >4 was considered positive for the presence of a DIC. A logistic regression was applied to the individual parameters and a predictive Z-score was derived to assess the diagnostic utility of each parameter.

Results: The predictive value of the individual parameters of the DIC panel is depicted in Table 1. Only 18 of the 143 patients diagnosed with DIC had a fibrinogen below the normal reference range (2-4g/dl). Only 8 of these patients had a fibrinogen level below 1g/dl to qualify for ISTH DIC point allocation.

TABLE 1 Z-scores for individual parameters on the CMJAH DIC screen:

Parameter:	Z-score:	p value:
PI (s)	2.57	0.01
aPTT (s)	3.44	0.001
Platelet count (x10^9/l)	-6.88	0.000
Antithrombin (%)	-5.47	0.000
Thrombin Time (s)	1.02	0.309
Fibrinogen (g/l)	-1.60	0.110
D-dimers (mg/l)	3.29	0.001

Conclusions: To improve cost-efficiency of DIC screening, omission of fibrinogen and thrombin time analysis in the routine DIC panel could be considered in our setting. This would have a minimal impact on the sensitivity of the assay but could improve cost efficiency.

PB 327 | Circulating Nucleosomes and Neutrophil Activation Predict for Disseminated Intravascular Coagulation but Not for Mortality in Patients with Severe Sepsis or Septic Shock

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Background: Disseminated intravascular coagulation (DIC) is a heterogeneous syndrome characterized by bleeding and thrombosis.

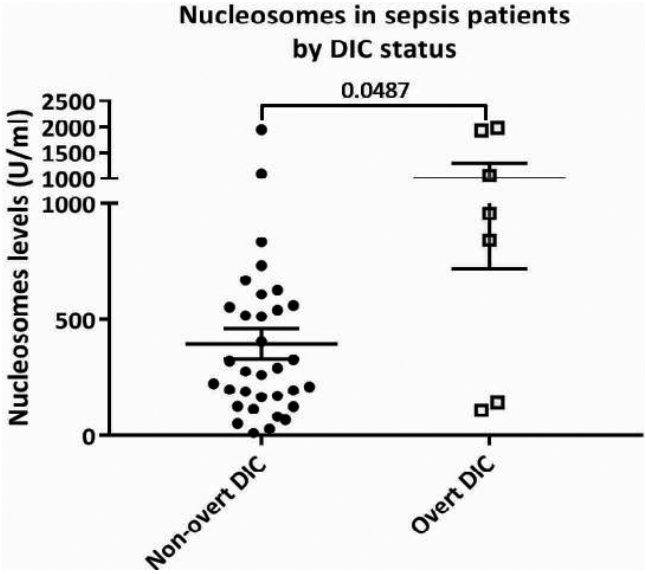

FIGURE 1A

Recently, damage-associated molecular patterns, e.g. extracellular cell-free DNA, DNA binding proteins and neutrophil activation, were shown to play a crucial role in the pathogenesis of DIC.

Aims: To explore the predictive value of extracellular DNA and neutrophil activation for DIC and mortality.

Methods: We used a well-described cohort of 40 patients admitted to the intensive care unit of University Hospital Bern, Switzerland (1998-1999) with severe sepsis or septic shock according to the American College of Chest Physicians consensus. Patients were followed for 90 days or until death. Blood samples taken at inclusion and at 24, 48, 72, and 96 hours were analyzed for extracellular DNA (nucleosomes) and neutrophil activation (elastase-a1antitrypsin (EA) complexes). DIC scores were calculated according to ISTH guidelines. D-dimer levels were categorized based on quartiles of the cohort (0 pts=1st quartile; 2 pts=2nd-3rd quartile; 3 pts=4th quartile). Predictive value for DIC (ISTH DIC score ≥5) and mortality were assessed by receiver-operating characteristic curves and Cox regression analysis.

Results: 7 of 40 patients developed DIC and 13 died. Nucleosomes and neutrophil activation at baseline were significantly higher in patients with DIC compared to those without (Fig. 1A and 1B).

Levels did not differ between survivors and non-survivors (p=0.3307; and p=0.1216, re spectively). With an area under the curve (AUC) value of 0.74 (95% CI 0.5-1.0; p=0.048) and 0.74 (95% CI 0.4-1.0; p=0.048), nucleosomes and neutrophil activation significantly predicted for DIC but not for mortality (AUC 0.598; 95% CI 0.4-0.8, and AUC 0.654; 95% CI 0.5-0.8).

Conclusions: Nucleosomes and neutrophil activation are predictive of DIC, suggesting an important role of neutrophil extracellular traps (NETs) formation. Their additive value to existing DIC or mortality prediction scores requires further investigation.

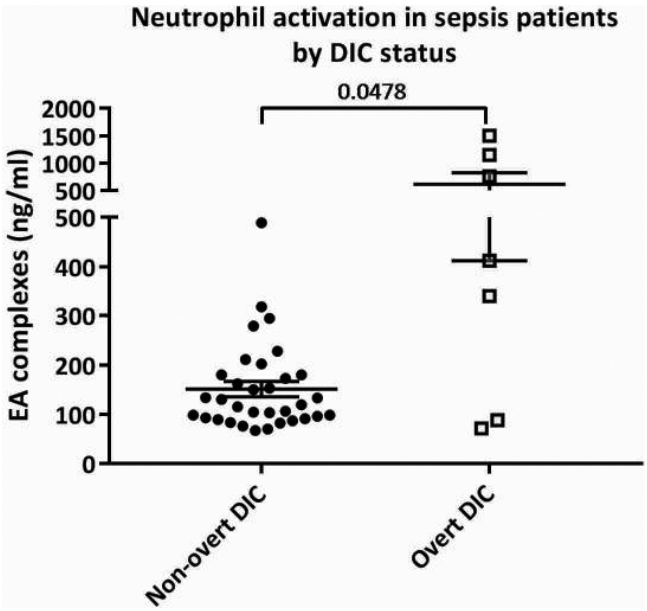

FIGURE 1B

PB 328 | A Prospective Observational Study for the Evaluation of New Diagnostic Criteria for Disseminated Intravascular Coagulation by the Japanese Society on Thrombosis and Hemostasis

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Background: The prognosis of sepsis is affected by the presence of disseminated intravascular coagulation (DIC). Several criteria have limited application in an appropriate phase to improve the outcome of sepsis-induced DIC. The Japanese Society on Thrombosis and Hemostasis (JSTH) has proposed new DIC criteria using molecular markers and antithrombin (AT) activity to overcome problems encountered with DIC diagnosis.

Aims: The objective of this study was to evaluate the JSTH criteria for DIC in patients with sepsis.

Methods: We prospectively enrolled 28 patients with sepsis, who had platelet counts of < $120X10^3$ /μL and/or FDP levels of >10 μg/mL. Blood coagulation parameters, soluble fibrin (SF), thrombin-AT complex (TAT) and AT activity were measured on days of 1 (at the time of enrollment), 5 ,3 ,2 and 7. The JSTH criteria was validated by comparing with overt DIC criteria of ISTH and DIC criteria of Japanese Ministry of Health and Welfare (JMHW). The APACHE II score was calculated on day 1, and the outcome measures were organ dysfunction by a SOFA score and the -28day mortality.

Results: Patients who fulfilled the JSTH DIC criteria included almost all those whose DIC was diagnosed by the ISTH and JMHW scoring

systems. The SF levels on Day 1 in DIC patients by the JSTH criteria were significantly higher than those of non-DIC patients (99.1±71.9 vs 18.1±21.1 µg/mL, p=0.030). The overall mortality was 42.8%, and APACHE II score of non-survivors was significantly higher than survivors (20.3±6.8 vs 14.7±5.4, p=0.031). Higher SOFA scores on Days 2, 3, 5 and 7 were observed in the non-survivors than in survivors. Although JSTH scores did not associate with the mortality rate, the recovery of platelets counts was an important predictor of the outcome.

Conclusions: The JSTH criteria has an acceptable property for the diagnosis of DIC, as the scoring system identified most of the patients diagnosed by the ISTH and JMHW criteria.

PB 329 | A comparison of Disseminated Intravascular Coagulation (DIC) Screening Parameters with and without Human Immunodeficiency Virus Infection in an African Academic Hospital Setting

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Background: HIV infection is highly prevalent in South Africa. HIV causes CD4+ T-cell lymphopaenia and predisposes to opportunistic infection and malignancies which may trigger DIC. DIC is results in dysregulation of coagulation homeostasis and systemic generation of fibrin clots with consumption of coagulation factors and platelets and the development of a bleeding diathesis. HIV positive patients show endothelial dysfunction which may cause a hypercoagulable state and affect DIC presentation in this population.

Aims: To compare the DIC presentation of HIV positive and HIV negative patients in an African Academic Hospital

Methods: All DIC screens performed over a 1 year period were assigned an ISTH DIC score. All screens with a score >4 were considered positive for a DIC. Data were accessed from the laboratory information system. All continuous variables were normalised and expressed as a mean. A 2 data-set mean comparison was performed. A level of significance of p< 0.05 was used.

Results: Of the 304 screens performed over the period, 144 were positive for a DIC. Of these, 33 had an unknown HIV status. More patients were HIV positive than negative (n=67 and n=44 respectively). HIV infected patients showed significantly higher D-dimer levels than HIV negative patients. (5.6ml/ml and 3.4 mg/ml respectively, p=0.03). The prothrombin time showed a trend to increased prolongation in HIV positive compared with HIV negative patients (28.1s vs 22.4s, p=0.06). No other parameters showed significant differences. (Table 1)

TABLE 1 Mean values for DIC screen parameters in HIV infected and uninfected patients with DIC

	HIV-infected patients (n=67) Mean Value	HIV-uninfected patients (n=44) Mean Value	Difference p-vale
D-dimer (mg/l)	5.6	3.4	0.03
Fibrinogen (g/l)	4.1	4.7	0.10
Prothrombin Time (s)	28.1	22.4	0.06
Platelets (10^9/l)	59.8	59.8	0.5
Antithrombin (%)	72.3	74.5	0.36

Conclusions: HIV positive patients with DIC showed significantly higher D-dimer levels. Higher D-dimer levels correlate with poor prognosis in patients infected with HIV and may be increased in asymptomatic HIV-infected individuals. This may act as a confounding variable and prevents accurate DIC diagnosis. he The prognostic significance of strongly raised D-dimers in patients with HIV and DIC should be evaluated.

PB 330 | International Normalized Ratio Relevance to the Observed Coagulation Abnormalities in Warfarin Treated and Those with Disseminated Intravascular Coagulation

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Background: Disseminated intravascular coagulation (DIC) is characterized by an ongoing systemic activation of the coagulation and fibrinolytic systems, which can exhaust coagulation factors. Patients receiving warfarin for anticoagulation also have a prolonged PT/INR.

Aims: The purpose of this study was to compare the coagulopathy observed in patients with DIC and in patients in the initial phase of warfarin therapy.

Methods: Citrated de-identified plasma samples were collected at baseline from patients diagnosed with sepsis-associated DIC (n= 100) and from patients in the initial phase of warfarin therapy (n=100). These plasma samples were evaluated for PT/INR (IL, Bedford, MA), APTT (Stago, Parsippany, NJ), fibrinogen, (IL, Bedford, MA) and functional (Aniara, West Chester, OH) and antigenic levels (Hyphen Biomed, Paris, France) of Factors VII, IX and X.

Results: The PT/INR values were significantly higher in warfarinized patients (WP) compared to DIC patients (p< 0.001). The APTT showed no correlation with the INR in WP but increased with an increase in the INR in the DIC group (p< 0.001). Similarly, the fibrinogen levels showed no correlation with INR in WP; however, in DIC patients, the fibrinogen levels decreased with an increase in the INR. In the factor assays, both the functional and antigenic levels of factors IX and X were decreased in the WP as INR increased, but showed no variation with INR in the DIC patient group. Factor VII levels were decreased with an increased INR in WP but not the DIC patients.

Conclusions: In the DIC patients, there was no decrease in factor levels with increase in INR. In contrast warfarin patients, showed a decrease in factor levels with an increasing INR. However, in DIC greater changes in global coagulation parameters were observed. These results suggest that the coagulopathy observed in a patient with INR ≥ 1.4 is fundamentally different in a patient receiving warfarin for anticoagulation than in patients with DIC.

PB 331 | Epidemiological Evaluation of Patients Presenting with Disseminated Intravascular Coagulation (DIC) at an Academic Hospital in an African Middle-income Country

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Background: DIC is a serious pathophysiological condition resulting from a number of different triggers including infection and malignancy. DIC results from dysregulation of coagulation homeostasis with inappropriate generation of systemic fibrin clots and eventual consumption of platelets and coagulation factors. DIC results in significant mortality and accurate diagnosis is essential for appropriate treatment.

Aims: To evaluate the underlying causes and patient demographic characteristics in patients diagnosed with a DIC at an African academic hospital.

Methods: All DIC screens over a 1 year period were included in this analysis. An ISTH DIC score was allocated to each screen and a score of >4 was considered positive. Assignment of an underlying cause was performed using results derived from the laboratory information system. All continuous variables were described as median values.

Results: 304 DIC screens were performed at our hospital of which 144 were assigned an ISTH DIC score above 4. The median age of the patients at diagnosis was 37 year (range of 0-85 years). The male:female ratio was 0.8:1. In 111 patients, an infection was demonstrated (77%). 67 patients were HIV positive of which 43 showed

FIGURE 1 Pathogenic causes of DIC

an additional infectious agent on culture. Of the 33 patients where HIV status was unknown, 16 were culture positive. 22 HIV negative patients were diagnosed with an infection. The second commonest cause was malignancy (in 31 patients) with or without concomitant infection. In 24 patients, no cause could be demonstrated.
(See figure 1).

Conclusions: In our setting, infection continues to be the highest demonstrable trigger for development of DIC. This, in part, reflects the high HIV prevalence which predisposes to malignancy and opportunistic infection.

PB 332 | Real Word Difficulties in the Diagnosis and Management of Hyperfibrinolysis

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Background: Hyper-fibrinolysis (HF) is challenging to treat/diagnose manifesting with bleeding & thrombosis with no standard approach. Often patients may have co-existing bleeding and thrombosis creating treatment dilemmas.

Aims: We present 5 cases with management & diagnostic issues to share experience.

Methods: Case series.

Results: Patient age range was 51-84 (median 69, 4 males & 1 female). Fibrinogen at presentation ranged from < 0.17 to 0.96g/L (ref. range 1.46-3.33g/L). Causes of HF were acute myeloid leukaemia (AML) in 2 cases (t(15;17) excluded by FISH), metastatic prostate cancer, carcinoma of unknown primary (CUP) and a thoracic aneurysm. 2 patients presented with simultaneous bleeding and venous thrombosis. 3 patients presented with solely bleeding manifestations (including skin, rectal and cerebral bleeding). In all but 1 case the cause of HF was apparent at presentation. A 79 year old female presented with haematuria, fibrinogen < 0.17g/L, D-dimer 19635ng/ml (0-230ng/ml) and extensive work-up for cancer was negative. At week 6 the patient developed cerebral bleeding and the white cell count rose from 4.7x10*9/l (normal blood count/film, range 3.6-10.5x10*9/l) to 44x10&9/L with 90% blasts typed as AML. The 3 patients presenting with bleeding were managed with combinations of cryoprecipitate, fibrinogen concentrate, tranexamic acid and aprotinin. 2 patients (1 CUP & 1 AML) were treated for both thrombosis and bleeding with dalteparin along with combinations of

tranexamic acid, cryoprecipitate and fresh frozen plasma. The patient with AML had an inferior vena cava filter as therapeutic anticoagulation was not possible due to bleeding risk. Prognosis was determined by the driving disease.

Conclusions: We conclude that management of HF is treating the predominant clinical problem (bleeding v thrombosis) and the driving disease (which are predominantly malignant). Diagnosis may be obscure and HF can precede an occult malignancy so bone marrow biopsy in cases of intractable HF may reveal myeloid disease.

PB 333 | Fulminant Disseminated Intravascular Coagulation as Initial Manifestation of Pulmonary Adenocarcinoma

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Background: Fulminant disseminated intravascular coagulation (DIC) is characterized by laboratory abnormalities with simultaneous widespread microvascular thrombosis and profuse bleeding from various sites.

Aims: Report a case of cardiac tamponade with fulminant DIC as an initial manifestation of primary lung cancer diagnosed post-mortem.

Methods: 57-year old patient admitted to the emergency room with signs of respiratory distress and hypoxia. Shortly after, cardiac arrest occurred. Due to unsuccessful advanced life support measures, extracorporeal membrane oxygenation (ECMO) was initiated. Echocardiogram revealed cardiac tamponade but only 70 cc of blood was withdrawn by pericardiocentesis. On admission laboratory tests results were: hemoglobin (Hb= 9,7g/dL); platelet count (plt): $40x10^9$/L; aPTT= 133,8 s; PT= 73,2 s; Fibrinogen < 10 mg/dL. Correction of coagulation parameters was attempted with fresh frozen plasma, fibrinogen, tranexamic acid and prothrombin complex concentrates. Pericardial hematic fluid was drained through open surgical approach. Abruptly shock ensued and vasopressor support was necessary. Even though repeated coagulation tests showed some improvement, profuse bleeding from various sites started. Efforts to correct hemostasis were unsuccessful.

Results: The cardiac tamponade relapsed and the patient condition deteriorated. Thoracic computed tomography (CT) angiography showed pulmonary thromboembolism in the left pulmonary artery. Head CT had evidence of tetra-ventricular hemorrhage, extensive ischemia with diffuse cerebral edema. At this point coagulation results were: aPTT= 48 s; PT= 21,3 s; Fibrinogen 142 mg/dL. There were no clinical improvements and the patient died 18h after admission. The autopsy revealed a pulmonary adenocarcinoma with pericardial and myocardial invasion.

Conclusions: This report sustains that DIC remains a syndrome of difficult approach due to its different etiologies and varied clinical presentations. Without treatment of the underlying cause it is predestined to fail.

PB 334 | Platelet-mimicking Synthetic Nanoparticles (SynthoPlateTM) Improve Hemostasis and Survival Following Uncontrolled Hemorrhage

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Background: Hemorrhage is the leading cause of preventable death after trauma. Platelet transfusions, while critical to hemostatic resuscitation following hemorrhage, are limited by availability, portability, storage requirements, contaminations risks, and a limited shelf-life. A synthetic nanoconstruct that mimics platelet-mediated mechanisms of hemostasis while resolving the above limitations can be of significant value in treating hemorrhage.

Aims: To test a novel, synthetic platelet-mimetic nanoparticle (SynthoPlate^{TM}) as a platelet substitute after uncontrolled hemorrhage.

Methods: SynthoPlate^{TM} is a biocompatible liposomal nanoparticle heteromultivalently surface-decorated with VWF-binding (VBPs), collagen-binding (CBPs) and fibrinogen-mimetic peptides (FMPs). Hemostatic capabilities were validated using ex vivo microfluidic assays and viscoelastic testing from trauma patients. SynthoPlate^{TM} vesicles (30mg/kg) were transfused in a validated murine model of uncontrolled hemorrhage (liver laceration). Mean arterial pressure and blood loss were quantified. 72-hr survival was analyzed using Kaplan-Meier method.

Results: Ex vivo clot formation was significantly enhanced with the addition of SynthoPlate^{TM} vesicles from human trauma patients. Following murine liver laceration, blood loss was significantly decreased in mice transfused with SynthoPlate^{TM} vesicles (1.6g control vs. 0.65g SynthoPlate^{TM}, $p < 0.05$). Mice transfused with SynthoPlate^{TM} vesicles took significantly longer to develop hypotension (166.7s control vs 433.3s SP, p=0.01). 72 hour survival was significantly improved in SynthoPlate^{TM}-treated mice (82% SP vs. 60% control, $p< 0.05$).

Conclusions: SynthoPlate^{TM}, a novel synthetic platelet-mimicking liposomal nanoparticle technology, demonstrates improved hemostasis, improved hemodynamics, and reduced mortality following uncontrolled hemorrhage in mice. This technology shows significant promise in hemostatic resuscitation of traumatic hemorrhagic shock.

PB 335 | Early Fibrinolytic Activation and Hypofibrinogenaemia Are Associated with Mortality in Isolated Traumatic Brain Injury

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Background: Traumatic brain injury (TBI) is among the leading causes of death and disability worldwide. Whilst the acute traumatic coagulopathy

associated with haemorrhage has been characterised in detail, the pathophysiology of TBI-induced coagulopathy remains poorly defined.

Aims: To characterise the derangements in coagulation and fibrinolysis in the hyperacute phase after TBI, and to identify patterns associated with adverse clinical outcomes.

Methods: Patients were prospectively recruited into the Activation of Coagulation and Inflammation in Trauma (ACIT) study. Blood samples were drawn in the emergency department for ROTEM, clotting factors and markers of fibrinolysis. Patients with isolated TBI were compared to minimally injured controls.

Results: Within two hours of injury, patients with isolated TBI (n=102) had markedly elevated prothrombin fragments (2,312pmol/L) which greatly exceeded the levels observed in controls (n=173; 449pmol/L, p< 0.01). Fibrinogen levels were lower in TBI patients compared to controls (2.1vs 2.6g/L, p< 0.01) and were proportional to clot amplitude on ROTEM (r^2=0.39, p< 0.01). In contrast, all other clotting factors were maintained at normal or supranormal levels, and ROTEM clotting times were similar to controls (55 vs 56 seconds, p=0.51). D-dimer levels in TBI patients were also greatly elevated (18,446 vs controls, 1,134ng/ml, p< 0.01) and were highest in non-survivors (68,250 vs survivors: 13,952ng/ml, p< 0.01). However, levels of tissue plasminogen activator (10.7ng/ml) and urokinase (3.5ng/ml) were similar to controls (tPA 9.0, p=0.39; uPA 3.3, p=0.73).

Conclusions: The principal functional impairment in coagulation early after TBI is hypofibrinogenaemia and reduced clot strength, without prolongation of clotting time. Concomitant fibrinolytic activation is associated with poor outcome and appears to be independent of the principal activators of plasminogen. Further investigation into the mechanisms of fibrinolysis and the role of fibrinogen supplementation in TBI is required.

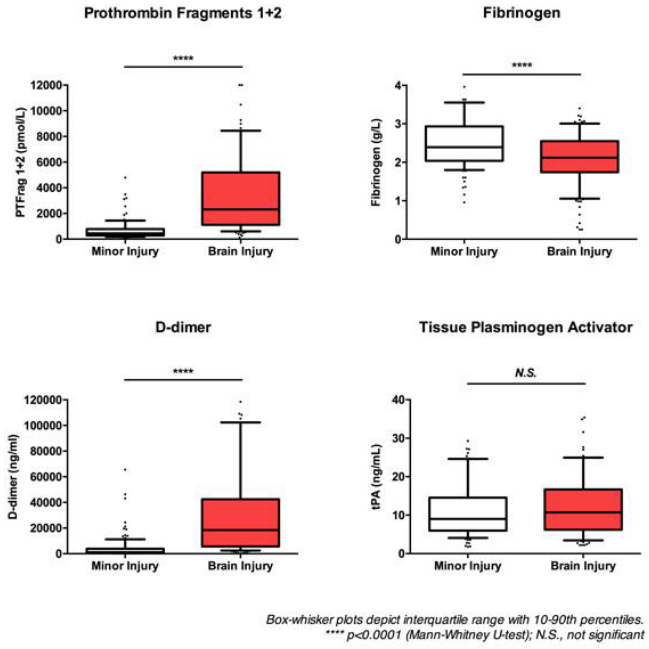

FIGURE 1 Traumatic brain injury results in increased thrombin generation, hypofibrinogenaemia and tPAindependent fibrinolysis

PB 336 | Platelets Loaded with Thrombin-encapsulated Liposomes Have Increased Coagulability

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Background: Platelets are an integral part of primary hemostasis and platelet transfusions are often used to reduce bleeding during severe hemorrhage. However, transfusions of blood components are insufficient to stop the most severe cases of hemorrhage, particularly when complicated by platelet dysfunction.

Aims: Although simple nanoparticle-based systems have been developed to replace or augment platelet transfusions, they inherently lack important aspects of platelet function. In a step towards increasing the efficacy of platelet transfusions during acute severe bleeding, we asked whether the natural coagulability of transfusable platelets could be enhanced by loading them with liposomal thrombin.

Methods: Thrombin was encapsulated into nanoliposomes and delivered to platelets isolated from fresh whole blood. Platelet coagulability was determined by measuring platelet activation, clot contraction, and the time to generate endogenous thrombin from plasma (thrombin generation time).

Results: Encapsulating thrombin into nanoliposomes shielded the enzymatic activity of thrombin and caused minimal background platelet activation. Delivery of liposomal thrombin into platelets led to an increase in the sensitivity and responsiveness of platelets to agonists, enhanced clot contraction, and faster thrombin generation times in plasma. This increased procoagulant activity of the modified platelets persisted in the presence of antiplatelet drugs and in acidotic conditions, which normally inhibits platelet function.

Conclusions: Platelet coagulability can be enhanced by the delivery of liposomal thrombin, even in conditions where platelets are dysfunctional. This study is a first step towards engineering transfusable platelets that have increased efficacy for treating severe hemorrhage, and may have future applications in delivering other proteins to platelets and other cells or tissues.

PB 337 | S100B in Suspected Intracranial Hemorrhage in Patients after Minor Head Injury Older than 65 Years and on Antiplatelet Therapy

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Background: After minor head injury (MHI), in older patients and patients with platelet aggregation inhibitors (PAI) cranial computed tomography (CCT) and hospital admission are increasingly performed to rule out intracranial hemorrhage. This leads to high radiation exposure and financial burden.

Aims: Aim of this study was to determine whether the astroglial-derived protein S100B released into blood can be used as a reliable negative predictive tool for intracranial bleeding in patients after MHI older than 65 years or on PAI (low-dose aspirin, clopidogrel).

Methods: We conducted a prospective observational study in two trauma hospitals. 782 patients with MHI (Glasgow Coma Scale score 13-15) with PAI or aged over 65 years were included. Clinical examination, observation and CCT were performed in the emergency department or on the ward. Medium age was 83 years, 69% were female. Sensitivity, specificity, positive and negative predictive values of S100B with reference to CCT findings were calculated.

Results: Of the 782 patients, 50 (6.4%) had intracranial bleeding. One CCT positive patient showed an S100B level below 0.105 µg/L. Of all patients 33.1% were below the cut-off. S100B showed a sensitivity of 98.0% (CI 89.5%-99.7%), a negative predictive value of 99.6% (CI 97.9%-99.9%), a specificity of 35.3% (CI 31.9%-38.8%) and a positive predictive value of 9.4% (CI 7.2%-12.2%).

Conclusions: S100B levels below 0.105 µg/L can accurately predict normal CCT after MHI in older patients and those on PAI. Combining conventional decision rules with measurement of S100B can reduce the CCT and hospital admission rate by approximately 30%.

PB 338 | Antifibrinolytic Therapy to Prevent Oral Bleeding in People on Anticoagulants Undergoing Oral or Dental Procedures: A Cochrane Systematic Review

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Background: Patients on continuous treatment with oral anticoagulants (OACs) such as vitamin K antagonists (VKAs) or direct oral anticoagulants (DOACs) are at increased risk of bleeding complications during and after oral and dental procedures. However, OAC discontinuation is associated with an increased thrombo-embolic risk. The use of antifibrinolytic agents could eliminate the need for dose reduction or discontinuation.

Aims: To assess the efficacy of antifibrinolytic agents to prevent bleeding complications in patients on continuous treatment with OACs undergoing oral or dental procedures.

Methods: We searched PubMed, Embase and the Cochrane Library for randomized and quasi-randomized controlled trials (RCTs and quasi-RCTs, date of last search 12 November 2016) and reference lists of relevant publications. Additional searches were performed, including ClinicalTrials.gov and abstract books of international meetings. For meta-analysis we extracted the proportion of patients with postoperative bleeding episodes requiring intervention. Estimates were pooled using the random effects model.

Results: No eligible trials in patients on continuous treatment with DOACs were identified. Three RCTs and two quasi-RCTs discussing the use of TXA in 385 patients on continuous VKA treatment were included. Two studies comparing locally administered tranexamic acid (TXA) with placebo showed a statistically significant beneficial effect of TXA on postoperative bleeding. Three studies comparing TXA with an alternative pro-hemostatic measure showed a small non-statistically significant beneficial effect of TXA [Table 1]. There were no side effects of TXA requiring treatment withdrawal.

Conclusions: The small number of identified RCTs and included participants and differences in treatment regimens do not allow to conclude definite efficacy of antifibrinolytic therapy in patients on continuous treatment with VKAs undergoing oral or dental surgery. No eligible trials in patients on continuous treatment with DOACs were identified.

TABLE 1 Results of the meta-analysis on the number of postoperative bleedings needing intervention

Postoperative bleedings	Studies	Participants	Risk difference (95%CI)	Number needed to treat (NNT)**
TXA versus placebo	2 (Ramström 1993; Sindet-Pedersen 1989)	128	-0.31 (-0.53 to -0.09)	3.2
TXA versus standard care*:	3 (Soares 2015; Bublitz 2000; Blinder 1999)	257	-0.07 (-0.22 to 0.09)	14
Total (TXA versus placebo and standard care)	5 (Ramström 1993; Sindet-Pedersen 1989; Soares 2015; Bublitz 2000; Blinder 1999)	385	-0.16 (-0.31 to -0.00)	6.3

Abbreviations: 95% CI: 95% confidence interval ; NNT: number needed to treat. *Standard care consisted of resorbable gelatin sponge and sutures, collagen fleece, mucosal flap, and dry gauze compression. **NNT to prevent 1 postoperative bleeding episode needing intervention

PB 339 | Management of Rivaroxaban or Apixaban Associated Major Bleeding with Prothrombin Concentrate: A Prospective Cohort Study

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Background: There is uncertainty regarding the efficacy of emergency bleeding management and risk of thromboembolic events in patients treated with prothrombin complex concentrates (PCC) for major bleeding events (MBE) related to rivaroxaban or apixaban.

Aims: We aimed to assess the effectiveness of PCC for the management of major bleeding events on rivaroxaban or apixaban.

Methods: Between 1/1/2014 and 1/10/2016, we prospectively included patients treated with PCC for emergency management of MBE on rivaroxaban or apixaban. The effectiveness of bleeding management with PCC was assessed using the International Society of Thrombosis and Hemostasis' Scientific and Standardization Subcommittee criteria for the assessment of effectiveness of major bleeding management, with the categories Good, Moderate and Poor. Safety outcomes were thromboembolic events and death during 30 days after treatment with PCC.

Results: A total of 86 patients received PCC for the urgent reversal of the anticoagulant effect of rivaroxaban or apixaban due a MBE. PCC was given at a median (IQR) dose of 2000 IU (1500-2000). There were 46 MBE on rivaroxaban and 40 MBE on apixaban. Intracranial hemorrhage (ICH) was commonest site of bleeding requiring reversal (60, 69.8%), followed by GI bleeding in 14 (16.3%). The bleeding was classified as traumatic in 27 (31.4%) of the patients. The effect of PCC was assessed as good or moderate in 60 (69.8%), and poor in 26 (30.2%). Most patients with poor effect of PCC had ICH (n=17, 65.4%). Two patients developed ischemic stroke occurring five and ten days after treatment with PCC. A total 26 patients died within 30 days after MBE.

Conclusions: The administration of PCC for the emergency reversal of MBE associated with rivaroxaban or apixaban was found to be effective and is associated with a low risk of thromboembolism. Further studies are needed to confirm these findings.

PB 340 | Favorable Thrombogenicity Profile of a Prothrombin Complex Concentrate (4F-PCC) in Animal Models of Venous and Arterial Thrombosis

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Background: Indications of 4-factor prothrombin complex concentrate (4F-PCC) include the prophylaxis and on-demand treatment of bleeding due to congenital or acquired deficiency of PCC factors such as the urgent reversal of anticoagulation with vitamin K antagonists. 4F-PCCs have also been shown to be of potential benefit in patients presenting with acute major bleeding related to the use of direct oral anticoagulants (DOAC). As with other procoagulant agents, PCC products carry the potential risk of exaggerated pharmacology resulting in arterial and venous thromboembolic complications.

Aims: This open-label, placebo-controlled in vivo study characterized the thrombogenic potential of a 4F-PCC, including a direct comparison to activated PCC (aPCC) and recombinant activated factor VIIa (rFVIIa), based on animal models of venous stasis and arterial thrombosis.

Methods: Anesthesized rabbits were treated with 4F-PCC (Beriplex® P/N, Kcentra®, CSL Behring) at doses of 0-500 IU/kg followed by induction of venous stasis over a period of 30 minutes at multiple time points post dosing (modified Wessler model). Anesthesized rats received 0-150 IU/kg 4F-PCC followed by $FeCl_2$ induced arterial injury. Venous thrombus formation (score and wet weight) and arterial occlusion (time and frequency) were used as primary thrombogenictiy parameters. A direct comparison was conducted to treatment with aPCC (FEIBA, Shire) and rFVIIa (NovoSeven RT, Novo Nordisk).

Results: A dose- and time- dependent increase in thrombogenic potential was observed after treatment with 4F-PCC. While there was no increase in thrombus formation seen up to the maximum clinically recommended dose level of 4F-PCC (i.e. 50 IU/kg) over placebo treatment, both aPCC (10-100 U/kg) and rFVIIa (50-300 µg/kg) elicited prothrombotic activity within their established therapeutic dose range.

Conclusions: Animal models of venous and arterial thrombosis support a favourable thrombogenicity profile of the 4F-PCC product tested.

PB 341 | fProthrombin Complex Concentrate for Major Bleeding on Factor Xa Inhibitors

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Background: Oral factor Xa inhibitors are increasingly used for anticoagulation but there is no approved reversal agent.

Aims: Prothrombin complex concentrate (PCC) for management of Xa-inhibitor-associated bleeding has been described in case reports and small case series. We evaluated PCC in a prospective cohort study.

Methods: Patients on apixaban or rivaroxaban and suffering a major bleed were treated as per existing hospital protocol with a fixed dose of PCC 2000 units. They were subsequently recruited for a 30-day follow-up. The treating physician evaluated the hemostatic effectiveness as Good, Moderate or Poor/None, using an Assessment Guide. Safety outcomes were death or thromboembolism.

Results: We recruited, as planned, 35 patients with major bleeding associated with rivaroxaban (54%) or apixaban (46%). The effectiveness was assessed as Good in 66%, Moderate in 17% and Poor/None in 17% with similar results when restricted only to patients with laboratory evidence of anticoagulant effect in plasma. For the 18 patients with intracranial hemorrhage the corresponding ratings were 72%, 11% and 17%, and for 11 patients with gastrointestinal bleeding they were 64%, 9% and 27%, respectively. There were 5 deaths (14%) by 30 days, all due to intracranial hemorrhage, one confirmed and one suspected but not confirmed ischemic stroke on day 25. Half of the patients with intracranial or intraspinal hemorrhage and effectiveness rated as Good had neurological deficit at the end of follow-up. In a post-hoc analysis of 18 evaluable cases with intracrania or intraspinall bleeding, using the adjudication rules of the ANNEXA-4 study (with andexanet alfa for reversal), 83% were rated as Excellent/Good outcome.

Conclusions: For major bleeding associated with oral Xa-inhibitors and management with PCC, treating physician assessed the effectiveness as good or moderate in 66% and 17% of cases, respectively. Thus, PCC is an option for management of critical bleeds on oral Xa-inhibitors.

PB 342 | Reversal of Dabigatran-associated Major Bleeding with Activated Prothrombin Concentrate: A Prospective Cohort Study

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Background: The reversal of dabigatran-associated major bleeding can now be achieved with the antidote idarucizumab.

Aims: We evaluated activated prothrombin complex concentrate (aPCC) as an alternative for this purpose.

Methods: Patients treated with dabigatran and suffering a major bleed were treated as per existing hospital protocol with aPCC. They were subsequently recruited for a 30-day follow-up. Effectiveness was evaluated by the treating physician, using an Assessment Guide. Safety outcomes were arterial or venous thromboembolism or death. A comparison was also made with historic cases with dabigatran-associated major bleeds treated with supportive care, by matching 1:2 for type of bleed, age and sex.

Results: We aimed at 32 evaluable cases but the study was prematurely discontinued after 14 cases due to the availability of the approved antidote. The effectiveness of aPCC was assessed as Good in 9 (64%), moderate in 5 (36%) and poor in none. There were no thromboembolic events and one death. In the secondary adjudication of effectiveness, using the same criteria and by the same adjudicators as previously done for the historic cases, the outcome was graded for the current cases versus the historic cases as Good, Moderate, or Poor in 10 (71%) versus 16 (57%), 3 (21%) versus 4 (14%), and 1 (7%) versus 8 (29%), respectively.

Conclusions: Although supportive care is sufficient to manage many patients with dabigatran-associated bleeding, aPCC might provide an additional benefit to control life-threatening bleeding in selected cases and does not appear to cause an excess of thromboembolic events.

PB 343 | A randomized study of 4-factor prothrombin complex concentrate and tranexamic acid on bleeding, thrombin generation, and pharmacodynamics after punch biopsies in rivaroxaban treated subjects with supratherapeutic drug levels

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Background: Currently, there are no therapies approved for reversing oral factor Xa inhibitors. Although prothrombin complex concentrate (PCC) restores thrombin generation (TG) in healthy volunteers, and tranexamic acid (TXA) is often given in emergent bleeding, the effect of these therapies on bleeding reversal is still uncertain.

Aims: To assess the effects of intravenous (IV) 4 factor PCC (Kcentra/Beriplex) or TXA on prothrombin time (PT), endogenous thrombin potential (ETP), prothrombin fragment 1.2 (F1.2), and D-dimer as well as bleeding duration (BD) and bleeding volume (BV) after a 5 mm skin biopsy (Bx) in volunteers given rivaroxaban (RIVA).

Methods: After protocol approval by an independent ethics committee and obtaining informed consent, a total of 147 healthy volunteers were enrolled and baseline Bx performed. Volunteers were then given RIVA 20 mg twice-daily (off-label regimen) for 3

days to obtain a steady-state. After receiving their last 20 mg dose of RIVA on day 4, volunteers were then randomized to receive PCC (50 IU/kg), TXA (1.0 g), or saline (n=49/group) 4 hrs prior to performing a second Bx. Investigators were blinded to treatment allocation.

Results: Neither PCC nor TXA reduced BD or BV following the Bx. PCC partially reversed PT and completely reversed ETP with increases in F1.2. TXA had no effect on these parameters. Additionally, neither therapy affected D-dimer. All treatments were well tolerated, and there were no major bleeding events or thrombotic complications.

Conclusions: PCC partially reversed PT and completely reversed ETP in volunteers given a supratherapeutic dose of RIVA, whereas TXA did not. Neither intervention reduced skin Bx BD or BV. The clinical relevance of these findings are uncertain, however the pharmacodynamic changes observed with PCC administration are consistent with previous studies. Assessing PCC on bleeding associated with lower therapeutic doses of RIVA may be warranted.

PB 344 | Idarucizumab for Dabigatran Reversal in Daily Practice - First Experiences from Slovenia

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Background: Idarucizumab is used for reversal of anticoagulant effects of dabigatran in the emergency setting.

Aims: To evaluate first experiences of idarucizumab in daily practice in Slovenia.

Methods: We analysed consecutive cases with idarucizumab use from January till October 2016. The indication for idarucizumab , assessment of clinical outcomes and the decision on blood sampling for coagulation tests (partial thromboplastin time, thrombin time and diluted thrombin time) before and after idarucizumab use were made by treating clinicians.

Results: Idarucizumab was used in 17 cases. One patient was treated with the antidote twice in the interval of two months. The median age of the patients was 83 (58-94) years, 10/16 patients were females. The median time from the last dose of dabigatran was 12 (1-72) hours. The indications for idarucizumab and clinical outcomes are presented in Table 1. In 7 cases dabigatran was reinitiated. Among cases with laboratory data available, baseline coagulation times were prolonged 12/13 cases with bleeding or emergency surgery. After idarucizumab administration normal coagulation parameters were confirmed in 10/11 cases within 16 hours, however later on re-occurrence of dabigatran effect was noted 4 patients with creatinine clearance < 30 ml/min and one patient with persistent bleeding required re-treatment with idarucizumab. In two patients with acute ischemic stroke and thrombolysis subsequently analysed baseline samples revealed dabigatran concentration < 30 ng/ml.

TABLE 1 Indications for idarucizumab and clinical outcomes

Indication for idarucizumab	No. of cases	Outcome	Death and cause of death
Emergency surgery			
- acute apendicitis	2	2 uneventful surgery	-
- stomac perforation	1		1 (septic shock)
- leg gangrene	1	1 uneventful surgery	-
Bleeding			
- intracranial	7	4 recovery and discharge 1 recovery and ischemic stroke on 6th day	2 patients (one new intracranial bleeding, the other multiorganic failure)
- gastrointestinal	2	1 recovery and discharge	1 patient (cardiac arrest)
- trauma or invasive procedure	2	1 recovery and discharge	1 patient (multiorganic failure)
Thrombolysis for ischemic stroke	2	2 recovery and discharge	-

Conclusions: Our first experiences with idarucizumab use in daily-care settings are supporting rapid and efficient decrease in anticoagulant effect of dabigatran in emergency situations. Late re-occurrence of dabigatran effect was noted in a subset of patients with severe renal failure. High mortality rate is not unexpected due to severity of the index events and associated comorbidities.

PB 345 | The Influence of Histones on the Generation of Factor XIa in Trauma Patient Blood

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Background: We previously reported that exogenous histone H4 can efficiently initiate thrombin generation (TG) in blood from healthy donors which is not affected by the addition of an inhibitory α-H4 mAb. In a current study, similar lack of an effect on TG by α-H4 was observed in trauma patient blood. It was also observed that FXIa is generated in citrated blood in the absence of corn trypsin inhibitor (CTI).

Aims: To evaluate/compare histone dependent FXIa generation in citrated blood from both trauma patients and healthy volunteers.

Methods: Three blood samples were collected in sodium citrate from 8 trauma patients and healthy donors matching in sex and similar in age. Anti-H4 mAb was added immediately to sample 1 and subsequently to sample 2 at 15 min post-draw. No additions were made to sample 3 and all samples were quenched with CTI at 15 min post-draw. FXIa concentrations in samples treated with α-H4 were quantitated based

on the response of whole blood TG and clot formation to an inhibitory α-FXIa mAb.

Results: Anti-H4 mAb added at 0 min inhibited FXI activation and consequentially delayed TG and clot formation in blood from both trauma patients and healthy donors. Based on thromboelastometry, FXIa levels in trauma patient blood treated with α-H4 at 15 min were approximately 12-fold higher (11.6 ± 6.2) than in that same blood treated with α-H4 at 0 min. In healthy donors, however, FXIa concentration increased by roughly only 3-fold (2.7 ± 1.4) in corresponding samples. Less pronounced differences in FXIa generation between healthy donors and trauma patients were observed in the TG assay, although the trend still held.

Conclusions: FXIa generation observed in citrated blood is endogenous histone-dependent and is partially inhibited by the α-H4 mAb. Furthermore, FXIa generation in trauma patient blood occurs at a more robust rate than in blood from healthy donors.

PB 346 | Markers of Platelet Activation Are Elevated Specifically on Leukocytes after Major Injury

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Background: Critical injury is associated with reduced platelet function, coagulopathy and immune dysfunction. The mechanisms for these remain unclear.

Aims: Our aim was investigate basal activation of whole platelets and platelet derived material bound to leukocytes in trauma patients *vs.* healthy controls, and to explore its impact on patient outcomes.

Methods: Adult trauma patients at a major trauma centre were recruited and underwent a baseline blood draw. Whole blood was incubated with a platelet marker (CD42b) and a panel of platelet activation markers; CD62P, CD63 and PAC1. Samples were fixed and processed using flow cytometry. Expression of platelet activation markers were quantified by CD42b positivity (CD42b+), either as a whole population or whether they were free or bound to leukocytes. Healthy volunteers served as controls.

Results: Expression of platelet activation markers on the total CD42b+ population was not significantly different from healthy controls. Leukocyte bound CD42b+ material had increased CD62P (MFI, 3894 patients *vs.* 1338 controls, $p = 0.08$) and CD63 expression (MFI 2149 patients *vs.* 448 controls, $p < 0.01$). Coagulopathic vs. non coagulopathic patients (MFI, 5342 vs. 3894) and patients that developed multiple organ failure (MOF) or died (MFI, no MOF 3109 *vs.* MOF 3743 *vs.* dead 4864) also trended towards greater platelet activation marker expression on leukocytes.

Conclusions: Total CD42b+ events in trauma patients did not significantly differ in their expression of activation markers from healthy controls; CD42b+ material bound to leukocytes exhibited markedly elevated expression of CD62P and CD63 in trauma patients. How this affects functional capacity of trauma patient platelets is unclear, but owing to the role platelets play in haemostasis and their potential to alter subsequent inflammatory responses, the reasons behind this phenomenon may warrant further examination.

PB 347 | Evaluation of Early Metrics of Clot Amplitude from New Generation Thromboelastogram (TEG6s) in Capturing Trauma Induced Coagulopathy

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Background: Maximum amplitude (MA) of clot from viscoelastic hemostatic assays like Citrated Kaolin (CK), Rapid kaolin (CRT) in TEG is the result of platelet-fibrin interaction while from functional fibrinogen (CFF) assay it is primarily the contribution from fibrin. Increased MA (idicative of hypercoagulability) is associated with thromboembolic complications, while decreased MA is indicative of hypocoagulable state, and can be used in tailoring transfusion of platelets or fibrinogen concentrate in hemorrhagic patients.

Aims: This study assesses the reliability of early variables like A10 and A15 (amplitude after 10 and 15 mins respectively of defining R, where R is the time in minutes to clot initiation) in predicting MA. Our objective was to understand their correlation, and agreement in stratifying hypo and hypercoagulable states within CK, CRT, and CFF assays.

Methods: We retrospectively analyzed data from trauma patients admitted at three trauma centers (n=300). Using linear regression model

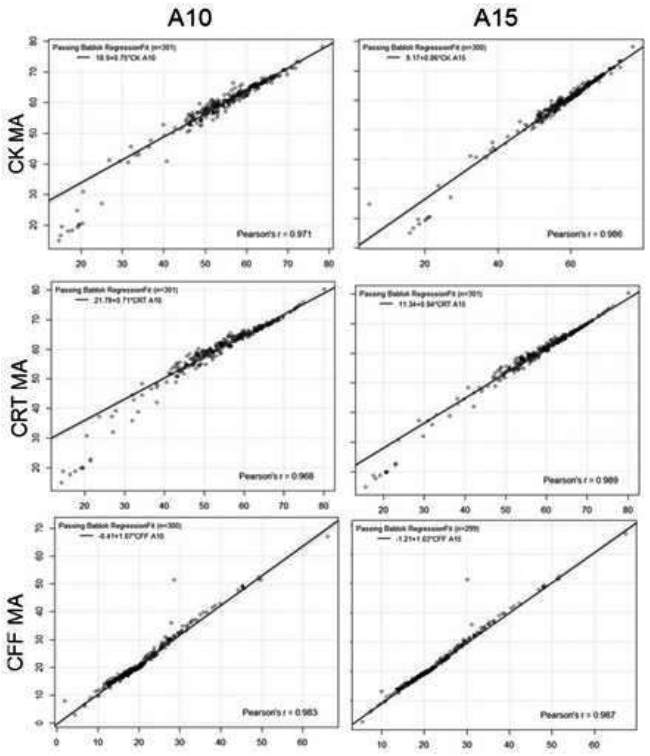

FIGURE 1 Plots showing strong correlation between MA and A10/A15 for CK, CRT, and CFF assays

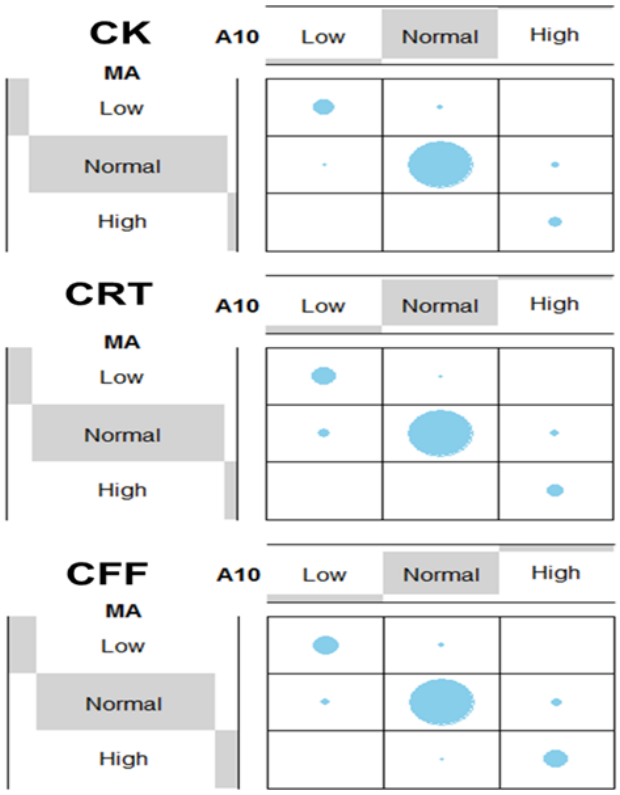

FIGURE 2 Contingency tables for A10 and MA represented using dots (size reflective of magnitude of agreement within categories)

we evaluated the correlation between MA, and the early variables. To understand the agreement between parameters A10/ A15, and MA, we categorized into Low (below range), Normal (within range), and High (above range) based on the reference ranges for each parameter within each assay and evaluated using Kappa. We compared time to reach A10 (mins) to the time to reach MA using paired Student's t.

Results: We observed excellent correlation between A10/A15 with MA (r>0.96) in each assay(Fig 1). The agreement between the early variables and MA in identifying the three levels of clot strength (Fig 2) are very strong (Kappa, A10: CK-0.92, CRT-0.85, CFF-0.89, A15: CK-0.96, CRT: 0.85, CFF-0.90). In each of the assay, the time to reach MA was significantly greater than the time to reach A10 (p< 0.001).

Conclusions: Early values of clot amplitude are fast, and reliable in predicting maximum clot strength. They may be used to get an advanced notice on coagulopathic condition like an increased risk of ischemic or bleeding events.

PB 348 | Implementation and Migration of Haemophilia-treatment-diaries from Paper Based to Electronic Documentation at Haemophilia CCC at the University of Bonn

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Background: According to §14 of TFG (law of transfusion in Germany) all usage factor concentrates and blood-products have to be ducumented by the treating physican. In order to enable a home based tratment this obligation is usually passed to the patient. The duty of documentation mostly has been fulfilled as paper based diaries which were mostly sent by post to the treatment-center to file them for the statutory period of 30 years.The rapid progress in the electronical market now offers new abilities to fulfill the legal requirements. Two app-based protocol projects launched in the last years into the german market: a web-based application and Haemoassist 2, a native smartphone app & web-based procuct, which made it the solution of choice in Bonn.

Aims: The project should simplify the documentation obeing german legislation and proove if such a system may improve data quality and quantity provided to the physican.

Methods: The center in Bonn built a team of physicans, data entrists and IT who implemented a secure redundant server system as well as the logical registration process whithin three months. The basic preparation was followed by the implementation of an interface to the existing database containing patient-information to exchange treatment and medication information. The real test drive took about 4 months whith a selected group of patients to avoid unwanted behaviour of the App or any connected system.

Results: Since start of the daily routine in April 2016 the center was able to register about 30 patients weekly in average in Haemoassist 2. The on demand SAE-email alerts make it possible to react whithin a short period of time and reducees the risk of late complications.

Conclusions: It is already obvious that hospitalization, bleedings and fluctuation in patients amount of substituion help in decision making. Finally we can say that patients are open minded about the electronic documentation. Additionally the physicans have a mighty tool to monitor the patients tratment including automated SAE alerts.

PB 349 | Development and Validation of the Warfarin-Aspirin Bleeding Assessment Tool (WA-BAT)

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Background: Bleeding assessment tools (BAT) are developed to screen and estimate bleeding risk in patients with bleeding disorders. A useful application of such tools would be as a standardised measure to compare rates of bleeding in patients on long-term thromboprophylaxis with either antiplatelet therapy or anticoagulation. To date, BAT have not been validated in this scenario.

Aims: To develop a self-administrable BAT to assess bleeding in patients undergoing long-term TP with aspirin or warfarin.

Methods: An online warfarin-aspirin-BAT (WA-BAT) was created based on the "Molecular & Clinical Markers for the Diagnosis & Management of Type 1 von Willebrand disease" BAT (Rodeghiero 2005). Items were refined and language adapted to be appropriate for

participants above 10 years. Items were removed (e.g. umbilical stump bleeding) and added as appropriate (e.g. bleeding post blood test).
The WA-BAT underwent validation by four experts (2 pediatric hematologists and 2 anticoagulant nurses).
Eligible participants were identified from our anticoagulation clinic database (Royal Children's Hospital, Melbourne) as patients aged above 10 years and receiving long-term aspirin or warfarin. For test-retest validation, the WA-BAT was administered twice to participants and Cohen's kappa analysis was used to determine intrarater reliability (IRR).
Results: Seven of the ten invited participants completed the WA-BAT. Of these, four completed the WA-BAT for a second time. Table 1 demonstrates the agreement between subsequent WA-BAT administrations. Substantial IRR agreement was determined.

TABLE 1 WA-BAT intrarater reliability coefficients

	Agreement (%)	Cohen´s Kappa	Days between adminstrations
Participant 1	82	0.65	91
Participant 2	100	1.00	41
Participant 3	82	0.60	135
Participant 4	94	0.85	43
Overall	90	0.77	78 (Mean)

Conclusions: The WA-BAT is the first self-administrable tool developed to assess bleeding in patients receiving aspirin or warfarin as TP. The WA-BAT showed substantial IRR, and assesses major and minor bleeding associated with long-term warfarin or aspirin use.
WA-BAT standardized questions make it suitable for longitudinal evaluation of patients. Further, this tool may be used to compare bleeding rates\types in populations on aspirin or warfarin.

PB 350 | Prothrombin Concentrate Complex for Major Bleeding in Patients Receiving Direct Oral Anti-coagulants: A Single Center Retrospective Study

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Background: The usage of direct oral anticoagulants (DOAC) has expanded over the last years. Major bleeding under anticoagulant treatment, including DOAC, is a life-threatening event, requiring rapid reversal of drug effect. However, in a real-world setting where direct antidotes are not yet available, administration of a prothrombin complex concentrate (PCC) is a reasonable therapeutic option.
Aims: To evaluate the efficacy of PCC administration for major bleeding in patients receiving DOAC.
Methods: A retrospective study evaluating all consecutive patients receiving PCC for major bleeding under DOAC, between 12.2014 and 12.2016. The primary outcome was 30 -day mortality. The secondary outcomes were need for blood transfusion, need for surgical intervention and thrombotic complications.
Results: 53 patients treated with PCC due to DOAC related a major bleeding were assessed. The median age at time of treatment was 81 years. 50% were male. 5 patients received dabigatran (9%), 29 received rivaroxaban (54%) and 19 received apixaban (36%). Intracranial hemorrhage (ICH) was the commonest event (29, 54%), followed by gastrointestinal bleeding (4,8%). 30-day mortality rate was 26%. Of the patients who died, 1 received dabigatran (1,7%), 10 received rivaroxaban (71%) and 3 received apixaban (4,22%). 30-day mortality in patients with ICH was 33%. Of the patients with ICH, 72% were treated with rivaroxaban and 28% with apixaban. There were no differences in primary or secondary outcomes between the groups. 2 patients (4%) only had thrombotic complications after PCC administration.
Conclusions: The rate of 30-day mortality in our cohort is slightly higher than the mortality rate demonstrated in previous studies after PCC administration for warfarin-associated bleeding. This may due to a high rate of ICH in our cohort. In current real life setting, PCC administration for ICH under DOAC therapy still seems a reasonable option.

PB 351 | Chemical Composition of Malian Medicinal Plants Used to Treat Bleeding Events

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Background: In first access to treatment for all diseases, 80 - 90 % of Malian population frequently uses medicinal plants. However bleeding diseases treatment remained a big challenge. Here, we investigate the chemical properties of 10 medicinal plants traditionally used to treat bleeding events.
Aims: Identify chemical components having hemostatic activity in the Malian flora.
Methods: Extracts from stems barks of *Annona senegalensis*, *Baissea multiflora Entada african,Carica papaya,Pteleopsis myrtifolia*, leaves of *Cassia sieberiana*, *Guiera senegalensis*, *Detarium microcarpum*, roots of *Carica papaya*, roots barks of *Erythrina senegalensis* and seeds of *Gossypium barbadense* were used. The chemical composition of dried and powdered plant material has been characterized using chemical groups' specific detection reagents and TLC. Water, dichloromethane and methanol extracts have been prepared using a 10% weight/volume proportion of powdered mater in 24 h maceration in solvents. These extracts have been tested for their hemostatic property in human blood.
Results: All plants parts were very rich in catechic tannins, sterols and terpens derivatives, flavonoids (mostly anthocyans type) and in sugars (oses and holosides). *Pteleopsis myrtifolia* contained both catechic and gallic tanins. *Pteleopsis myrtifolia* (leaves and stem bark), *Entada africana* and *Erythrina senegalensis* have shown a high content of coumarins. Water extracts from *Pteleopsis myrtifolia* (leaves and stem

bark), *Baissea multiflora* (stem bark) and *Entada africana* (stem bark) have shown potent haemostatic activity.

Conclusions: Medicinal plants traditionally used to treat bleeding event in Mali contain substances known for their pro-coagulant activity; other compounds having anticoagulant activity have also been found in smaller amount. These results when confirmed could open a new research area in the field of hemostasis in Mali.

PB 352 | First Italian Experience of Clinical Use of Obizur, Recombinant Porcine Sequence Factor VIII (RPFVIII), for Acquired Haemophilia A (AHA)

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Background: A recombinant porcine factor VIII B-domain-deleted product (rpFVIII; OBIZUR, Shire plc) was recently approved for treatment of AHA in some countries, but not yet used in Italy.

Our aim is to describe the first Italian experience using rpFVIII for AHA patient.

Aims: Our aim is to describe the first Italian experience using rpFVIII for AHA patient.

Methods: A 68-year-old man with lung cancer and a recent coronary angioplasty (PTCA) accessed our emergency department with left chest intramuscular haemorrhage, right periorbital haematoma onto the chin: bleeding diagnosed as associated to dual antiplatelets therapy (DAPT) and patient was discharged. Six days later he was re-admitted for clinical worsening and AHA was diagnosed with FVIII 2%, FVIII inhibitor 55UB/mL. DAPT was stopped, blood components transfused and a prohaemostatic therapy with aPCC (80U/kg bid) and tranexamic acid (10U/kg bid) from day 1-4 was administered, with no clinical benefit. We switched to rpFVIII.

Results: rpFVIII initial dosage was 100U/kg and FVIII activity (one-stage clotting assay) 30' after infusion increased from 2 to 162%. We sustained FVIII trough level at 55±5% and at 116±16% (mean values +/- SD in %) after infusion. In 36-48 hours bleeding stopped and rpFVIII was definitely discontinued after 6 days. Anti-pFVIII antibodies were stable at 1 UB/mL. Patient was discharged on day 11. Concomitant immunosuppressive therapy with full dose of prednisone was administered.

Conclusions: RpFVIII resulted as efficient therapy with no thrombotic adverse event in AHA patient at high thrombotic risk for recent PTCA.

PB 353 | The Effect of PCC on ROTEM Parameters in Patients with Coagulopathy of Liver Disease

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Background: As an alternative to platelet transfusion for the prevention and treatment of bleeding due to thrombocytopenia, we speculated that increasing the concentration of procoagulant factors by the addition of prothrombin complex concentrate (PCC) in thrombocytopenia associated with liver disease may improve thromboelastometry (ROTEM) parameters and contribute to normalising haemostasis.

Aims: To evaluate the effect of PCCs on the parameters of ROTEM analysis in patients with coagulopathy of liver disease and thrombocytopenia.

Methods: NATEM ROTEM (TEM Innovations GmbH, Munich Germany) analysis was performed on whole blood samples from patients with liver disease and thrombocytopenia (Child Pugh Score B and C, INR >1.5, platelet count range 26-79 x 10^9/L, n=11). 10mL blood was collected into sodium citrate and corn trypsin inhibitor. A 0.12pM tissue factor/0.2M $CaCl_2$ trigger was used at baseline and after spiking with a four factor PCC at doses corresponding to 0.15, 0.30 and 0.60 IU prothrombin/mL final concentration. 0.60 IU/mL is equivalent to a therapeutic PCC dose of 25 IU/kg.

Results: Spiking with PCC significantly shortened the clot time (CT) and time to maximum velocity of clot formation (MaxV-t), with one way ANOVAs across all concentrations giving $p< 0.0001$ and 0.05 respectively. Addition of 0.15IU/mL shortened CT (M=464 to 287) and MaxV-t (M=556 to 361) compared to baseline (paired t-test $p< 0.05$ in both instances), and alpha angle was significantly increased compared to baseline (paired t-test $p< 0.001$). MCF (maximum clot firmness) showed a non-statistically significant increase (M=33 to 40, p=0.08).

Conclusions: Platelet dependent ROTEM parameters CT and MaxV-t show a significant shortening and alpha angle a significant increase in response to sub-therapeutic concentrations of PCC in samples from patients with liver disease and varying degrees of thrombocytopenia. This could infer a potential for therapeutic application for PCC in liver disease.

PB 354 | Factor VIII and von Willebrand levels at Desmopressin (DDAVP) Challenge Test: A Single Center Results

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Background: DDAVP is useful in the treatment of vW factor type 1 qualitative defects and, mild A haemophilia, is used to determine an increase in Factor VIII and vW, to diagnose coagulation disorders and to plan therapeutic decisions.

Aims: Describe the behavior in factor levels with the administration of desmopressin.

Methods: A retrospective cohort study in patients older than 18 years who underwent DC, known to have factor VIII, vW or ristocetin cofactor (RC) deficiency, to describe factor VIII and vWF behavior during the challenge. The test starts with a baseline sample collection to determine factor VIII, vWF and RC, followed by a subcutaneous DDAVP, and samples at hours 1, 4, 6 hours after the injection.

Results: 25 Patients with von Willebrand or factor VIII deficiency diagnosis were included. 6 men and 19 women. Median age 37 years (Range 18-65). 18 baseline samples had low FVIII levels, median activity of 37%. Median factor level were 89 (2.1 times the basal levels), 75,1 and 88,9% at 1, 4 and 6 hours respectively. 14 baseline samples had abnormal vWF values, median activity 22,5%. Median factor levels were 80 (3,5 increase from baseline levels), 84 and 63.4% at 1, 4 and 6 hours respectively. 3 baseline samples had RC abnormal levels, median 13,3%. Median factor levels were 83 (6.38 increase from baseline levels), 59 and 46% at 1, 4 and 6 hours respectively. No serious adverse event was detected during the 6-hour clinical surveillance or in the 24-hour telephone follow-up.

Conclusions: The response criteria for DC has not been clearly defined, until now 2 to 3 times increase in baseline values has been considered acceptable, however a detailed evaluation of the clinical situation of each patient should be done, as there may be an acceptable increase in levels in terms of the response criteria without achieving a safe hemostatic ranges. In this cohort of patients, the vast majority demonstrated adequate responses achieving hemostatic level for measured factors; with minimal side effects.

PB 355 | Correlation between Platelet Count and Platelet Component in Thromboelastometry

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Background: The difference between two ROTEM tests (EXTEM and FIBTEM) represents platelet contribution to blood clot strength i.e. platelet component. Estimation of platelet component (PC) should be done based on Clot Elasticity (CE) according to Solomon et al. It is not a routinely calculated parameter in thromboelastometry but it could be clinically useful in management of major hemorrhage. We hypothesized that platelet number correlates better with PC Maximum Clot Elasticity (MCE) then with Maximum Clot Firmness (MCF).

Aims: To establish if the platelet count correlates better with MCE PC or with MCF PC.

Methods: Routinely performed thromboelastometry results (N=45) were collected and analyzed retrospectively from laboratory database. Maximum Clot Elasticity was calculated as follows MCE= (100*MCF)/(100-MCF). PC MCE and PC MCF were calculated as follows: EXTEM MCE-FIBTEM MCE, EXTEM MCF-FIBTEM MCF. Thromboelstometric measurements EXTEM and FIBTEM were performed on ROTEM delta (TEM International GmbH, Munich, Germany). Platelet count (n=45) averaged 104±76x10^9/L was measured using Sysmex XN1000 (Sysmex Corporation, Kobe, Japan). Statistical analysis was performed using MedCalc Statistical Software demo version 14.12.0. Pearson correlation coefficients were calculated and p value < 0.05 was considered significant.

Results: Platelet count positively correlated with MCF PC (r=0.495, p=0.0005) and MCE PC (r=0.718, p< 0.0001).

Conclusions: Much higher correlation coefficient was obtained between platelet count and MCE PC then with MCF PC, as we hypothesized. However, obtained correlation coefficient is not very high since blood clot formation does not depend only on the platelet number but also on the platelet functionality. MCE Platelet Component could be a better predictor of bleeding or a better parameter in platelet transfusion management. It should be clinically validated for management of major hemorrhage.

PB 356 | Favorable Effect of Combined Use of Prothrombin Complex Concentrate and Plasma in in vitro-Induced Coagulopathy

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Background: Uncontrollable bleeding is a major cause of death in emergency rooms. Transfusion to secure intravascular volume with crystalloid liquids may induce dilutional coagulopathy that requires additional treatment with clotting factor concentrates, such as fibrinogen (Fbg) and prothrombin complex concentrate (PCC). For plasma resuscitation, single donor fresh frozen (FFP) or solvent/detergent (S/D)-treated plasma can be used. The use of oral anticoagulants worsens trauma- or surgery-induced coagulopathy.

Aims: To investigate the effect of resuscitation fluids, clotting factor concentrates and combinations thereof on the hemostatic profile of rivaroxaban-anticoagulated whole blood.

Methods: We used rivaroxaban spiked whole blood and tissue factor triggered thromboelastography in the presence of tissue-type plasminogen activator to induce fibrinolysis. We mimicked a resuscitation approach that resulted in a drop in hematocrit from on average 39 to 24% and a 50% reduction in rivaroxaban concentration.

Results: Spiking whole blood with 50-300 µg/ml rivaroxaban resulted in a hypocoagulabe state that remained upon subsequent dilution with plasma or saline. Combined use of FFP and PCC, however, did not only strongly improve clot formation but also clot stability. S/D-treated plasma was less efficient than FFP that is most likely due to a low alpha-2-antiplasmin level, and required tranexamic acid (TXA) in addition to PCC to compensate for induced hyperfibrinolysis. Saline also induced a profibrinolytic state as a component of dilutional coagulopathy, as such worsening the hemostatic potential of rivaroxaban-anticoagulated blood. Subsequent joint PCC and Fbg supplementation

required additional factor V and TXA for correcting clot formation and stability.

Conclusions: In the setting of rivaroxaban anticoagulation, a combination of plasma and PCC may provide the most effective resuscitation approach with the notion that S/D-treated plasma requires antifibrinolytic drug support.

PB 357 | Towards a Clinically Relevant Hybrid Adenovirus-Sleeping Beauty Transposon Vector for Gene Therapy for von Willebrand Disease

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Background: Gene therapy offers potential for a long-term treatment of severe von Willebrand disease (VWD). Using non-viral *Sleeping Beauty* transposons (SBT), we previously achieved sustained expression of von Willebrand factor (VWF) in VWF-deficient (*Vwf*$^{-/-}$) mice. To circumvent clinical limitations associated with hydrodynamic delivery, high-capacity adenoviral vectors (HC-AdV) were combined with the SBT technology.

Aims: To develop a clinically applicable gene therapy platform for VWD based on HC-AdV that deliver the SBT system to hepatocytes.

Methods: Two HC-AdV were constructed, comprising the two-component SBT system. A first HC-AdV contained the VWF transgene under control of a liver-specific promoter, flanked by FRT sites and SBT inverted repeats. A second HC-AdV carried the Flp recombinase and SB100X transposase. After intravenous co-injection of the vectors in *Vwf*$^{-/-}$ mice, VWF antigen levels and VWF multimer pattern were regularly determined in plasma for up to 1 year. Correction of the bleeding phenotype was evaluated using tail-clip and saphenous vein bleeding models.

Results: Functionality of this hybrid adenovirus-SBT vector was assessed in *Vwf*$^{-/-}$ mice and resulted in very high and stable VWF levels, still 2072±383% of wild type levels 1 year after gene transfer. Moreover, FVIII activity was restored to physiological levels (102±33%, 1 year after gene transfer). Both tail-clip bleeding at 12 weeks and saphenous vein bleeding at 1 year resulted in small, but not significant reductions of bleeding time. The reduced fraction of high molecular weight multimers observed in hepatocyte-produced VWF might account for the partial corrected bleeding phenotype.

Conclusions: The hybrid adenovirus-SBT vectors efficiently delivered VWF transposons into hepatocytes, resulting in very high and sustained VWF transgene expression. Despite no full correction of the bleeding diathesis, this powerful vector system shows great promise towards a clinically applicable gene therapy for VWD e.g. for targeting endothelial cells.

PB 358 | Comprehensive Functional and Structural Studies to Characterize von Willebrand Factor Propeptide Novel Missense Variants with Unknown Pathological Significance

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Background: Six novel missense variants were identified in von Willebrand factor (VWF) propeptide either as homozygous (p.V86Q and p.C608W) in patients with type 3 von Willebrand disease (VWD) or as compound heterozygous in VWD patients with ambiguous phenotype (p.G55E, p.W191R, p.N211D and p.G344Q).

Aims: This study aimed to determine the pathological consequence of these novel variants on the structure, biosynthesis and function of VWF.

Methods: The wilde-type (wt) or mutant VWF cDNA was expressed in HEK293 cells. The levels both VWF and propeptide secreted into medium were determined and qualitative assessments including VWF binding functions and multimer analysis were performed. The subcellular localization and storage of VWF was evaluated by immunofluorescence confocal microscopy and transmission electron microscopy imaging. Protein models of the discrete propeptide (D1-D2) and D´-D3 domains were generated. The joined models were docked multimerically to generate putative tubular structures. The variants were then mapped on these models and MD simulation was performed.

Results: Transfection of the four out of six mutants (p.G55E, p.V86Q, p.W191R and p.C608W) demonstrated severely impaired VWF secretion and defect in intracellular storage. Their co-transfections with the VWF-wt resulted in correction of the expression phenotype but still showed reduction of secreted VWF, loss of big multimers and diminished binding functions of the VWF. The generated models illustrated that these mutated residues either directly disturb the core fold of the individual domains (for e.g. p.C608W), or indirectly influence the surface properties of the domains by disturbing the designated allosteric disulfide bonds (for e.g. G55E).

Conclusions: Using a combination of in vitro gene expression, modeling and MD simulation studies we have been able to expand insights into the significance of VWF propeptide missense mutations in VWF intracellular processing and pathophysiology of VWD.

PB 359 | Triggering Receptor Expressed on Myeloid Cells Like Transcript-1 (TLT-1) is a Novel Ligand for von Willebrand Factor

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Background: Von Willebrand Factor (VWF) binds to platelets via interaction of its A1 domain with platelet Glycoprotein Ib and then

subsequent interaction between the RGD sequence in the VWF C4 domain and platelet glycoprotein IIbIIIa. However it has recently been shown that Vimentin present on platelets also binds to VWF and therefore other platelet receptors may act as ligands for VWF. To this end we performed a binding screen of known platelet membrane proteins and identified TLT-1 as novel VWF binding partner. TLT-1 is exclusively expressed in platelets where it is stored in alpha-granules and is presented on the platelet surface following platelet activation. A soluble form of TLT-1 is also released into the circulation.

Aims: To characterise the interaction of VWF with TLT-1 and define its functional significance.

Methods: Soluble TLT-1 (sTLT-1) and recombinant VWF fragments were expressed in HEK293T cells and purified by Nickel affinity chromatograph. Binding assays were performed using plates coated with sTLT-1 and incubation with VWF and its fragments. Flow assays were carried out perfusing whole or plasma free blood over VWF or collagen surfaces in the absence or presence of an anti-TLT-1 antibody.

Results: Using plate binding and immunoprecipitation assays we showed that VWF binds to sTLT-1 with high affinity (5.7 nM) and the binding is dependent on calcium ions and involves the VWF A3 and D4 domains. Furthermore beads coated with the extracellular portion of TLT-1 were able to interact with VWF under shear stress. Using an antibody directed against TLT-1 we were able to block the interaction with VWF and in flow assays observed a marginal reduction in platelet capture under shear stress, but a significant reduction in thrombus size and volume, suggesting that targeting TLT-1 may be a novel, safe anti-thrombotic strategy.

Conclusions: TLT-1 is a novel ligand for VWF and targeting TLT-1 maybe a potentially safe route to preventing excessive thrombus formation.

PB 360 | A2 Domain Unfloding Might Significantly Increase Shear and Elongation-meadiated vWF Unfolding in the Bloodstream

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Background: Multimeric vWF molecules circulating in blood plasma are unique mechanosensitive glycoproteins experiencing significant conformational changes in response to high shear and elongation rates within the blood flow. VWF A2 domain unfolding is known to be crucial for ADAMTS13-dependet regulation of vWF multimers size distribution, however, the role of A2 domain flexibility in overall conformational dynamics of vWF multimers is not clear.

Aims: The basic goal of this work was to study the impact of A2 domain unfolding on conformational dynamics of vWF molecule using the coarse-grained computational model of vWF.

Methods: In order to account for A2 domain unfolding the classical coarse-grained model of vWF was modified to have variable inter-dimer distance. The kinetic characteristics of A2 domain unfolding in response to external force were taken from single-molecule measurements reported earlier. Conformational dynamics of vWF was analyzed for a wide range of hydrodynamics conditions, including stationary shear and elongation rates, their combinations and non-stationary conditions corresponding to local flow disturbances by vessel stenosis or platelet thrombus.

Results: The results of our simulations show that A2 unfolding might influence the conformational dynamics of larger vWF multimers, increasing their unfolding probability under both shear and elongational flows. For shear rates exceeding 3000 s-1 unfolding probabilities obtained with the modified model are significantly higher comparing to the old model, which does not take into account of A2 domain unfolding. Simulations of vWF dynamics under non-stationary conditions show significant dependencies of vWF unfolding probability on the obstacle passage time, predicting high unfolding rates for longer stenosis regions (bigger thrombus).

Conclusions: Our results show that vWF A2 domain unfolding can influence the conformational dynamics of vWF multimers under a wide range of hydrodynamic conditions, especially those expected in stenosed arteries.

PB 361 | Acquired von Willebrand Disease (aVWD) in Plasma Cell Dyscrasias (PCD): Variable Underlying Pathophysiology as Evidenced by the Analysis of Proteolytic von Willebrand Factor (VWF) Cleavage Fragments

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Background: PCD may be associated with hemorrhagic diathesis and aVWD, found in some PCD patients, is one of several possibly predisposing factors. The mechanism leading to aVWD may be variable and is partly speculative.

Aims: We investigated proteolytic VWF fragments from 3 published patients with PCD-associated aVWD (Dicke et al. Ann Hematol 2016; cases 1,5,6) to test whether enhanced proteolytic degradation may be a causal mechanism.

Methods: VWF from citrated plasma was immunoadsorbed in the presence of a universal protease inhibitor to rabbit anti-VWF IgG coupled

to CNBr-activated Sepharose 2B beads. Reduced and alkylated beads were applied to SDS-PAGE and immunoblotting using polyclonal anti-VWF IgG, monoclonal antibodies against N- or C-terminal VWF subunit domain (M7 and M31, respectively, Berkowitz et al. JCI 1987) or against a neoepitope on the N-terminal fragment generated by ADAMTS13 cleavage of the Y1605-M1606 bond (Kato et al. Transfusion 2006).

Results: 1 patient with decreased VWF activity (< 10%) and -antigen (12%) and another with elevated VWF levels but a paraprotein inhibiting VWF-glycoprotein Ib interaction showed, besides the intact 250 kD subunit small amounts of ADAMTS13 induced N-terminal 140 kD and C-terminal 176 kD fragments as seen in normal plasma. One patient with AL amyloidosis, VWF activity (20%) and antigen (126%) and loss of large VWF multimers showed an increased proportion of cleavage fragments. In addition to the physiologic ADAMTS13 induced fragments there was an N-terminal 176 kD and C-terminal 145 kD band, detected by M7 and M31, respectively, compatible with a plasmin induced VWF cleavage, strongly supported by elevated plasmin-antiplasmin complexes in this patient.

Conclusions: Analysis of in vivo VWF fragments revealed an aberrant cleavage pattern, presumably caused by plasmin, as cause for a PCD (AL amyloidosis)-associated aVWD. Whether fibrinolysis inhibition may correct the acquired VWD remains to be studied.

PB 362 | Axial Structure and Collagen Binding of the von Willebrand Factor Multimer

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Background: The von Willebrand factor (VWF) is a multimeric plasma glycoprotein with size ranging between 0.5 and >20MDa. VWF binding to collagen (VWF:CB) decreases with the pathological loss of HMW multimers. Whether the accumulation of high molecular weight (HMW) multimers leads to increasing VWF:CB and changes in the VWF axial structure (e.g, length), is currently unknown.

Aims: Here we investigated the correlation between VWF:CB ratio and VWF length measured with atomic force microscopy (AFM), and the correlation between AFM-based VWF length and the SDS-agarose ELFO-based MW. Furthermore, we explored whether VWF length is affected by sample storage.

Methods: VWF from plasma-derived therapeutic specimens or platelet cryoprecipitate was fractionated according to MW and heparin affinity (Sepharose CL2B, HiTrap Heparin HP). VWF concentration (VWF:Ag) and collagen-binding capacity (VWF:CB) were measured with ELISA. HMW multimer proportion (measured with SDS-agarose ELFO followed by immunoblot and densitometry) was expressed with M_{MW25} that corresponds to the MW that demarcates the upper 25% of the densitogram integral. VWF length was measured in AFM images by tracing the contour of mica-adsorbed individual multimers.

Results: VWF multimers appeared as 100 to 2410-nm-long strings of 15-30 nm globules. In HMW fractions, long contour length chains (800-2410 nm) dominated. In this HMW fraction the VWF:CB/VWF:Ag was between 1.5 - 0.5, and the M_{MW25} was 8.6 - 3.1 MDa. We found a correlation between both VWF:CB/VWF:Ag and VWF length (r=0.9, p=0.001), and VWF:CB/VWF:Ag and M_{MW25} (r=0.9, p< 0.0001). We also found that large multimers gradually vanished after several days of storage at -20°C. After one hundred days of storage the maximum length became shorter by 50%, and the entire length distribution shifted to smaller lengths.

Conclusions: In summary, our results indicate that the elevation of the VWF:CB/VWF:Ag ratio can be a good indicator for the appearance of HMW VWF multimers in the human blood plasma.

PB 363 | Safety and Efficacy of Recombinant von Willebrand Factor (rVWF) in Patients with Severe von Willebrand Disease (VWD) Undergoing Major and Minor Elective Surgical Procedures: A Prospective Clinical Trial

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Background: Surgical hemostatic management in VWD is critical for patient safety. rVWF (VONVENDI) is safe and effective for bleed treatment, allows independent control of VWF and FVIII, and is not restricted to co-administration of FVIII, thus minimizing the risk of thrombotic events.

Aims: Evaluate hemostatic efficacy and safety of rVWF with or without rFVIII in patients with severe VWD undergoing major and minor elective surgical procedures.

Methods: Intraoperative and overall hemostatic efficacy rating using a 4-point nominal scale; overall efficacy was assessed 24 hrs after the last rVWF infusion or at day 14, whichever occurred earlier. A priming dose of rVWF, was given 12-24 hrs pre-surgery to raise endogenous FVIII:C; if target FVIII:C was not reached, a preoperative loading dose of rVWF and rFVIII was given to raise FVIII:C to recommended levels. Peri- and postoperative rVWF and rFVIII were infused to maintain target trough levels.

Results: All 15 subjects (Table 1) treated with rVWF with or without rFVIII for major (10), minor (4), and oral (1) surgical procedures had overall hemostatic efficacy ratings of excellent (73.3%) or good (26.7%). Intraoperative hemostatic efficacy ratings were also excellent (86.7%) or good (13.3%) for all subjects. Subjects received 121 infusions of rVWF with or without rFVIII, with the majority of subjects receiving rVWF alone: 100% (15/15) for the priming dose, 80% (12/15) for the loading dose, 80% (12/15) postoperatively, and 2 subjects received no additional postoperative rVWF or rFVIII. The median overall surgical dose of rVWF was 220.4 IU/kg (63.8 - 648.4 IU/kg). No treatment-related AEs occurred, and none were due to a severe allergic reaction. No subjects developed neutralizing antibodies to rFVIII or rVWF.

Conclusions: These data support the safe and effective use of rVWF with or without rFVIII in achieving peri- and postoperative hemostasis in subjects with VWD undergoing major, minor, and oral elective surgery.

TABLE 1 Demographics (N = 15)

Age (years, range)	Median (range)	40 (20–70)
Sex, n (%)	Male / Female	7 (46.7) / 8 (53.3)
Weight (kg)	Median (range)	73.5 (52.0–127.2)
VWD type, n (%)	1	3 (20)
	2A	2 (13.3)
	2B	1 (6.7)
	2M	1 (6.7)
	3	8 (53.3)
Surgical Procedure Classification, n (%)	Major	10 (66.7)
	Minor / Oral	4 (26.7) / 1 (6.7)

PB 364 | Recombinant Activated Factor VII-Induced Correction of Bleeding Tendency in Genetically-engineered von Willebrand Disease Type 2B Mice Evaluated Using New Tail Transection Bleeding Models

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Background: Von Willebrand disease subtype 2B (VWD2B) is characterized by von Willebrand factor (VWF) and platelet abnormalities related to enhanced VWF/platelet interactions. Treatment of patients with VWD2B can be challenging.

Aims: Our goal was to evaluate the haemostatic efficacy of recombinant human activated factor VII (rhFVIIa) using two novel tail transection bleeding models in a VWD2B murine model (genetically-engineered mice carrying the p.V1316M mutation in the *VWF* gene (V1316M mice)).

Methods: Tail artery transection (TAT) or tail vein transection (TVT) were performed in a standardized manner (depth and diameter: 0.7 and 0.3 mm respectively). Bleeding time (BT) and blood loss (BL) were measured in wild-type (WT) and V1316M mice with or without rhFVIIa infusion (3 mg/kg).

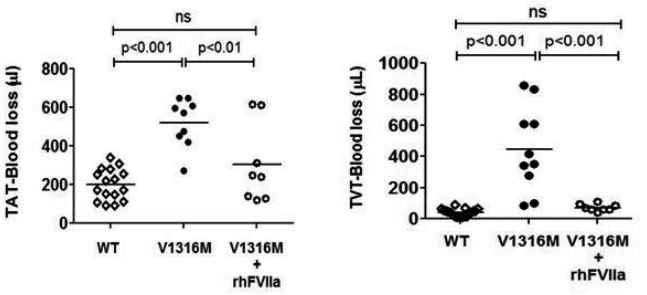

FIGURE 1 Effect of rhFVIIa on blood loss obtained in TAT and TVT bleeding models in von Willebrand disease type 2B mice (V1316M)

Results: In WT mice, BT was similar in both models (2.9±0.2 min *vs* 2.2±0.1 min for TAT and TVT respectively) whereas BL was significantly more important in the TAT model than in the TVT (201±20 µl *vs* 40±5 µl, p< 0.001). In V1316M mice, both parameters were significantly increased compared to WT mice: for TAT: 18.1±2.6 min (BT) and 522±42 µl (BL) and for TVT: 24.6±2.5 min (BT) and 448±86 µl (BL) (p< 0.001 for all parameters measured). After rhFVIIa infusion, BL was reduced in V1316M mice both in the TAT and TVT, to levels comparable to those obtained in WT mice: TAT: 303.2±72 µl (p=0.19 *vs* WT); TVT: 72±8 µl (p=0.86 *vs* WT) (Fig 1). For the BT parameter, a similar correction was observed in the TVT: 5.1±1.0 min (p=0.31 *vs* WT) but not in the TAT model: 14.1±2.8 min (p=0.004 *vs* WT).

Conclusions: In conclusion, we show that TAT and TVT are promising tools to evaluate bleeding tendency in murine models. It allowed us to show that a single infusion of rhFVIIa induces an immediate and complete correction of the bleeding tendency in V1316M mice. These results suggest that rhFVIIa should be considered as a treatment option in the clinical management of severe hemorrhagic episodes in patients with VWD2B.

PB 365 | VWF D'D3 Mutations Associated with Type 1 von Willebrand Disease Demonstrate Impaired Gp1Bα-VWF Binding under High Shear Stress

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Background: Traditionally, type 1 von Willebrand disease (VWD) mutations are thought to decrease von Willebrand factor (VWF) quantity but not to affect VWF function. However, the incomplete penetrance and variable expressivity of type 1 VWD suggests that other mechanisms, such as qualitative defects in VWF, may explain the discrepancy between VWF levels and clinical bleeding.

Aims: The hypothesis of this proposal is that some Type 1 VWD mutations may demonstrate qualitative defects in VWF function and it is evaluated by assaying the in vitro platelet dependent - VWF function of various mutants.

Methods: Transfection: Conditioned supernatant from HEK293T cells transfected with varying DNA ratios of VWF expression vectors with and without Type 1 VWD mutations are evaluated in a platelet rolling assay. Mutations are noted in Table 1.

TABLE 1 List of Type 1 VWD Mutations

Mutation	Domain	VWD Association	Multimer Pattern
R782Q	D´D3	Type 1	Normal
V1229G	D´D3	Type 1	Normal
R1374C (Positive Control)	A1	Type 2M	Normal

Rolling Assay: 20 µg/mL of an anti-VWF antibody is affixed in a microfluidic chamber. After blocking, 10 IU/dL VWF is incubated for 1 hour and fixed platelets are then perfused ($1x10^5$ cells/µL) at shear rates $100s^{-1}$ to $4500s^{-1}$ ($1500\text{-}4500s^{-1}$ shown). High speed movies are captured and total/average pause time (the aggregate/average time of non-movement/binding time of platelets) is calculated via ImageJ/FIJI.

Results: At high shear rates, there was a significant impairment of pause time for the V1229G and R782Q mutations in terms of total pause time (seen at $3500s^{-1}$ and $4500s^{-1}$) (Figure 1A) and for average pause time (seen only at $4500s^{-1}$) (Figure 1B) suggesting impaired VWF-A1-Gp1Bα binding at high shear rates. The finding of decreased total and average pause time suggest that the impaired VWF-A1-Gp1Bα binding is an aggregate finding across multiple platelets (total pause time) but also occurs at the level of the individual platelet (average pause time).

Conclusions: Both V1229G and R782Q demonstrate qualitative deficiencies of VWF-A1-Gp1Bα binding at high shear rates. This finding may suggest an additional mechanism that may influence bleeding in patients with type 1 VWD.

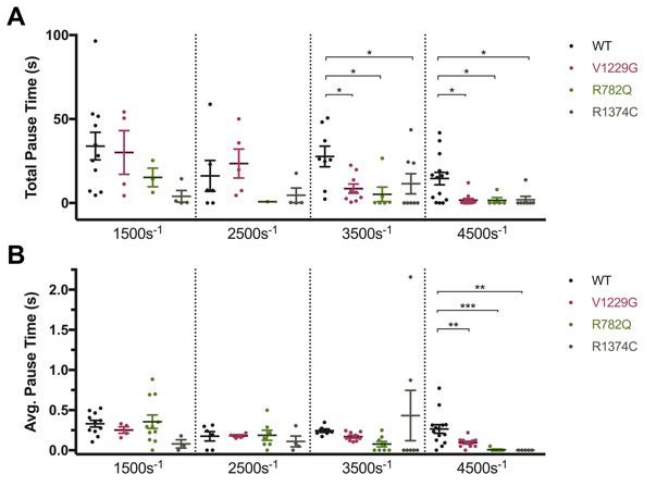

FIGURE 1 At high shear rates there is significant impairment of platelet pause times for V1229G and R782Q. * = P<0.05, **P<0.01, *** P<0.001

PB 366 | Diagnostic Challenges of Acquired von Willebrand Syndrome

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Background: Acquired von Willebrand syndrome (aVWS) is associated with several different clinical conditions, thereby involving a variety of medical disciplines like cardiology and hematology. The most important factor causing aVWS is enhanced shear stress on VWF, which propagates VWF proteolysis, resulting in the loss of the largest VWF multimers. One problem in diagnosing aVWS accompanying these disorders, is due to the highly variable levels of von Willebrand factor (VWF:Ag) and a frequently inconsistent correlation to VWF functional activities like collagen binding (VWF:CB). Therefore, aVWS may remain undiagnosed even when combining VWF quantitative and functional assays.

Aims: To assess the diagnostic value of VWF multimer analysis in aVWS.

Methods: From 2002 until 12-2017, a cohort of 4,737 patients was diagnosed with aVWS type 2A by assessing the ratio of VWF:CB/VWF:Ag and in addition by VWF multimer analysis for comparison. Clinical data were available for 2097 patients. All patients with a reduction of VWF high molecular weight multimers were considered as having aVWS, irrespective of their VWF:Ag or VWF:CB.

Results: By multimer analysis, patients were diagnosed with aVWS. Many patients had normal or even elevated VWF:Ag and/or VWF:CB and 39% had even a normal VWF:CB/VWF:Ag ratio. The percentages of undiagnosing aVWS by using only the VWF:CB/VWFAg ratio at a cut off >0,79, is 19-52% overall in the different groups (s. Table 1).

TABLE 1 Number of patients and rate of diagnostic failure by the VWF:CB/VWFAg ratio

Conditions	n	Ratio >0.8
Cardio-vascular	918	52 %
Myelo-proliferative	677	29 %
Lympho-proliferative	294	19 %
others	208	43 %
All	2097	39 %

Conclusions: The results of our study show that multimer analysis is superior in detecting even minor deficits of the largest and most active VWF multimers. Depending on the underlying disorder, a significant number of patients with aVWS would remain undiagnosed by conventional quantitative and functional assays without multimer analysis, which is further on an indispensable tool for a valid diagnosis or exclusion of aVWS.

PB 367 | Multimer Size Distribution and Multimerization Defects of von Willebrand Factor Investigated at the Single-molecule Level by AFM Imaging

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Background: The multimeric plasma glycoprotein von Willebrand Factor (VWF) is crucial for hemostasis. As large multimers are hemostatically more active than shorter ones, mutations in VWF that impair multimerization cause von Willebrand disease (VWD) type 2A. Multimerization occurs in two steps: First, monomers dimerize at their C termini via the three disulfides Cys2771-Cys2773', Cys2773-Cys2771', and Cys2811-Cys2811'. Subsequently, dimers are linearly linked at their N termini via disulfides Cys1099-Cys1099' and Cys1142-Cys1142', yielding an exponential multimer size distribution, with short multimers being more abundant than larger ones.

Aims: We aimed to investigate the multimer size distribution of wildtype VWF and specific mutants, in particular to gain further insights into the distinct roles of the cysteine residues involved in dimerization.

Methods: We employed atomic force microscopy (AFM) to directly visualize recombinant VWF samples at the single-molecule level. For proof-of-principle we compared our results with electrophoretic multimer analysis.

Results: For wildtype VWF and the N-terminal mutant p.Cys1099Tyr (VWD type 2A/IIC Miami), we confirm complete dimerization and exponential size distributions, with a markedly reduced extent of multimerization for p.Cys1099Tyr. For the C-terminal mutants p.Cys2771Arg and p.Cys2773Arg (VWD type 2A/IID), we find a severe loss of large multimers and observe a large fraction of ‚non-native' multimers that exhibit N-terminally attached monomers (see Fig. 1). We quantify the degree of dimerization abolishment to be 87% and 73%, respectively. Mutant p.Cys2811Ala also exhibits a significant fraction of non-native multimers, but only a mild loss of large multimers.

Conclusions: We show that AFM is a powerful approach to assess the size distribution of VWF that can help to gain a quantitative understanding of the processes involved in VWF's multimerization and in multimerization defects. Thus, AFM is a powerful complementarily method to multimer analysis.

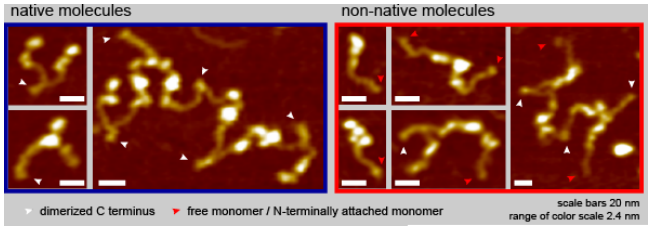

FIGURE 1 Single-molecule AFM imaging on wildtype and mutant VWF multimers

PB 368 | Exploration of Interim Bleeding Scores in VWD Subjects from Irish LoVIC, Canada and the US Zimmerman Program

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Background: The ISTH bleeding assessment tool (ISTH-BAT) bleeding score (BS) includes all bleeding episodes in a patient's history.

Aims: We sought to explore an interim BS collected in 489 subjects from the US Zimmerman Program, Canada and Irish LoVIC cohort to determine its potential utility in patient management by evaluating bleeding symptoms over time in relation to VWD type, levels, age and gender.

Methods: Original ISTH-BAT scores (BS) included entire history at time of enrollment and interim bleeding scores (1BS) represented bleeding that had occurred since the BS was obtained. The original BS/year (BS/yr) and interim BS/year (1BS/yr) were calculated as a means to standardize the scores over time.

Results: Index case (IC) BS correlated with VWF levels in subjects with VWF:RCo< 30 IU/dl (8) followed by 30-50 (7) and >50 (5) ($p< 0.05$). There was a significant difference in 1BS and 1BS/yr in VWD subjects with levels < 30 (5, 1.2) and 30-50 (4, 0.8) ($p< 0.05$), but no difference between levels 30-50 and >50 (4, 0.8). 1BS in type 3 IC (13) was greater than type 2 (7) and type 1 (4) ($p< 0.01$). Type 1 IC females had higher BS (7) than males (5) ($p< 0.01$), however the 1BS and 1BS/yr were similar between males (4, 0.8) and females (4, 0.9). Original BS were lower in pediatric patients (5) than adults (8) ($p< 0.0001$), however there was no significant difference in 1BS or 1BS/yr. When type 1 IC (VWF< 50) were separated by abnormal or normal BS, there was a difference in 1BS (4, 3.5) ($p< 0.05$), but not 1BS/yr (1, 0.7) nor differences in VWF levels.

Conclusions: Higher interim 1BS in subjects with VWF:RCo < 30 show correlation between low VWF levels and increased bleeding. There was no difference in 1BS between males and females nor pediatric vs. adults. Having a positive or negative original BS did not predict a difference in interim 1BS/yr in type 1 patients. The interim 1BS may be a valuable tool to help monitor the success of treatment and bleeding symptoms over time in VWD patients.

PB 369 | Does Ageing Modify Hemostatic Parameters in Type 1 von Willebrand Disease (VWD-1)?

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Background: Plasma levels of clotting factors increase with ageing in healthy subjects but the influence of ageing on haemostatic parameters in VWD-1 is uncertain.

Aims: To assess changes in VWF:Ag, VWF:RCo, FVIII:C and RIPA in patients with VWD-1 repeatedly evaluated at our Center over a 25 yrs period.
Methods: Between Jan 1990 and Dec 2015 195 subjects (55.4% women, median age 28,7 yrs, range 0.4-74.5 yrs) with VWD-1 (Sadler JE, JTH 2006), were repeatedly evaluated at our Center for VWF:Ag, VWF:RCo, FVIII and RIPA. Plasma levels of VWF:Ag, VWF:RCo, FVIII:C and RIPA were measured by standard methods.
Results: Median follow-up was 6.6yrs (0.4-24.7); each patient was studied on average 3 times (range 2 to 14). Median VWF:Ag, VWF:RCo, FVIII:C and RIPA baseline levels were 0.45IU/ml, 0.35IU/ml, 0.57IU/ml and 1.4mg/ml, respectively. Between first and last measurement median VWF:Ag increased by 0.14±0.03IU/ml, VWF:RCo by 0.096±0.02IU/ml, FVIII by 0.094±0.035IU/ml and RIPA decreased by 0.3±0.1mg/ml (all p< 0.05).
The variations of VWF:RCo, VWF:Ag and FVIII:C were positively correlated with the length of the time interval between first and last measurement (increase rate: VWF:RCo=0.047IU/ml/10yrs, VWF:Ag=0.062IU/ml/10yrs, FVIII:C=0.102 UI/10yrs and RIPA=-0.1mg/ml/10yrs). Neither gender- nor blood group-related differences were observed. However, when patients were subdivided in mild (baseline VWF:Ag and VWF:RCo>0.3< 0.5IU/ml, n=143) and moderate (baseline VWF:Ag and VWF:RCo≤0.3IU/ml, n=52), a significant increase of VWF antigen (+0.2IU/ml, p< 0.05) and activity (+0.17IU/ml, p< 0.05) was confirmed only in mild VWD-1 with no changes in moderate (-0.001IU/ml and -0.01IU/ml, p=ns).
Conclusions: An age-related increase of VWF levels is evident only in patients with mild type 1 VWD, while in the more clear-cut cases this increase is not apparent. Our data suggest that the milder patients initially labelled as VWD-1 may be false diagnoses and changes of VWF-related laboratory parameters may be restricted to healthy individuals.

PB 370 | Common Single Nucleotide Variants in the von Willebrand Factor Gene Associate with "Mutation Negative" Type 1 VWD

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Background: The von Willebrand factor (*VWF*) gene is highly polymorphic. Previous analysis of the 1000 Genomes data identified 91 non-synonymous exonic single nucleotide variants (SNVs) within the *VWF* gene. There is also evidence that sequence variations within the *VWF* locus correlate with VWF antigen levels, activity, FVIII binding and half-life in the general population. Studies investigating the mutational landscape in >500 index cases of Type 1 VWD have consistently revealed that no clear pathogenic *VWF* variant is documented in ~35% of cases.
Aims: To investigate the association between Type 1 von Willebrand disease (VWD) and common non-synonymous SNVs at the *VWF* locus in a cohort of 42 "mutation negative" patients from the Canadian Type 1 VWD Study.
Methods: The proximal promoter (1300 bps), exons 1 to 52, and approximately 70 base pairs of the donor and acceptor sites of the *VWF* gene were sequenced. Copy number variation (CNV) was previously evaluated in cases with a VWF: Ag < 0.35 IU/ML. For the remaining 42 "mutation negative" patients, allele frequencies of the 91 non-synonymous SNVs were compared to American frequencies within the 1000 Genomes. Analysis was performed using Chi-square. Alamut software analyzed changes for variant pathogenicity, Grantham distance and degree of conservation.
Results: Variation was documented in 8 of 91 non-synonymous codons. We identified 3 significant differences between the 1000 Genomes cohort and this Type 1 VWD cohort: p.Asn318Lys (c.954T>A, rs1800387, p=0.001); p.His484Arg (rs1800378, c.1451A>G, p< 0.0001), and p.Gln852Arg (rs216321, c.2555A>G, p< 0.0001).
In silico analysis (SIFT, Polyphen2) indicated the variants are tolerated, with p.Asn318Lys being possibly damaging. All variants had a small to moderate physiochemical difference (Grantham distance) and moderate amino acid conservation.
Conclusions: Common non-synonymous *VWF* variants are over-represented in "mutation negative" Type 1 VWD and may contribute to the phenotype.

PB 371 | Functional Polymorphisms of the von Willebrand Factor Gene in Patients with Mild to Moderate Bleeding Tendency and Low von Willebrand Factor Antigen or von Willebrand Factor Ristocetin Cofactor

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Background: The diagnosis of von Willebrand disease (VWD) is backed by molecular analysis. Functional single nucleotide polymorphisms (SNPs) of the coding and promoter sequences in the von Willebrand factor (VWF) gene are correlated with VWF antigen and ristocetin cofactor levels.
Aims: Evaluation of the frequency of functional polymorphisms in the VWF gene in a Vienna Bleeding Biobank (VIBB) subgroup.
Methods: From November 2009 to January 2016, 431 patients (female=354, 82.1%) were included in the VIBB. Of these, 40 (female=34, 85%) were diagnosed with low (30-50%) or very low (0-30%) VWF:Ag or VWF:RiCo levels respectively. Sequencing analysis of the VWF gene revealed mutations associated with VWD in 8 patients, who were excluded from the subsequent evaluation of functional polymorphisms in exons 18, 20, and 28 of the VWF gene. The frequency in our cohort was compared to that of the European population of the 1000 genome project provided by dbSNP.

TABLE 1 Patient characteristics (n=32)

Female, n (%)	28 (87.5)		
Blood group 0, n (%) *	23 (71.9)		
	median	25th - 75th percentile	Range
Bleeding Score (Vincenza)	5	3.25-7.75	2-15
Age [years]	32	22-40.75	16-73
BMI [kg/m2]	22.7	20.3-26.1	18.6-32.5
vWF:Ag [%]	50	45.75-57	12-91
vWF:Rico [%]	44	40.25-48	13-56
FVIII [%]	72	59.25-82	8-138

* Blood group was not determined in 3 patients

TABLE 2 Frequency of functional polymorphisms in the VWF gene

SNPs	Allele Frequency (VIBB)	Allele Frequency (EUR)
E18: c.2365A>G,p.Thr789Ala	0.156	0.367
E20: c.2555G>A,p.Arg852Gln	0.125	0.0865
E28: c.4141G>A,p.Ala1381Thr	0.203	0.399
E28: c.4693G>T,p.Val1565Leu	0.125	0.076

VIBB=Vienna Bleeding Biobank. EUR= European population of the 1000 genome project provided by dbSNP.

Results: Patient characteristics are given in Table 1. Twenty-four patients (66%) had the following functional polymorphisms in Exon 18, 20 or 28: p.Ala1381Thr, p.Arg852Gln, p.Val1565Leu, and/or p.Thr789Ala. Associations between p.Arg852Gln, p.Val1565Leu and reduced VWF levels and increased proteolysis by ADAMTS13, respectively, and between p.Thr789Ala and higher VWF levels have been described.

The frequency of the polymorphisms is listed in Table 2. We detected a higher frequency of the p.Arg852Gln (13% vs. 9%) and the p.Val1565Leu SNP (13% vs. 8%) when compared to the European population of the 1000 genome project. In contrast, the p.Thr789Ala and the p.Ala1381Thr polymorphisms appear with a lower frequency (16% vs. 37% and 20% vs. 40%) in our patient subgroup.

Conclusions: In the group of patients from the VIBB study characterized by low VWF levels, we detected a shift of polymorphisms, which might partially explain the decreased VWF levels in this very specific group of patients without disease defining mutation in the VWF gene.

PB 372 | Expression of the von Willebrand Factor C-domains in E.coli

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Background: Von Willebrand Factor (VWF) is a large multidomain protein that performs essential roles in normal haemostasis. While crystal structures of the A1, A2 and A3 domains and the cysteine knot domain have been resolved, limited structural data exists on the rest of the molecule that is comprised of D and C domains. Such studies are hampered in part due to the large number of cysteine residues located in the D and C-domains. Recently the FVIII binding region in the VWF D' domain was expressed in e.coli and used to derive structural NMR data suggesting that other cysteine rich domains of VWF could be produced in this way.

Aims: To express and purify the individual VWF C-domains

Methods: The cDNA for the C1, C2, C3, C4, C5 & C6 domains was cloned into the bacterial expression vector pET32-TRX creating fusions proteins with an N-terminal thioredoxin tag and a C-terminal His-tag. Proteins were expressed in *E.coli* Origami cells following induction with IPTG and proteins were purified by Nickel affinity and ion-exchange chromatography.

Results: With the exception of the C5 domain, all the VWF C-domains could be expressed at high levels in *E.coli* after 6 hours of expression and were found in both soluble and insoluble fractions. In vectors lacking the thioredoxin tag no protein was found in the soluble fraction and only minimal protein was observed in inclusion bodies. Proteins were purified by two passages over a nickel affinity column and following cleavage of the TRX tag, were purified to homogeneity using ion-exchange. All proteins demonstrated good expression levels ~1mg/100ml of culture. Finally the C4 domain which contains the RGD sequence was able to bind to immobilised GPIIbIIIa confirming that the proteins were properly folded.

Conclusions: The isolated VWF C-domains can be expressed at high levels in e.coli cells and purified to homogeneity. Using this system we can now obtain structural information about this region of the VWF molecule.

PB 373 | Quantitative ELISA Assay for in vivo Proteolysis of von Willebrand Factor andBbleeding: A Pilot Study in Type 2A (IIA) von Willebrand Disease

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Background: Mutations within the A2 domain of VWF inducing type 2A(IIA)VWD, are classified according to their mechanism: abnormal

assembly and secretion (group 1), excessive proteolysis (group 2) or unspecified (group 3). The characterization of the mechanism for type 2A(IIA) VWD could help evaluating the bleeding tendency.

Aims: To investigate the relation between the bleeding tendency and VWF-proteolysis in patients with 2A(IIA) VWD.

Methods: We have developed an ELISA assay to measure VWF-proteolysis. Results are expressed as the proportion of proteolysed-VWF (%). VWF-proteolysis in normal plasma was 6±2% (mean±SD). This quantification was performed in 87 patients of the French cohort of VWD identified with a molecular defect in the VWF-A2 domain resulting in type 2A(IIA) VWD (group 1, n=14; group 2, n=39; group 3, n=34). The bleeding score (BS), a history of epistaxis or gastro-intestinal (GI) bleeding was available for 38 of them. Results are expressed as median [min-max].

Results: There was a significant difference in VWF-proteolysis between groups (106% [0-243] group 1, *vs* 80% [21-143] group 2 *vs* 48%[0-153] group 3, ANOVA-p < 0.01). A high degree of proteolysis was associated with a high bleeding score (Mann-Whitney p=0.05). No difference in BS was observed between groups. GI-bleeds (Item > 3 of the BS) were reported in 13/38 patients (34%) with no difference between groups. A history of epistaxis was found in 22/38 patients (58%) and appears to be more frequent in group 1 patients (4/4-100%) compared to groups 2 (8/14-57%) and 3 (11/20- 55%), respectively.

Conclusions: This study underlines the phenotypic heterogeneity of the proteolysis associated with the mutations of VWF-A2 domain in type 2A(IIA)VWD. These results confirm the data previously obtained with mutant recombinant VWF pointing the combination of several mechanisms. Further studies involving more patients, especially patients of group 1 in whom the bleeding tendency appears the most severe, are needed to confirm these preliminary results.

PB 374 | Bleeding Symptoms in Patients Previously Diagnosed as Type 3 von Willebrand Disease: Results from a Multicenter, Multinational Cross-sectional Study

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Background: Patients with type 3 von Willebrand Disease (VWD) usually have markedly reduced FVIII/VWF levels and very severe bleeding manifestations. Because of the rarity of type 3 VWD, the clinical phenotype of these patients has been however poorly evaluated in relation to demographical factors.

Aims: We aimed at evaluating age and sex distribution of bleeding symptoms in patients with type 3 VWD and comparing them with previously available data from a cohort of type 1 patients.

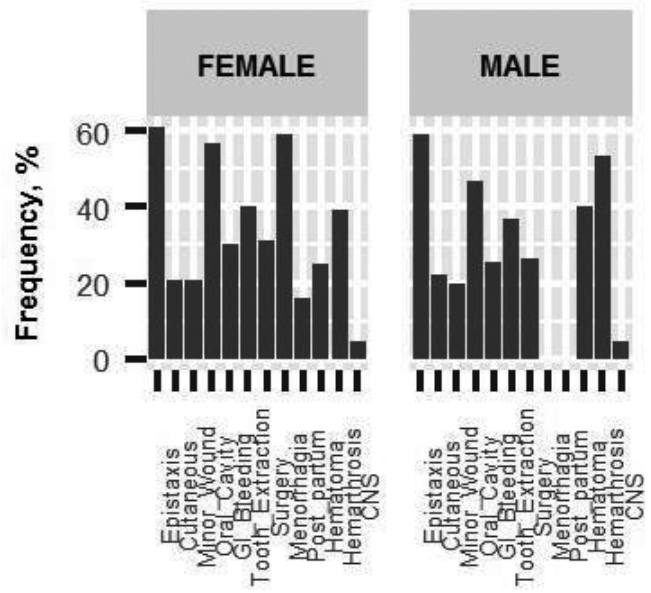

FIGURE 1

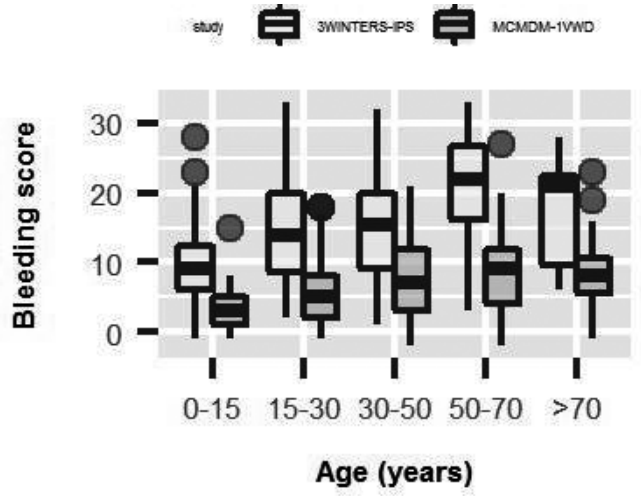

FIGURE 2

Methods: We analyzed patients enrolled in the 3WINTERS-IPS study, a multicenter, multinational cross-sectional study that analyzed clinical and phenotypical data on patients historically diagnosed as type 3 VWD. Retrospective information on bleeding symptoms at presentation was collected using the MCMDM-1 VWD bleeding questionnaire, and bleeding severity summarized as bleeding score. Individual bleeding symptoms were considered as relevant when having a score >1 (hence requiring medical attention). Data was compared with that retrieved from the MCMDM-1 VWD study database on patients affected by type 1 VWD (index cases and affected family members).
Results: We analyzed a total of 215 patients for whom bleeding symptoms history was available up to recruitment. The median age at study inclusion was 27 (interquartile range, 28); 125 were females. There were 105 of Iranian descent, while the remaining of patients were from Europe. Figure 1 shows that epistaxis was the most frequent relevant symptom, followed by menorrhagia in females.
Males had a higher frequency of hemarthroses and hematomas than females (53% vs. 39% and 40% vs. 25%, respectively). When comparing the clinical presentation of type 3 vs. type 1 VWD, increased bleeding scores were evident for all age-classes and even in pediatric cases (Figure 2).
Conclusions: This study shows that the bleeding phenotype in type 3 VWD patients is remarkably different from that of type 1 patients; hemarthrosis in males and menorrhagia in females are prevalent causes of morbidity.

PB 375 | Fluorescence Intensity-based Analysis of the Shear-dependent Internalization of Von Willebrand Factor Mutants by Macrophages

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Background: After secretion from endothelial cells and platelets elongated VWF multimers are cleaved proteolytically by ADAMTS13 producing smaller circulating VWF multimer fragments. It has been suggested that macrophages contribute to removal of these VWF fragments from the circulation. Clearance of VWF critically contributes to the clinical phenotypes of von Willebrand disease type 1 and 2A/IIC Miami.
Aims: Aim of this study is to investigate the mechanism by which mutations alter the shear-dependent clearance of VWF by macrophages. This is also of interest with respect to half-life of coagulation factor VIII, because it is internalized together with VWF.
Methods: Human macrophages were seeded in channel slides and perfused with 75 µg/ml wtVWF or VWF mutants for 30 min at 10 dyne/cm^2. Subsequently the cells were fixed, permeabilized and intracellular VWF was detected by indirect immunofluorescence. To assess the clearance rate, the fluorescence intensities were quantified by a modified protocol using the ImageJ software.
Results: We investigated the VWD 2A/IIC Miami mutants p.Ser58Arg, p.Met1051Thr, p.Cys1099Tyr and p.Glu333_385dup and showed that the clearance by macrophages of all analyzed mutants is significantly reduced compared to wtVWF. We can now further use this method to investigate the receptor proteins which are involved in binding and internalization of VWF.
Conclusions: We successfully established and validated a quantitative fluorescence intensity-based method to quantify cellular uptake of VWF into macrophages. This method can now be used to gain deeper insights into the clearance mechanisms contributing to VWD.

PB 376 | Von Willebrand Factor Multimer Analysis in 255 Patients Previously Diagnosed as VWD Type 3 from the Iranian Republic and Nine European Countries (3WINTERS-IPS Study)

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Background: The phenotypic variation in type 3 Von Willebrand disease (VWD) is still poorly understood due to the rarity of the disease.
Aims: The 3WINTERS-IPS study evaluates for the first time the phenotypes and von Willebrand Factor (VWF) parameters in a large cohort of Iranian and European VWD patients. The VWF multimers were evaluated.
Methods: The patients stem from seven treatment centers located in the Iranian Republic (111 out of a population of ~ 84,000,000), and 14 treatment centers located in nine European countries (144 out of a population of ~ 360,000,000). All patients were previously diagnosed as type 3 VWD. Accordingly the multimers should be lacking. Last treatment with VWF concentrates was preferentially commenced more than one week before blood sampling.
Results: After exclusion of ten patients with normal (3), mildly reduced VWF (4) and so far ill defined subtypes (3), the following types of multimer patterns were revealed: 1= type 3 with visible protomer and 2 sharp single bands; 2= treated patient with residual VWF with smeary material and no banding pattern; 3= severe type 1 in whom multimers were present with additional ultralarge multimers plus a faint triplet structure; 4= severe type 2 with missing largest multimers, while the remaining multimers showed a relative decrease. Sharp central bands were visible with triplets or amorphous material.
In the patients from the Iranian Republic, 92 (83%) fell into category 1, 9 (8%) into category 2, and 10 (8%) into category 3.
In the patients from Europe, 67 (47%) fell into category 1, 38 (27%) into category 2, 17 (12%) into category 3, and 11 (8%) into category 4.
Conclusions: Thus the patients from the Iranian Republic are seemingly more homogenous compared to the patients from Europe. One reason for this diversity is the number of patients treated with VWF concentrate. But also different diagnostic procedures in the more centralized Iranian Republic compared to the heterogeneous European countries may play a role.

PB 377 | Interaction between the A3 and A2 Domains of von Willebrand Factor Reduces the Binding of the A1 Domain to Glycoprotein Ibα

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Background: The A3 domain in the A1A2A3 domains complex in the glycoprotein von Willebrand factor (VWF) is the major collagen-binding domain. Interestingly, few antibodies for the A3 domain, and, thus far, two mutations in A3 domain that cause von Willebrand disease (VWD) significantly reduce the binding of VWF to platelet glycoprotein (GP)Ib. The mechanism by which the A3 domain impacts the function of the A1 domain remains unknown.
Aims: Since we and others have demonstrated that the A1-A2 binding inhibits the interaction of A1 domain to GPIb, we examined whether a direct contact between A2 and A3 domains influences on the A1-GPIb binding.
Methods: We employed purified plasma VWF and recombinant A1, A3, A1A2, A2A3, wild type A1A2A3, and gain-of-function A1(R1450E) A2A3 mutant proteins. We also used antibodies against A2 domain of human VWF. The binding analyses were performed utilizing ELISA, surface plasmon resonance (SPR), and fixed platelets were used in other binding assays. We also used urea denaturation and differential scanning calorimetry (DSC).
Results: The A3 domain bound to isolated A2 domain or A2 in full length VWF with a comparable half-maximal binding obtained at 250 nM. This interaction was inhibited by monoclonal antibodies against the A2 domain, and isolated A3 protein. Using urea denaturation and DSC, we have demonstrated that the A3 domain associates with the A2 domain and stabilizes not only the structure of A2 domain but the overall A1A2A3 complex. Moreover, using A1A2A3 protein, the impairment of A1-A2 interaction concurrently dissociates A2 from A3 domain. Lastly, isolated A3 domain protein was capable of inhibiting the binding of purified A1A2 domains protein to platelet GPIb in solution.
Conclusions: The interactions between the A1-A2-A3 domains are essential in stabilizing the structure of the A1A2A3 complex, and in inhibiting the binding to GPIb in solution. Natural mutations in A3 domain may affect the classification of VWD.

PB 378 | Incidence and Economic Burden of Thromboembolic Events (TEEs) among US Patients with von Willebrand Disease (VWD) - A Large Claims Database Analysis

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Background: VWD patients face the risk of developing TEEs, especially those with risk factors for thrombosis or excessively high FVIII levels.
Aims: To estimate incidence, healthcare utilization and economic burden of TEEs in VWD patients.
Methods: VWD patients were identified from Truven databases (01/2008-09/2015). Patients were continuously enrolled for ≥12 months with no TEEs since eligibility start date, and followed from index date (12 months post-eligibility start date) to a TEE event or end of plan eligibility. Patients with TEE post-index date were matched based on age and gender with non-TEE patients. Healthcare cost/resource use in the 12-month post-TEE period for TEE patients and over a similar 12-month period for matched non-TEE patients were compared using regression analyses for matched pairs, adjusting for baseline covariates.
Results: A total of 17,549 VWD patients were identified, 74.7% were female and mean age was 33.8±19.9 yrs. During a median follow-up of 2.6 yrs, 1,076 (6.1%) patients developed ≥1 TEE type. The top 3 most prevalent TEEs were deep vein thrombosis (2.8%), ischemic stroke (1.8%) and acute cerebrovascular event (1.2%). Incidence rate for all TEEs was 2.2/100 person-year. Identified matched pairs (n=534) had mean age of 52.1±18.4 yrs. Patients with TEEs were significantly (p< .001) more likely to have an inpatient admission (OR=8.1; 95%CI=5.9-10.0), ER visit (OR=1.7; 95%CI=1.3-2.3), longer inpatient stay (IRR=6.8; 95%CI=5.0-9.1), more frequent inpatient admissions (IRR=4.2; 95%CI=3.3-5.4), ER (IRR=1.6; 95%CI=1.3-2.1) and outpatient (IRR=1.6; 95%CI=1.5-1.8) visits compared to those without TEE. Patients with TEEs also incurred significantly (p< .001) higher total healthcare cost (adjusted mean diff.=$54,095; 95%CI=$37,037-$71,152) than those without TEE.
Conclusions: Although the incidence is low, the economic burden of TEE in VWD patients is high. Clinicians need to monitor VWD patients for early signs of thrombosis, especially those receiving VWF concentrates that also contain FVIII.

PB 379 | oPerioperative Management of Replacement Therapy in von Willebrand Patients: Steps towards Individualization of Treatment?

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Background: Von Willebrand disease (VWD) patients are regularly treated with replacement therapy in cases of acute bleeding, trauma or surgical procedures.
Aims: To evaluate current perioperative management with von Willebrand factor (VWF)/Factor VIII (FVIII) (Haemate P®) concentrate in VWD patients.
Methods: VWD patients undergoing minor or major surgery between 2000-2015 and treated with VWF/FVIII concentrate (Haemate P®) were included. Achieved VWF:Act/FVIII:C during FVIII:C-based treatment regimens were compared to predefined target levels in National guidelines. A waiver for informed consent was granted on behalf of the Medical Ethics Committee based on the observational nature of the study.
Results: In total, 103 VWD patients (148 surgeries) were included: 54 type 1 (73 surgeries), 43 type 2 (67 surgeries) and 6 type 3 (8 surgeries). Overall, treatment resulted in high VWF:Act/FVIII:C levels, defined as ≥0.20 $IUmL^{-1}$ above predefined levels. In type 1 VWD patients, respectively 65% and 91% of trough VWF:Act and FVIII:C levels were higher than target levels. In type 2 VWD and type 3 respectively, 53% and 57% of trough VWF:Act and 72% and 73% of trough FVIII:C levels were higher than target level. Prevalence of low FVIII and VWF:Act was rare. Furthermore, FVIII accumulation over time was observed, with significantly higher levels than VWF:Act. Occurrence of bleeding complications (3.4%) was not associated with a low trough VWF:Act and/or low FVIII (p=0.95 and 0.25). Blood group O was associated with high VWF:Act levels, explained by lower endogenous baseline levels resulting in administration of higher dosages of VWF/FVIII concentrates.
Conclusions: High VWF:Act and accumulation of FVIII:C levels was observed after perioperative FVIII:C-based replacement therapy in VWD patients, without additional hemostatic effect. Alternative more individualized dosing regimens not only based on body weight may be able to optimize treatment in VWD in the near future.

PB 380 | Deficiency of von Willebrand Factor (VWF) High Molecular Weight Multimers (HMWM) in the Presence of Normal/Increased Activity of both VWF Cofactor Activity (VWF:RCo) and VWF Antigen (VWF:Ag) in Patients with Severe Aortic Stenosis

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Background: Recurrent bleeds in patients with aortic valve stenosis may be caused by acquired von Willebrand syndrome (AVWS) characterized by deficiency of high molecular weight multimers of VWF resembling type 2A VWD.
Aims: The study was designed to analyse correlation between functional results of VWF (VWF:RCo and VWF:CB) and VWF multimer structure in patients with severe aortic valve stenosis.
Methods: Our study was performed in a group of 17 patients (71-87 years old) with severe aortic-valve stenosis (mean transvalvular gradient >40 mmHg and aortic valve area < 1,0cm2. The following tests were performed: factor VIII coagulation activity (VIII:C), VWF

ristocetin cofactor activity (VWF:RCo) and antigen (VWF:Ag) by Siemens, VWF collagen binding acivity (VWF:CB) by Technoclone, VWF propeptide (VWFpp) by Sanquin; multimers studies were carried out according to Krizek et al 2000.

Results: In 7/17 patients (41%) with aortic stenosis deficiency of HMWM of VWF (similarly to VWD 2A) was determined; VIII:C, VWF:RCo, VWF:Ag were in normal range or elevated; VWD:CB was decreased in 3/7 patients. The pathological ratios (< 0,6) of VWF:RCo, VWF:CB to VWF:Ag were observed respectively in 3/7, 1/7 patients; VWFpp and VWFpp/VWF:Ag ratio were in normal range in 7/7. In all patients 7/7 with deficiency of HMWM VWF fraction non specific bleeding episodes were observed.

Conclusions: Results of our study showed that in patients with severe aortic stenosis the use of VWF functional methods and ratios of VWF:RCo/VWF:Ag; VWF:CB/VWF:Ag are insufficient to discriminate cases with deficiency of VWF HMWM fraction. Lack of bleeds in patients with HMWM VWF deficiency suggest that the multimers with lower molecular weight can support hemostasis.

PB 381 | Usefulness of VWF Propeptide in the Differential Diagnosis between Patients with VWD and AVWS

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Background: An increased von Willebrand factor propeptide (VWFpp) to von Willebrand factor antigen ratio (VWFpp/VWF:Ag) indicates an enhanced clearance of VWF. This finding has been described in von Willebrand disease (VWD) and acquired von Willebrand syndrome (AVWS). If family history is not available, it is difficult to distinguish between VWD and AVWS because the laboratory tests rarely can discriminate. Therefore, there is a need for diagnostic biomarkers that may facilitate the differential diagnosis.

TABLE Baseline characteristics of the study population

Variables	VWD (type 1 Vicenza excluded) (n=111)	type 1 Vicenza (n=14)	AVWS (n=28)
Sex (male/female)	42/69	5/9	15/13
Age at visit (yrs) *	26 (13-42)	45 (22-62)	56 (43-71)
Bleeding score*	10 (4-16)	17 (9-19)	10 (3-14)
VWF:Ag (IU/dL)*	35 (22-46)	10 (9-13)	34 (15-65)
VWFpp (IU/dL)*	63 (46-92)	99 (89-121)	116 (89-143)
VWFpp/VWF:Ag ratio*,†	2 (1.5-2.7)	10 (8-14)	4 (1.5-8.7)

* median values (IQR), † Normal range VWFpp/VWF:Ag 0.6-1.6

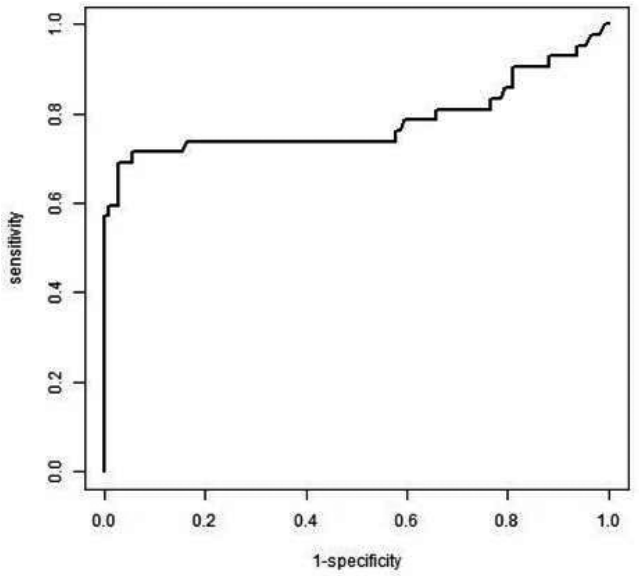

FIGURE 1 Receiver operating characteristic curve for discrimination between patients with lower VWF versus patients with higher VWF clearance

In this cross-sectional study we assessed the VWFpp/VWF:Ag in a group of 153 patients,
125 with VWD and 28 with AVWS.

Aims: To evaluate the ability of VWFpp/VWF:Ag in the differential diagnosis between VWD and AVWS.

Methods: VWF:Ag and VWFpp levels were determined by ELISA methods. In the frame of a logistic regression, a receiver operating characteristic curve was used to assess the optimal cut-off of VWFpp/VWF:Ag for discrimination between VWD and AVWS. This cut-off was identified as the value with the best compromise between sensitivity and specificity. An internal validation of the predictive model containing VWFpp/VWF:Ag, age and sex was performed with 1.000 bootstrap replicates to correct for optimism.

Results: Higher VWFpp/VWF:Ag were mainly associated with AVWS and type 1 Vicenza VWD diagnosis (Table).

The best cut-off value of VWFpp/VWF:Ag for the discrimination of patients with lower VWF clearance (most VWD) versus patients with higher VWF clearance (AVWS and type 1 Vicenza) was 3.9 (VWFpp/VWF:Ag normal range:0.6-1.6) (Figure), corresponding to a sensitivity of 70% and a specificity of 97%. The AUC of the full predictive model was 0.88 (95% CI, 0.80 to 0.95) and became 0.87 after correction for optimism.

A further evaluation of mutation p.R1205H will be necessary to discriminate between type 1 Vicenza and AVWS patients.

Conclusions: VWFpp/VWF:Ag is helpful to discriminate patients with a higher VWF clearance (AVWS or type 1 Vicenza) from those with lower VWF clearance (most VWD).

PB 382 | Lack of Discontinuous von Willebrand Factor Multimer Organization Associated with the c.2269_2270del Mutation in the von Willebrand Factor Gene

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Background: Reduced levels or abnormalities of von Willebrand factor (VWF), a multimeric glycoprotein involved early in hemostasis, are associated with von Willebrand disease (VWD). We describe a case of VWD lacking in discontinuous VWF multimer organization, except for two oligomers: an ultralarge band and the normal VWF protomer.

Aims: To clarify the disease's etiopathogenesis and genotype/phenotype relationship. sociated with von Willebrand disease (VWD).

Methods: We investigated the patient's hemostatic and genetic profile using DNA and RNA analyses.

Results: The proband, with a history of severe bleeding (bleeding score 30 vs normal 0-3), showed a marked reduction in VWF antigen, function and platelet content (Table 1), and no anti-VWF antibodies. Multimer analysis revealed the absence of most VWF oligomers, with only two bands detectable, the lower one (VWF dimer) and an unusually ultralarge band. DDAVP infusion induced an approximately 4-fold increase in VWF antigen and function, a stronger representation of the two VWF oligomers identified beforehand, but no other multimers became apparent. VWF gene sequencing revealed the homozygous c.2269_2270del mutation in exon 17. Three different RNA species were found associated with this mutation: the r.2269_2270del RNA, giving rise to a truncated VWF protein (p.Leu757Valfs*22); the r.[2269_2270del;2281_2282insAG] RNA, in which the acceptor splicing site of exon 18 is retained; and the r.[2269_2270del;2282_2288del] RNA, resulting in altered residues 757-763 of the VWF propeptide, a condition inducing persistence of the VWF propeptide, accounting for the factor VIII binding defect of the patient's VWF. The proband's parents were heterozygous for the c.2269_2270del mutation and consanguineous (Table 1).

Conclusions: Since the multimers are normally assembled (the ultralarge band was identified), the c.2269_2270del mutation seems to affect the discontinuous, ADAMTS13-dependent, multimer organization.

PB 383 | Surgeries in adults and Children with von Willebrand's Disease - Results from the Ongoing Study Wilate-STATE

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Background: wilate® is a double virus inactivated VWF/FVIII concentrate with a physiological 1:1 proportion of FVIII:VWF. With the introduction of filling sizes 500/1000 IU in 2012, a non-interventional study (NIS) was started.

Aims: In this study we are aiming to confirm consistency of efficacy and safety data of wilate® used in routine clinical practice with previous study data. The predominant reason for administration of wilate® in this study was to prevent bleeding during and after surgery. For this reason, the subgroup of patients that had surgical procedures was evaluated.

Methods: After obtaining informed consent, patients with hereditary or acquired VWD of any age requiring replacement therapy are eligible to be included in the study. A thorough documentation of anamnestic data is done before study entry. Details on surgical procedures including an efficacy assessment are documented. Surgeries are categorized as "minor" or "major" according to pre-defined criteria considering the haemostatic challenge.

Results: 55 surgeries were performed in 54 patients. The patients' age ranges from 8 months to 74 years, including 37 paediatric patients (≤14 years). All types of VWD were present: 42 (77%) patients have type 1, 10 (19%) type 2, 1 (2%) type 3 and 1 (2%) acquired VWD. Adenotomy and tonsillectomy are the most frequently documented surgeries (58%), which are classified as major operations due to their high bleeding risk. In total, 339,000 IU were administered on 161 exposure days (ED) for surgeries. Per ED, a median of 35.1 and 31.8 IU/kg, for minor and major surgeries were administered respectively. The efficacy was rated excellent/good in 96% of cases based on all surgeries. None of the patients experienced an ADR.

Conclusions: The results of this interim analysis confirm the efficacy and safety of the investigated VWF/FVIII concentrate wilate® in managing bleeding prophylaxis during and after surgical procedure.

TABLE 1 Main hemostatic and genetic findings in the proband and his parents

Patients	PTT sec	PFA100 sec	FVIII U/dL	VWF:Ag U/dL	VWF:CB U/dL	VWF:FVIIIB U/dL	VWF:FVIIIB ratio	Platelet VWF U/dL	Mutation
Proband	54.6	>300	3.44	1.25	1.95	0.55	0.44	1.70	c.2269_2270del homozygous
Mother	22.8	160	209.6	114.3	104.9	118	1.03	97.6	c.2269_2270del heterozygous
Father	31	198	89.1	39.3	40.4	38.2	0.97	103.0	c.2269_2270del heterozygous
Normal range	24-36	94-193	60-160	60-160	65-150	65-150	≥0.75	70-140	

PB 384 | Laboratory Diagnosis of von Willebrand Disease Type 2A versus LVAD-Induced Acquired von Willebrand Syndrome

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Background: Patients suffering from the bleeding disorder von Willebrand disease (VWD) type 2A are diagnosed by severely reduced ratios of VWF:CB/VWF:Ag (< 0.7) and VWF:RCo/VWF:Ag (< 0.7) and severely decreased high molecular weight (HMW) VWF multimers (0-15%). Patients with implanted left ventricular assist devices (LVAD) have a bleeding diathesis diagnosed as acquired von Willebrand syndrome (aVWS). Laboratory diagnosis of a defect in VWF in these patients is less obvious as their VWF:CB/VWF:Ag, VWF:RCo/VWF:Ag ratios and HMW VWF multimers do not always meet the criteria for clear VWD diagnosis.
Aims: Side by side comparison of VWF:CB/VWF:Ag, VWF:RCo/VWF:Ag and HMW VWF multimers of patients with VWD type 2A and LVAD-induced aVWS.
Methods: Plasma samples from 9 VWD type 2A and 14 LVAD patients were analysed for VWF:Ag, VWF:CB and VWF:RCo using ELISA and for VWF multimers using SDS agarose gel electrophoresis and compared to plasma of healthy subjects (NHP). Ratios and percentages are represented as median (with interquartile ranges).
Results: All VWD type 2A patients had a clear laboratory diagnosis of VWD. VWF:CB/VWF:Ag (0.0 (0.0-0.31)) and VWF:RCo/VWF:Ag (0.15 (0.12-0.64)) ratios were clearly below 0.7 and HMW VWF multimers were severely reduced (0.0% (0.0-12.29%) versus 31.00% (29.88-35.73%) in NHP, p< 0.001). In contrast, VWF defects were less pronounced in LVAD patients. These patients had slightly reduced VWF:CB/VWF:Ag (0.76 (0.71-1.03) versus 1.00 (0.98-1.02) in NHP) and normal VWF:RCo/VWF:Ag (1.00 (0.87-1.21) versus 1.01 (0.99-1.04) in NHP) ratios. HMW VWF multimers were moderately decreased (19.66% (16.05-20.43%) compared to 31.00% (29.88-35.73%) in NHP, p< 0.05).
Conclusions: Identifying bleeding linked to defects in VWF is less obvious in LVAD patients as laboratory analysis did not reveal VWF:CB/VWF:Ag and VWF:RCo/VWF:Ag ratios below 0.7. Only HMW VWF multimers are significantly reduced. Hence careful analysis of VWF parameters in LVAD patients is important to recognize aVWS in these patients.

PB 385 | Elevated Levels of vWF Antigen and Activities, Factor VIII and Fibrinogen Inversely Correlated with the Estimated Glomerular Filtration Rate in Patients with Non-dialysis Chronic Kidney Disease

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Background: Elevation of von Willebrand factor (vWF) antigen and activity has been shown in end-stage kidney disease and is linked to increase thrombotic risk. However, the data regarding hemostatic derangement in non-dialysis chronic kidney disease (CKD) is limited. Moreover, the relationship between vWF as well as other hemostatic markers and the estimated glomerular filtration rate (eGFR) is unknown.
Aims: To investigate the association between the hemostatic markers and the change of eGFR in non-dialysis CKD patients.
Methods: Patients with stable CKD stage 1 to 5 and healthy controls were prospectively enrolled. Blood was collected to determine the hemostatic parameters and blood groups. Patients with the conditions known to interfere with the test results were excluded. Informed consents were obtained. The study was approved by the ethic committee.
Results: The total of 252 subjects were recruited and categorized into 6 groups (CKD stage 1-5 and the controls). The mean age of CKD patients and the controls were 57±16 and 35±9 years, respectively. Of 210 CKD patients, 109 (51.9%) were female. Diabetic nephropathy was the most common cause of CKD. The mean eGFR by CKD-EPI formula in CKD stage 1-5 and the controls were 105.9±10.5, 74.2±8.2, 45.6±8.5, 23.5±4.2, 9.5±3.3 and 111.2±9.5 ml/min, respectively. Twenty-three (10.9%) and 14 patients (6.7%) with CKD stage

TABLE 1 Hemostatic parameters in patients with chronic kidney disease and healthy controls

Hemostatic Parameters	Healthy Control	CKD stage 1	CKD stage 2	CKD stage 3	CKD stage 4	CKD stage 5	P value
vWF:Ag (IU/dL), mean (SD)	104.9 (35.2)	133.7 (59.6)	141.8 (61)	149.5 (47.1)	144.9 (41.9)	170.2 (62.4)	<.001
vWF:RCo (IU/dL), mean (SD)	84.1 (21.5)	85.0 (29.6)	95.4 (23.8)	92.4 (20.2)	98.1 (18.6)	99.9 (29.8)	.009
vWF:CBA (IU/dL), mean (SD)	83.9 (25.5)	90.2 (35.7)	97.9 (42.6)	107.3 (34.6)	112.0 (40.2)	117.5 (45.4)	<.001
FVIII (IU/dL), mean (SD)	121.2(32.9)	210.7 (107.7)	214 (124.9)	218.7 (51.9)	246.9 (74.5)	290.1 (129.1)	<.001
Fibrinogen activity (mg/dL), mean (SD)	345.4 (102.5)	396.3 (132.7)	393.8 (121.2)	397.2 (145.6)	471.9 (155.5)	490.7 (167.8)	<.001
P-selectin (%), median (range)	30.4 (0.3-70.8)	19.8 (0.52-82.2)	25.6 (1.6-75.3)	7.29 (0.1-72.5)	13.44 (0.3-49.9)	27.94 (0.2-68.0)	<.001
Bleeding time (min), median (range)	4.0 (3.0-9.0)	5.0 (3.0-8.0)	5.0 (3.0-10.0)	4.5 (1.0-16.0)	5.0 (2.0-15.0)	7.0 (1.0-20.0)	<.001

2-5 had prolonged bleeding time (BT) and decreased platelet (plt) P-selectin expression by flow cytometry compared with the controls ($p< .001$ and $p=.021$, respectively). Elevated levels of vWF antigen (vWF:Ag) was found in 84 patients (40%). In addition, the mean levels of vWF:Ag, vWF activities, factor VIII (FVIII) and fibrinogen (FBG) were significantly increased in patients with CKD compared with the controls (p< .001) and inversely correlated with the decreased eGFR.
Conclusions: Elevated levels of vWF antigen and activities, FVIII and FBG were associated with decreased eGFR and suggested the prothrombotic tendency in non-dialysis CKD patients.

PB 386 | Measurement of Platelet von Willebrand Factor (VWF) Antigen Concentration and Activity Using Automated Latex-immunoturbidometric (LIA) Assays

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Background: Easy to perform LIA assays represent the state-of-the-art in measuring VWF antigen levels (VWF:Ag) and activity (VWF:Ac) in plasma. However, they have not yet been applied to analyze VWF in platelets (PLT) on a routine basis.
Aims: To establish a protocol allowing measurement of platelet (PLT) VWF:Ag and VWF:Ac based on commercially available assays (VWF Ag and Innovance VWF Ac, Siemens Healthcare Diagnostics, Marburg, DE) and to establish reference ranges.
Methods: PRP was obtained by centrifugation of ACD-anticoagulated blood at 1,550g x 2 min, followed by sedimentation of the cell pellet at 1,000g x 5 min. Platelets were resuspended in modified Tyrode's solution containing 0.35% BSA and washed twice in the presence of 30 µg/L bivalirudin. For platelet lysis 10% Triton X-100 was added to achieve 0.25% final concentration. After incubation for 1 h at 37°C the lysate was centrifuged at 12,000g x 30 min and the supernatant stored at -80°C before measuring VWF:Ag and VWF:Ac.
Results: The assay was feasible with $>10^8$ PLT obtained from 20 mL whole blood. In 3 measurements of 6 samples an intraassay coefficient of variation (CV) of 6.6% for platelet VWF:Ag (4.6% for VWF:Ac) and an interassay CV of 6.2% for VWF:Ag (4.4% for VWF:Ac) were demonstrated. Storage of ACD-anticoagulated whole blood samples < 4 h before processing did not affect measurement results. After the first washing cycle the supernatant did not contain measurable amounts of VWF. A preserved platelet function before lysis was confirmed using Born aggregometry. In samples from a healthy population (n=29) a platelet VWF:Ag of 0.768±0.144 U/10^9 PLT and VWF:Ac of 0.975±0.228 U/10^9 PLT were determined (mean ± SD). Platelet VWF:Ag and VWF:Ac ranged between 0.529-1.122, and 0.587-1.638 U/10^9 PLT, respectively. Obtained results lay within previously reported ranges.
Conclusions: The here presented method represents a clinically applicable approach to measure platelet VWF which might help to improve understanding and diagnosis of von Willebrand Disease.

PB 387 | Impact of Changes in Laboratory Diagnostic Criteria in the Classification of von Willebrand Disease

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Background: von Willebrand Disease (VWD) is the commonest inherited bleeding disorder. United Kingdom Haemophilia Centre Doctors´ Organisation (UKHCDO) has updated laboratory criteria for VWD diagnosis. Changes include classification of patients with "low von Willebrand Factor (VWF)" (activity 0.3-0.5IU/mL) and changes to VWF antigen:activity ratio in the classification of type 2 VWD. Desmopressin (DDAVP) provides an option for management in type 1 and 2 VWD and knowledge of response helps personalize care.
Aims: To audit compliance of laboratory testing and DDAVP response testing against UKHCDO guidelines.
Methods: Retrospective single center (St George's Hospital) review of all registered VWD patients. Classification, DDAVP testing and laboratory results (VWF activity, antigen, multimers and FVIII:C) were obtained from the electronic records.
Results: 234 patients were registered with VWD; 211(90%) had laboratory testing. Multimer analysis was performed in 75% (n=159), with testing to guide type 2 sub-classification in 88% (49/56). Multimer analyses in 110 non-type 2 VWD patients demonstrated loss of high molecular weight multimers in one patient. Review of results against current guidance changed classification in 41% (n=86); the most common reasons were change to "low VWF" (n=53, 62%) and change from type 1 to 2 VWD (n=9, 10%). Change from type 2 to 1 VWD due to antigen:activity ratios of 0.6-0.7 occurred in two patients. DDAVP response testing was performed and tolerated in 64% (n=136), with full results (pre, 60min and 4-6hours) available for 25% (n=34). Lack of compliance was mainly (83%, n=85) due to only one sample sent post DDAVP.
Conclusions: Changes in diagnostic criteria may affect classification of VWD patients. The impact of reclassifying patients as having "low VWF" is not clear and requires further study. Unselected multimer analysis is associated with appreciable cost, but minimal impact on classification. Closer adherence to sample testing post-DDAVP will identify those with rapid VWF clearance.

PB 388 | Combined Lenalinomide and Dexamethasone Treatment of Multiple Myeloma Cures Acquired von Willebrand Syndrome: A Case Report

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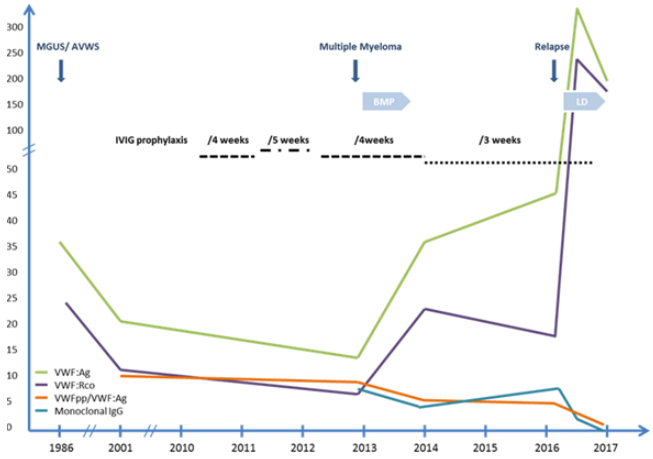

FIGURE 1 Clinical and biological follow up of our patient from 1986 to 2017

Background: Acquired von Willebrand Syndrome (AVWS) is an uncommon bleeding disorder due to acquired von Willebrand Factor (VWF) deficiency. It can be associated with monoclonal gammopathies, through an immunologic mechanism. Treatment of the underlying disorder is essential, and intravenous immunoglobulin (IVIG) proved efficient in IgG MGUS associated AVWS.

Aims: To report the effect of combined Lenalinomide and dexamethasone (LD) in a patient treated for a first relapse of IgG Multiple Myeloma (MM) who developed an AVWS thirty years earlier.

Methods: We report thirty years of clinical and biological follow up of our patient, from AVWS diagnosis to recovery.

Results: Upon diagnosis (in 1986), the patient had no personal or family history of bleeding disorder. His VWF propeptide (VWFpp) /VWF antigen (VWF:Ag) ratio showed an accelerated clearance. VWF concentrates were unable to control bleeding, whereas IVIG proved efficient for several surgeries. He developed 24 years after diagnosis several gastrointestinal bleeding episodes requiring a monthly IVIG prophylaxis. Two years later, he was diagnosed with MM. He received seven Bortezomib cycles combined with Melphalan and Prednisone (BMP) inducing a stable disease and persisting AVWS. IVIG prophylaxis was continued. MM relapsed 4 years later, and he received combined LD chemotherapy. After the first cycle, VWF levels were completely corrected, and a partial response was obtained. Lenalidomide and Dexamethasone therapy was stopped after a total of 8 cycles, achieving a very good partial response. As VWF levels correction was sustained, IVIG prophylaxis could be stopped. The VWFpp/VWF:Ag ratio was normalized.

Conclusions: In our patient, a spectacular plasma VWF level increase followed the first Lenalidomide and dexamethasone cycle, allowing us to stop IVIG prophylaxis. It was reported before in one case of MM and one MGUS. This could be an argument in favour of Lenalinomide as first line treatment of MM associated with AVWS, or even in MGUS with refractory AVWS.

PB 389 | Profile of Mutations Identified in the 3WINTERS-IPS Project on Iranian Patients with Previously Diagnosed Type 3 von Willebrand Disease

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Background: Patients with type 3 von Willebrand Disease (VWD3) have markedly reduced levels of von Willebrand factor (VWF) and very severe bleeding phenotype. Due to the recessive inheritance pattern, VWD3 is by definition a rare bleeding disorder (1:Million) but its prevalence may increase in countries like Iran with consanguineous marriages.

Aims: To identify the VWF genetic defects in a cohort of Iranian patients with previously diagnosed VWD3 enrolled into the *3WINTERS-IPS project*.

Methods: Patients classified locally as VWD3 were enrolled in the 3WINTERS-IPS study from 7 Iranian sites. Plasma/buffy-coat samples were sent to expert labs to confirm patients laboratory phenotype and to perform molecular analysis. DNA samples were extracted and next generation sequencing was used to search for VWF mutations. All defects identified were confirmed using Sanger sequencing.

Results: DNA samples from 66 patients (F/M=37/29) with a median age of 17 years (range 1-27) were analyzed. Median (range) Bleeding Score was 11.5 (1-33). In 65 cases VWF antigen levels were < 5 IU/

dL. All VWD3 showed 2 mutations, 61 homozygous and 5 compound heterozygous. Among the 132 mutated alleles the following types of variants were identified: 41 nonsense, 35 small deletions, 18 small insertion, 15 missense, 11 splice, 6 indel and 6 large (> 2 exons) deletions. 42 different variants were found. Seven mutations were recurrent and found in 25 patients (21 unrelated individuals). 26 mutations were found only once, each one in single cases in either homozygous and heterozygous state. Seven VWD3 were homozygous and one heterozygous for 5 different missense mutations. One of these mutations was previously reported in VWD3. 2 missense mutations result in loss of cysteine residues and 2 are located in the propeptide region of VWF.

Conclusions: The majority (92%) of the patients were found to be homozygous reflecting a high rate of consanguinity. Seven mutations were recurrent suggesting a common genetic background for a part (38%) of the cohort.

PB 390 | Self-reported Experiences with Menorrhagia in Women with von Willebrand Disease: A Patient-oriented Survey Study

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Background: Women with von Willebrand disease (VWD) often experience bleeding complications in the setting of menstruation. Patient-reported experiences in this setting have not been well characterized.

Aims: To elicit patient-reported menstrual health experiences in women with VWD.

Methods: We conducted a provider-administered survey of women seen at Mayo Clinic for workup and/or management of VWD. Patients were asked to discuss their diagnosis of VWD and questioned about menstrual health and how their VWD had impacted their experience.

Results: Of 103 women diagnosed with VWD, 68 (66%) agreed to participate. For those women, VWD data are shown in the Table. 61 (90%) of women self-reported a history of menorrhagia; for 17 (25%) menorrhagia was their first bleeding event. However, menorrhagia and other bleeding often occurred prior to a diagnosis of VWD (median age of first bleed = 8; overall median age at diagnosis = 24). 35 women (51%) used estrogen-containing oral contraceptives (OCPs) for treatment of menorrhagia. 26 (38%) ultimately underwent hysterectomy for menorrhagia. Other treatments included IUD (5 women, 7%), non-hysterectomy procedure (4, 6%), antifibrinolytic (3, 4%), and DDAVP (1, 1%). 29 (45%) of women reported that their menstrual health was impacted by VWD. 25 of the 29 (86%) discussed their diagnosis (once made) with their physicians, but many reported being “embarrassed” and “ashamed” about discussing menorrhagia and some were “told it was normal” before a diagnosis was made.

Conclusions: Menorrhagia is frequent in women with VWD, yet many women are not diagnosed until well after onset of menorrhagia. Women reported sensations of shame and perceived dismissal regarding menorrhagia. Physicians should be trained to recognize menorrhagia as a possible early symptom of VWD, and ideally, women with VWD should be diagnosed at an early age and managed by a multidisciplinary team to optimize reproductive health experiences and outcomes.

PB 391 | Primary Hemostasis in Severe von Willebrand Disease Patient’s Blood Is Reconstituted in vitro by Ultralarge von Willebrand Factor Multimers

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Background: High molecular weight (HMW) von Willebrand factor (VWF) multimers play a crucial role in primary hemostasis. It was shown that ultralarge MW (UL) are even more potent in VWF-platelet interaction. Severe von Willebrand disease (VWD) patients have to be treated with factor supplements.

Aims: We developed an in vitro blood circulation and simulated shear dependent processes of primary hemostasis and showed the influence of recombinant VWF (rVWF) containing UL on VWD patient’s blood in comparison to plasma derived products (pdVWF).

Methods: Blood from healthy donors and severe VWD patients was tested. Then rVWF/+UL or pdVWF products were added (1 IU/ml). First we determined the influence on the blood closure time in the PFA-200. Furthermore we observed the effects of high shear (30k s^{-1}) on VWF spiked citrate anticoagulated whole blood over collagen surface. Both techniques mimic a vascular leasion and measure, respectively visualize functional primary hemostasis and flow dynamic effects of platelet-VWF interaction.

Results: The healthy control group showed normal PFA results (ADP 81-112s; EPI 93-162s). The proportion of aggregate formation in the flow chamber was 5-25% of the surface. Patients lacking large VWF multimers had no closure in the PFA and no observable aggregation in the flow chamber. Adding rVWF/+UL to patient’s blood supra-normalized the blood closure time (ADP 50-70s; EPI 60-90s) and promoted up to 30% aggregate formation on the surfaces. On the contrary pdVWF products hardly changed results when added in the physiological concentration range to the blood samples lacking VWF.

Conclusions: We demonstrated and visualized that UL are important for primary hemostasis and promote VWF-platelet interaction in VWD patient blood leading to normal aggregate formation. UL decreases blood closure time to supra-normal levels irrespectively of the sample’s VWF:Ag value. Equimolar doses of pdVWF do not show these effects. Nonetheless all VWF products show good results in patient application.

PB 392 | Ristocetin Cofactor Latex Immunoassay, High Sensibility to Acquired von Willebrand Disease with Subtle Loss of High Molecular Weight Multimers

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Background: The diagnostic tests recommended for Acquired von Willebrand syndrome (AVWS) are the same as those used to diagnose VWD: VWF antigen assays (VWF:Ag), ristocetin cofactor assay (VWF:RCo), collagen binding capacity (VWF:CB), and multimer analysis. In AVWD, a reduced VWF:RCo/Ag ratio of <0.6 indicates inhibitory antibodies or a selective loss or decrease in HMW multimers (HMWM).

Aims: We discuss here 11 suspected AVWD associated with the following different clinical disorders:

2 hypothyroidism, 12 essential thrombocythemia (ET) and 2 autoimmune diseasaes (APS).

Methods: In order to investigate AVWD we performed the following laboratory assays: PFA-100 (Siemens), factor VIII (clotting assay, Werfen), VWF:Ag (latex immunoassay, Werfen), VWF:Activity (latex immunoassay, Werfen) and VWF:RCo [platelets agglutination assay, Bio-Data, Chemiluminescent assay (CliA) Werfen].

Results: After laboratory investigation we confirmed AVWD in both patients with hypothyroidism. On the contrary all ET and APS patients showed normal factor VIII, VWF:Ag and VWF:Act and discordant VWF:RCo depending on the laboratory method utilized for the analysis. In these subjects, VWFRCo latex/VWF:Ag ratio range was 0.2-0.5 while VWF Activity/VWF-Ag, CliA VWF Activity/VWF:Ag and VWF agglutination RCo/VWF:Ag had higher ratio ranges (0.4-0.9). The significant reduction of VWF:RCo/VWF:Ag ratios observed with the latex immunoassay in TE and APL positive patients suggested reduction of HMWM in these patients while the VWF:Activity/Ag ratios obtained with all the other VWF Activity assays utilised were only in some subjects consistent with a reduction.

Conclusions: As described in literature VWF:RCo is often inadequate for detecting AVW abnormality because most of AWD patients present abnormal VWF multimers profile with losses of HMWM and normal VWF:Ag, VWF:RCo and VWF:RCo/VWF:Ag ratios. On the contrary VWFRCo latex immunoassay in our hands showed high sensibility in detecting subtle loss of HMWM.

PB 393 | Von Willebrand Factor Antigen Levels in 255 Patients Previously Diagnosed as VWD Type 3 from the Iranian Republic and Nine European Countries (3WINTERS-IPS Study)

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Background: The phenotypic variation in type 3 von Willebrand disease (VWD) is still poorly understood due to the rarity of the disease.

Aims: The 3WINTERS-IPS study evaluates for the first time the phenotypes and Von Willebrand Factor (VWF) parameters in a large cohort of Iranian and European VWD patients. The variability in VWF:Ag was evaluated.

Methods: The patients stem from seven treatment centers located in the Iranian Republic (111 out of a population of ~ 84,000,000), and 14 treatment centers located in nine European countries (144 out of a population of ~ 360,000,000). All patients were previously diagnosed as type 3 VWD. Accordingly all should have ≤0.05 IU/ml VWF:Ag. Last treatment with VWF concentrates was preferentially commenced more than one week before blood sampling.

Results: In the Iranian patients the VWF:Ag ranged between < 0.01 IU/ml and 0.16 IU/ml. 96% showed values ≤0.05 IU/ml, and 99% ≤0.10 IU/ml. All real type 3 patients were below the lower limit of quantification (LLOQ) of our ELISA (0.008 IU/ml). Those with higher concentrations were either treated (n= 8) or were seemingly compound heterozygous (null-allele together with another VWD type; n= 9).

The European patients showed greater variability. 73% had VWF:Ag ≤0.05 IU/ml, 80% ≤0.1 IU/ml, while 20% were above 0.1 IU/ml. Again, the real type 3 patients (n= 67) with 3 exceptions were below the LLOQ of our ELISA (0.008 IU/ml). Those with higher concentrations were either treated beforehand

(n= 38) or were seemingly compound heterozygous (null-allele together with another VWD type; n= 28). In 3 patients VWF was normal, in 4 patients mildly reduced, and in 3 patients high VWF was unexplained.

Conclusions: The patients from the Iranian Republic are more homogeneous compared to the patients from Europe. The European variability is partly due to the number of patients receiving VWF concentrates. But also different diagnostic procedures in the more centralized Iranian Republic compared to the heterogenous European countries may play a role.

PB 394 | Clinical Characterization of 76 Families with von Willebrand Disease

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder and is classified into types 1, 2 and 3.

Aims: To characterize clinical and laboratory profile of families with VWD registered in a hemophilia treatment center.

Methods: This is a retrospective study. Patients were included at Hemocentro de Belo Horizonte, Minas Gerais, Brazil after signing an informed consent. Variables were obtained from medical records and interview of patients who were diagnosed from 1994-2012. Eligible patients had at least one family member who was also diagnosed with VWD. Laboratory tests included: platelet count, VWF ristocetin cofactor assay (VWF:RCo), VWF antigen level (VWF:Ag), VWF:RCo/VWF:Ag Ratio, FVIII:C and ristocetin-induced platelet aggregation (RIPA). Data was compiled in the Epidata 3.1 and statistical analyses were performed using SSPS 17.0. The study was approved by ethical committees.

Results: Among the 388 patients analyzed, 214 (55.1%) belonging to 76 families, had an average of three affected members per family. Median age was 33 (interquartil range, 19.5-48.0) and most (53.3%) were male. A total of 45 (59.2%), 18 (23.7%), 4 (5.3%) and in 9 (11.8%) families were classified as type 1, 2, 3 and undetermined VWD, respectively. A total of 189 (88.3%) individuals (96 male and 93 female) had personal history of bleeding. Defined as a bleeding score ≥3 for men and ≥5 for women, men accounted for 51.0%(n=49) and 20.4%(n=19) for women. A total of 61/82 (74.4%) women reported data from the first pregnancy, of whom 21/61 (34.4%) informed postpartum bleeding.

Conclusions: We classified 88% of the families with VWD using personal and familial history and routine blood tests for VWD. The remaining 12% will require specialized tests to confirm subtype. Postpartum bleeding was reported by about a third of included women.

Funding: FAPEMIG, CAPES.

PB 395 | Clinical Evaluation of the Sebia Hydragel von Willebrand Factor Assay in Comparison to Electophoresis and Blotting Based Multimer Analysis

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Background: Conventional multimer analysis is an extensive personal and time consuming process. However, for diagnosis and (sub)classification of von Willebrand disease (vWD) multimer analysis is required. Recently, a new rapid test (Hydragel 5, Sebia, France) has been developed and is now commercially available.

Aims: We investigated the impact of the Hydragel semi-automated system (Sebia, Lisses France) in comparison to conventional method.

Methods: The Hydragel 5 von Willebrand Multimer (H5vM) kit was used with the Hydrasys 2 Scan instrumentation to perform agarose gel electrophoresis, direct immunofixation, visualisation with peroxidase-labelled antibody and densitometry. Conventional multimer analysis involves preparation low- and intermediate-resolution gels combined with an optimized visualization system. We analyzed 101 patients with suspected or confirmed vWD. Clinical and laboratory phenotype was determined by standardized questionnaire and vWF parameter, respectively.

Results: Kits of H5vM were ready to use and reagents were provided. Results were received within one working day. In total, we found 61% of samples without vWD, 22% with vWD type 1 and 16% with type 2. Discrepant findings between both assays were found in 8%. In two cases H5vM probably failed diagnosing hereditary vWD type 2M and cardial acquired vWD type 2, respectively. In two other cases with discrepant findings, clinically and laboratory based diagnosing of vWD was not sufficient. False-positive results using the conventional assay were suspected likely due to transport artefact in four cases.

Conclusions: The new assay was easy and rapid to perform. The risk of false-positive results, e.g. due to transport, could be excluded. This study confirms the reliability of H5vM in lab's routine. It detects diminution or loss of multimers in the majority of samples, for more complex samples it helps the lab in orientating the decision towards more specialized laboratory tests (i.e. visualization of triplets, genotyping).

PB 396 | Can a One-time von Willebrand Testing Rule out Type 1 von Willebrand Disease in Young Women with Menorrhagia?

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Background: While there are guidelines as to what laboratory values are diagnostic for Type 1 VWD, there is little information regarding how many repeat tests are necessary to rule out the diagnosis.

Aims: The purpose is to determine: 1. the prevalence of Type 1 VWD among young women presented with menorrhagia; 2. the likelihood that Type 1 VWD can be predicted by history and clinical symptoms; 3. if a one-time VWF testing can rule out Type 1 VWD.

Methods: This retrospective observational cohort study from a single center hospital included consecutive post menarcheal females with menorrhagia (< 22 year-old) who had at least 2 VWF studies done between 2010 and 2016. VWF study included Factor VIII, VWF Antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo), and vWF:RCo/vWF:Ag ratio. Data including age, ABO blood group, family history, along with other bleeding symptoms were collected from medical records and analyzed for information regarding VWD status. Type 1 VWD was defined as having vWF:Ag and/or vWF:Rco < 30% with a ratio of >0.7 and/or normal multimer analysis.

Results: A total of 65 patients were included in this study. Only 6 patients (9%) eventually met laboratory criteria for Type 1 VWD. Family history, blood group, epistaxis, and other bleeding symptoms besides menorrhagia were not statistically associated with Type 1 VWD in the first or subsequent studies. There was no difference in number of repeat study between the two groups (p=0.73). Of note, among the 6 patients who met the laboratory criteria for Type 1 VWD, had the median value of 42% for VWF:Ag and 33% for VWF:RCo on all testing combined.

Conclusions: Our study suggested that there was a high prevalence (9%) of Type 1 VWD among young women with menorrhagia. However, history and clinical symptoms and even the number of repeat testing cannot predict the likelihood of Type 1 VWD. VWF:Ag >54% and VWF:RCo >42% makes Type 1 VWD unlikely and subsequent studies likely unproductive.

TABLE 1 VWF:Ag and VWF:RCo value for Type 1 VWD patients vs. non-VWD patients

	VWF Antigen, % Median(IQR)	VWF Activity, % Median(IQR)
Patients with Type 1 VWD diagnosis	42(35-54)	33(30-42)
Patients without Type 1 VWD diagnosis	72(57-110)	68(48-289)

PB 397 | Screening of von Willebrand´s Disease in a Region of High Consanguinity

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Background: Von Willebrand disease (VWD) is the most common constitutional abnormality of hemostasis. It is related to a quantitative abnormality (types 1 and 3) or qualitative Willebrand factor
(type 2) Autosomal dominant or recessive transmission • Extremely heterogeneous phenotypically and genotypically.
VWD may be more complicated to diagnose in women because abundant and prolonged menstruation is often wrongly attributed to a gynecological disorder and also the epistaxis attributed to a local cause, so this disease must absolutely be diagnosed.

Aims: Identification of people with Von Willebrand disease who have not yet been diagnosed or treated appropriately.

Methods: It is a transversal study in the maternity and consultations of ORL from 1 September to 30 January 2017. A standard haemostasis assessment with a study of the Willebrand complex (FVIII: C, VWF: Ag and VWF: RCo), blood grouping and hemogram are performed Diagnosis of type 2B is suspected in association with a combination of VWF: RCo / VWF: Ag < 0.7 with thrombocytopenia.

Results: Screening :26 cases of VWD are diagnosed including 17 women and 9 men The average age at diagnosis is 17 years [5-49 years]. The sex ratio M / F is 0.5, five patients are classified as type 3 (19.2%) 7 type 2 (26.9%) and 14 type 1(53.8%).

Conclusions: Von Willebrand disease screening is vital Knowing the prevalence, while knowing that Willebrand disease is the most common hereditary haemorrhagic disease, the complexity of the diagnosis partly explains why this disease remains less well known than hemophilia.

A second transvesal study is scheduled from1 May to 30 November, screening will be carried out as follows, patients will be identified through awareness or education activities or through active research by contacting patients already known to try whether the family members are suffering from disorders of hemostasis and have not been diagnosed yet.

PB 398 | Management of a Spontaneous Ileo-psoas Haematoma in a Girl with Type 3 von Willebrand Disease and Alloantibodies to von Willebrand Factor

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Background: Type 3 von Willebrand disease is caracterized by undetectable levels of von Willebrand factor (VWF) and very low levels of FVIII; affected patients experience severe spontaneous bleeding tendency since childhood. In 5-10% of patients alloantibodies can develop against VWF causing loss of haemostatic response to infused concentrates but also sometimes leading to anaphylactic reactions. In such cases a limited but favourable experience has been reported on the use of recombinant FVIII concentrates (rFVIII).

Aims: We describe the case of a 12 years old girl with type 3 von Willebrand disease and inhibitors.

Methods: At the age of 6 years old she presented an anaphylactic reaction after the infusion of FVIII concentrates rich in VWF, with arousal of inhibitors against VWF. She was then treated on demand with recombinant FVIII concentrates. On December 2016 she attended Emergency Room because of arousal of intense left inguinal pain, she denied any thrauma. Her PTTr was 1.7, VWF:Ag < 3 IU/dL VWF:RCo < 4 IU/dL and FVIII:C 3.9 IU/dL, FVIII inhibitors were 0.23 BU/ml and VWF inhibitors were 1.96 BU/ml.

The CT showed a large haematoma in her left ileo-psoas muscle.

Results: The patient was admitted in hospital, she was anaemic; rFVIII concentrate at 60 IU/Kg was started every six hours but in 24 hours anaemia worsened with the need of transfusion, so we started continuous infusion of octocog alfa: a bolus of 60 IU/Kg was followed by continuous infusion 30 IU/Kg/h. Haemoglobin promptly was stabilized, levels of FVIII were maintained around 40 IU/dL. Pain and leg function improved in few days. Infusion speed was reduced after three days to 20 IU/Kg/h, till the stop after two more days.

Conclusions: Alloantibodies against vWF in type 3 von Willebrand disease are a rare complication after the treatment with vWF rich concentrates. With this case report we can confirm that, as previously described in few cases, the continuous infusion of recombinant FVIII concentrates may be useful, as an alternative to bypassing agents.

PB 943 | Genetic Risk Stratification to Minimize Inhibitor Risk with the Use of Recombinant Factor VIII Concentrates: A SIPPET Analysis

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Background: The development of neutralizing antibodies against factor VIII is a serious complication of hemophilia A treatment. A recent randomized trial showed a higher risk of inhibitors with recombinant factor VIII (rFVIII) than plasma-derived (pdFVIII) concentrates (44.5% vs 26.8%, hazard ratio 1.87 (95% confidence interval (CI95) 1.17-2.96). Given the high risk with rFVIII, it has been suggested to restrict the use of rFVIII to low risk patients.

Aims: Utility of treatment with rFVIII or pdFVIII based on genetic risk factors.

Methods: SIPPET is a randomized trial in which 251 previously untreated or minimally treated patients were treated exclusively with a concentrate from the class of rFVIII or pdFVIII. Patients were tested for inhibitors before entry and at regular intervals during follow-up. Patients were classified at high risk when they carried a null mutation (inversion, large deletion, frameshift, nonsense mutation) in the F8 gene and as low risk when they carried another causative variant (missense, splice site, polymorphisms). We estimated cumulative incidences, hazard ratios and numbers needed to harm (NNH).

Results: Among 251 patients, 76 developed an inhibitor (all > 0.7 Bethesda Units (BU)) of which 50 were high-titer (> 5 BU). High and low risk patients were equally distributed over the two arms of the trial, i.e., 96 out of 126 treated with rFVIII were high risk, and 101 out of 125 treated with pdFVIII. Among high risk patients, CI was 30.7% with pdFVIII, and 46.5% with rFVIII (risk difference 15.8%). Among low risk patients, no inhibitors developed with pdFVIII, whereas the cumulative incidence was 43.2% with rFVIII (risk difference 43.2%). This implies that the Number Needed to Harm for rFVIII was 5.6 overall, 6.3 for high-risk patients, and 2.3 in low risk patients.

Conclusions: Risk stratification by the type of F8 mutation does not identify patients with hemophilia A who have a low inhibitor risk when exposed to rFVIII.

PB 944 | Pharmacokinetics of Recombinant Fusion Protein Linking Activated Factor VIIa to Human Albumin (rVIIa-FP), Eptacog Alfa and Plasma-derived Factor VII in Patients with Congenital FVII Deficiency

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Background: Patients with congenital FVII deficiency can experience increased bleeding after surgery or trauma and in severe cases may suffer spontaneous and life-threatening bleeds. Replacement of FVII is a mainstay of treatment but is limited by the short half-life of current FVII therapies. A novel, recombinant fusion protein linking coagulation FVIIa with albumin (rVIIa-FP) is being developed for congenital FVII deficiency.

Aims: To compare the pharmacokinetic (PK) profile of rVIIa-FP with rFVIIa and pdFVII.

Methods: A Phase I multicenter, randomized, open-label, parallel-arm, single-dose PK study was conducted in patients with congenital FVII deficiency. Patients received their routine FVII product (30 IU/kg pdFVII or 25 µg/kg rFVIIa ([eptacog alfa]), and were then randomly assigned to receive 100 or 300 µg/kg of rVIIaFP. Serial blood samples were taken for up to 24 h after rFVIIa injection, and for up to 48 h after rVIIaFP or pdFVII injection. FVII was measured by the FVII:C or FVIIa assays. Noncompartmental analysis was performed for derivation of PK parameters.

Results: PK analysis was completed for eight patients; three receiving pdFVII and five received rFVIIa prior to treatment with rVIIa-FP (n=5 at 100 µg/kg and n=3 at 300 µg/kg). The mean half-life of rVIIa-FP at doses of 100 and 300 µg/kg, respectively, was up to 3-fold longer compared to rFVIIa (3.87 and 5.10 vs. 1.69 h; FVIIa assay) and was comparable/longer compared to pdFVII (6.4 and 8.08 vs. 6.28 h; FVII:C assay). At 24 h post infusion, 8/8 rVIIa-FP patients had measurable FVIIa activity vs. 0/5 rFVIIa patients. At 48 h, both doses of rVIIa-FP had higher FVII activity than pdFVII.

Conclusions: rVIIaFP had an improved PK profile compared to rFVIIa in patients with congenital FVII deficiency. Due to rVIIa-FP's longer half-life, a bleeding episode may be treated with a single as opposed to multiple injections, and may facilitate prophylactic management of congenital FVII deficiency.

PB 945 | Bone Properties in Children with Hemophilia A: Influence of Target Joint and FVIII Inhibitor

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Background: Hemophilic children are prone to low bone mass accrual.

Aims: To assess bone material properties in hemophilic children, using peripheral quantitative computed tomography (pQCT) and to correlate findings with clinical data.

TABLE 1 impact of FVIII inhibitor in pQCT bone parameters

	Left tibia	Left tibia	Right tibia	Right tibia
	W/o inhibitors	With inhibitors	W/o inhibitors	With inhibitors
CC	251.4±34.1**	154.2±42.0	245.7±40.9**	162.9±37.4
CA	245.4±31.3**	160.0±40.0	240.8±36.8**	169.6±35.3
SSI	1335.7±241.0**	826.0±16.1	1318.8±207.9**	854.4±117.6
CBD	1027.5±29.8**	962.5±47.4	1020.3±30.4**	969.9±47.0
CTHC	3.39±0.32*	2.36±0.85	3.35±0.45*	2.44±0.78
	Left radius	Left radius	Right radius	Right radius
SSI	114.6±24.2*	81.3±14.1	119.8±23.5*	80.0±15.6

** denotes statistically significant differences at p<0.01 * denotes statistically significant differences at p<0.05

Methods: pQCT scan of both radii (at 4% and 60% site) and both tibiae (at 4% and 65% site) were performed in 31 hemophilic A children (severe 24, mean age 11.2 years). Seven subjects (22.6%) had history of inhibitor, 12 at least one leg target joint (6 right) and 5 in hand (3 left). The following parameters were measured: trabecular, total and cortical bone density and content (TBD, ToBD, CBD, TbC, CC), strength-stress index (SSI), and tibial cortical area (CA), inner and outer bone contour length (PERI, ENDO), cortical, periosteum and endosteum circumference (CTHC, PERIC, ENDOC) and mean cortical thickness (CTH).

Results: All parameters matched for age, height and weight. Right radii TBD values were significantly higher than the left (p< 0.015). In 5 elbow target joints subjects, radius TBD values were significantly lower ipsilaterally, than in non-target hand (186.6±60.4 vs 218.6±39.8, p< 0.05). Left hand target joint subjects had significantly lower left radius TBD values in comparison to subjects without hand target joints (155.4±50.3 vs 215.7±37.9, p< 0.05). There was no similar difference in leg target joints group. Bone quality and geometry parameters were significantly lesser in inhibitor versus non inhibitor group, matched for age and height (Table) with statistically significant side-to-side differences for legs and arms and left side predominance.

Conclusions: Our results suggest a low bone quality and strength in all hemophilic children extremities with FVIII inhibitors, compared to non inhibitor group. Moreover, target joint impact seems to be significant in radius TBD.

PB 946 | Longitudinal Modified Hemophilia Joint Health Scores (mHJHS) in Children, Adolescents, and Adults with Severe Hemophilia A with Long-term rFVIIIFc Prophylaxis

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Background: Hemophilic arthropathy is a challenging complication of hemophilia; prophylaxis has been shown to prevent chronic arthropathy.

Aims: To evaluate longitudinal change in joint health with rFVIIIFc prophylaxis during A-LONG (adults/adolescents) and Kids A-LONG and the ongoing extension study, ASPIRE (NCT01454739).

Methods: Joint health was assessed by mHJHS at A-LONG/Kids A-LONG baseline (BL), ASPIRE BL and annually thereafter. A-LONG subjects with mHJHS data at A-LONG BL, ASPIRE BL, and ASPIRE Year 1 [Y1], Year 2 [Y2], and Year 3 [Y3] were included (Kids A-LONG BL, ASPIRE BL, Y1 and Y2 available for kids). Change from A-LONG/Kids A-LONG BL to last visit was analyzed using a paired t-test.

Results: Mean (SEM) mHJHS total score for adults/adolescents with data at all time points (n=28) was 25.0 (2.9) at A-LONG BL. Mean (SEM) change from A-LONG BL was -2.0 (1.2) at ASPIRE BL,-3.8 (1.5) at ASPIRE Y1, -4.5 (1.6) at ASPIRE Y2, and -5.0 (1.5) at ASPIRE Y3 ($P< 0.01$). These improvements were observed regardless of prestudy regimen (**Figure**). Similar results were observed regardless of age or the presence/absence of BL target joints. Subjects were classified according to the median of mHJHS at BL. The half with higher BL mHJHS (>22) had greater mean (SEM) improvements vs A-LONG BL through ASPIRE Y3 (-8.4 [2.6]) than the half with lower initial mHJHS (-1.8 [1.2]). Mean (SEM) improvements at ASPIRE Y3 vs A-LONG BL were observed for both weight-bearing (-1.1 [0.5]) and non-weight-bearing (-3.0 [0.8]) joints. The mHJHS components that showed ≥25% score reduction from A-LONG BL to ASPIRE Y3 were joint instability (-89%), swelling (-46%), joint pain (-31%), and muscle atrophy (-25%). Results from the Kids A-LONG cohort (n=24) also showed mean (SEM) improvement from A-LONG BL to ASPIRE Y2 (-1.2 [0.6]; $P< 0.05$).

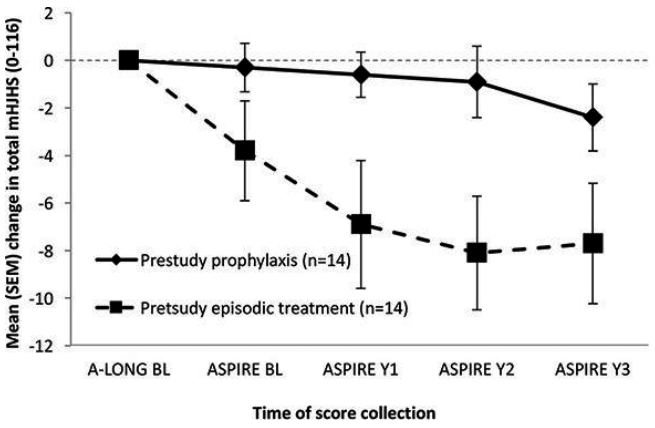

FIGURE 1 Mean (SEM) change from baseline in mHJHS total score and by prestudy regimen from A-LONG BL to ASPIRE Y3 in adults/adolescents

Conclusions: Among subjects receiving long-term rFVIIIFc prophylaxis, continuous annual improvement in mHJHS scores was observed, regardless of prestudy dosing regimen, even among those with reduced joint health at BL.

PB 947 | Obesity and Dosing of Replacement Therapy in Hemophilia; How to Optimize?

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Background: Factor VIII (FVIII) dosing in hemophilia A is based on body weight in kilograms (kg) and a FVIII *in vivo* recovery (IVR) of 2.0 which equals an increase of 2.0IUdL^{-1} per unit of FVIII per kg. Overweight/obese patients have significantly higher IVR values resulting in overestimation of FVIII. The use of other body size descriptors as an alternative to body weight has been discussed.

Aims: To explore the potential of PK-guided dosing based on FVIII population pharmacokinetic (PK) models in overweight/obese hemophilia A patients and to evaluate the use of the body size descriptors ideal-, adjusted- and lean body weight, instead of actual body weight.

Methods: Twenty-two hemophilia A patients (baseline FVIII< 0.05IUmL^{-1}) were classified according to body mass index (BMI), normal: < 25kgm^{-2}(n=5), overweight: 25-30kgm^{-2}(n=12) and obese: >30kgm^{-2}(n=5). All patients received ±50IUkg^{-1} FVIII, with FVIII:C sampling at 4, 24 and 48 hours. Population PK modeling was applied to calculate FVIII plasma concentration over time. Informed consent was obtained after study approval by Medical Ethics Committee.

Results: An earlier reported population PK model for FVIII (Bjorkman et al. 2010) was not able to correctly predict FVIII levels in overweight/obese patients. Therefore, a modified model was constructed, which described individual PK parameters and FVIII levels adequately. Using this model, it was demonstrated that median IVR was significantly higher in overweight (2.65) and obese (3.00) patients in comparison to patients with a normal BMI (2.17) (p< 0.05). IVR-based dosing (using IVR of 2.0) resulted in FVIII trough levels below set target levels for specific bleeding events, most importantly in case of life threatening bleeding.

Conclusions: When PK-guided dosing of factor VIII concentrate is applied it is important to ensure that the population PK model represents all patient groups, including obese/overweight patients. Alternative dosing based on body size descriptors is not equivalent to PK-guided dosing.

PB 948 | Safety of Nonacog Beta Pegol (N9-GP): Results from the paradigm™ Clinical Trials

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Background: N9-GP is a recombinant glycoPEGylated factor IX (FIX) with extended half-life, developed for haemophilia B (HB) therapy. Safety and efficacy of N9-GP in previously treated adults, adolescents and children were assessed in the paradigm™ clinical trials.

Aims: To report safety results from the paradigm™ trials:
1 (phase 1 pharmacokinetics);
2 (phase 3 pivotal);
3 (surgery);
4 (phase 3B, extension of paradigm™2 and 3); and the ongoing
5 (paediatric trial, including extension, data cut-off date 1 January 2016).

Methods: Previously treated individuals with HB (FIX ≤2%), without FIX inhibitors, received N9-GP prophylactically (10 or 40 IU/kg, once/week) or on-demand (40 or 80 IU/kg). Safety parameters included reporting of adverse events (AEs), serious AEs and clinical laboratory assessments.

Results: On these trials, 115 males (including 25 children aged 1-12 years) were exposed to N9-GP, with 205 total exposure years; longest trial participation at cut off was 3.75 years. No patients developed FIX inhibitors (≥0.6 Bethesda units) or reported thromboembolic events. 824 AEs occurred. Fourteen serious AEs occurred in 12 patients; all except one were judged unlikely to be related to N9-GP. The event judged as probably related to N9-GP was a severe hypersensitivity/allergic reaction in which the patient was subsequently treated with antihistamine and IV cortisone; the patient fully recovered and was withdrawn from the trial. One fatality was recorded (hepatocellular carcinoma), but judged unlikely to be related to N9-GP. Following evaluation of all AEs, hypersensitivity, pruritus and injection site reactions were considered AEs possibly related to N9-GP, reported in 1, 3 and 4 patients respectively. No systematic changes over time were seen for any haematological, hepatic or renal parameters.

Conclusions: Overall N9-GP was well tolerated and the AE rate was low. One severe hypersensitivity reaction was thought probably related to N9-GP; no other safety concerns were identified.

PB 949 | Perioperative Replacement Therapy in Hemophilia B: Should We "B" More Precise?

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Background: Hemophilia B is a deficiency of coagulation factor IX (FIX) and characterized by bleeding in muscles and joints. In the perioperative setting, patients are treated with FIX therapy striving for physiological FIX levels to secure hemostasis. Targeting of specified FIX levels is challenging and requires frequent monitoring and adjustment of therapy.

Aims: We conducted an international multicenter study to evaluate perioperative management in hemophilia B, including monitoring of FIX infusions and observed FIX levels, whereby predictors of low and high FIX levels were assessed.

Methods: Hemophilia B patients with FIX< 0.05 $IUmL^{-1}$ undergoing elective, minor or major surgical procedures (2000-2015) were included from ten centers in two countries. Patient, surgical and treatment characteristics were collected. Observed FIX levels were compared to target levels as recommended by guidelines. The study was approved by all Medical Ethics Committee and an opt-out consent procedure was used.

Results: A total of 255 surgical procedures were performed in 118 patients (median age 40 years, median body weight 79 kg). Sixty percent of FIX levels within 24 hours of surgery were below target with a median difference of 0.22 $IUmL^{-1}$ [IQR 0.12-0.36]; while > six days after surgery 59% of FIX levels were above target with a median difference of 0.19 $IUmL^{-1}$ [IQR 0.10-0.39]. Clinically relevant bleeding complications (necessity of a second surgical intervention or red blood cell transfusion) occurred in three procedures (1.2%) and were not associated with low FIX levels. Bolus infusion and minor surgical procedures were predictive of lower FIX levels (OR=5.4 95%CI 3.5-8.3, OR=2.0 95%CI 1.2-3.2).

Conclusions: This study demonstrates that targeting of FIX levels in the perioperative setting is complex and suboptimal, but despite this bleeding is minimal. Alternative dosing strategies taking patient and surgical characteristics, as well as pharmacokinetic principles into account, may help to optimize and individualize treatment.

PB 950 | Identification of Patient and Disease-related Characteristics Influencing the Risk of Bone Fracture in Individuals with Haemophilia

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Background: Increased prevalence of osteoporosis has been reported in individuals with haemophilia, but real-life data on incidence of fractures in these subjects are lacking.

Aims: To investigate incidence rate of fractures in a cohort of subjects with haemophilia treated in our haemophilia center. We also investigated several patient- and disease- related characteristics in attempt to identify characteristics which may represent risk factors for fracture in individuals with haemophilia.

Methods: In this retrospective cross-sectional study we included 148 male subjects with haemophilia
(57 with severe, 27 moderate and 64 mild haemophilia) mean age 41.2±16 years. All previous fractures were recorded over the last two decades (January 1996 - January 2016). Data was gathered from a total of 2960 patient-years of observation and incidence rate was calculated. The influence of type and severity of haemophilia, age, inhibitor presence, smoking habit, alcohol consumption, use of anti-inflammatory drugs, status of joints, bleeding frequency and body mass index on fracture occurrence have been investigated.

Results: During the observation period for whole cohort of subjects with haemophilia, 36 fractures have been recorded, giving total incidence of 12.2 fractures per 1000 patient-years. In our analysis smoking (p=0.003), frequency of joint bleeds (p=0.037) and number of joints with haemophilic arthropathy (p=0.049) appeared to be associated with increased risk for bone fracture in patients with haemophilia.

Conclusions: Incidence rate of bone fractures in our cohort of subjects with haemophilia is slightly higher than reported for general population (12.2 versus 9.6 per 1000 patients years). Smoking habit, frequency of bleeding and number of affected joints with haemophilic arthropathy were identified as factors which are associated with increased risk of bone fractures in subjects with haemophilia.

PB 951 | Post-partum Bleeding in Women who Are Carriers of and Who Have Bleeding Disorders: A Case-Control Study

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Background: Women with inherited bleeding disorders or who are carriers (WBD) have been previously reported as having higher rates of primary and secondary post-partum haemorrhage (PPH), even with optimal haemostatic management. In normal pregnancy, risk factors for PPH include age, BMI, parity, anemia, induction of labour (IoL), prolonged labour, mode of delivery, instrumentation and birth weight.

Aims: We hypothesized that higher rates of PPH may be due to higher rates of elective Caesarean section.

Methods: We audited the obstetric care of WBD who delivered at our centre between 2008 and 2016 and compared them to a control group, matched for mode of delivery (vaginal/elective Caesarean/emergency Caesarean). WBD were managed according to national and local guidelines during their pregnancy, puerpeurim and postpartum period. Data was collected from a database.

Results:

TABLE 1 Postpartum mean EBL and PPH in WBD and controls

	WBD (n=65)	Controls (n=130)
Mean EBL (all deliveries)	451ml	420ml
Mean EBL (vaginal deliveries without instrumentation)	316ml	326ml
Mean EBL (Elective Caesareans)	473ml	498ml
Mean EBL (Emergency Caesareans)	754ml	641ml
Primary PPH ((≥500ml for vaginal delivery, (≥1000ml for Caesarean)	11 (17%)	17 (13%)
Secondary PPH	4 (6%)	0 (0%)*

* p< 0.05

There were 65 pregnancies in this study, in 48 WBD. Of these, there were 18 pregnancies in Haemophilia A carriers, 10 in Haemophilia B carriers, 17 in Factor XI deficiency patients, and 20 in von Willebrand disease patients. All women were seen by a multi-disciplinary team and 40 had peri-delivery haemostatic treatment. There were 130 pregnancies in the control arm. There was no significant difference in BMI or parity between groups and no significant difference between groups in first trimester, pre-labour and post-partum haemoglobin. There was an increased rate of IoL in WBD (32% vs. 14%). There was no significant increase in estimated blood loss (EBL) or primary PPH between groups. WBD were more likely to have had a secondary PPH (6% vs. 0 %, P< 0.05).

Conclusions: We have shown that when WBD are matched for mode of delivery to controls, there is no difference in the EBL or rates of primary PPH, despite higher rates of IoL. In our group there is an increase in rates of secondary PPH. Further work is needed to understand how optimal management can reduce this.

PB 952 | Prophylaxis with rIX-FP Reduces Consumption Compared with Previous FIX in Both Adult and Pediatric Patients

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Background: rIX-FP, a fusion protein genetically linking recombinant human coagulation Factor IX with recombinant human albumin, was designed with an improved pharmacokinetic profile to extend the dosing interval during prophylaxis therapy (PT).

Aims: To evaluate the impact of an extended dosing interval on FIX consumption in adult and pediatric patients with hemophilia B enrolled into 2 clinical trials.

Methods: Study 1: Previously treated patients (12-65 years) with hemophilia B (FIX ≤2%) received either on-demand treatment with rIX-FP for 6 months before switching to 7-day PT (n=23) or received 7-day PT for 6 months and continued on 7-day PT or switched to 10- or 14-day PT (n=40). Study 2: Pediatric patients (< 12 years; n=27) received 7-day PT with rIX-FP. Consumption of FIX before and during study was compared.

Results: In adults, 7- and 14-day PT with rIX-FP reduced mean monthly FIX consumption compared with previous FIX therapy by approximately 37% and 51%, respectively (7-day: 202.7 IU/kg; 14-day: 157.4 IU/kg; previous FIX: 320.7 IU/kg). Mean dose for patients receiving 7-day PT (n=59) was 47.1 IU/kg versus a mean weekly dose prior to study entry of 69.9 IU/kg. Mean dose for 14-day rIX-FP PT (n=21) was 71.9 IU/kg. In the pediatric population, treatment frequency in those receiving PT prior to study entry was 2x weekly (n=15), 3x weekly (n=2), every 3 days (n=2), every other day (n=1) and weekly (n=4); 7day rIX-FP PT decreased injection frequency for 20/24 patients. Mean weekly dose was considerably lower with rIX-FP PT than with previous FIX for all patients (47.2 vs 107.1 IU/kg), for those aged < 6 years (49.1 vs 138.7 IU/kg) and for those 6 to < 12 years (45.6 vs 80.3 IU/kg).

Conclusions: Compared to previous treatment, prophylaxis with the prolonged dose interval afforded by rIX-FP resulted in a significant decrease in total FIX consumption and a subsequent reduction in the burden of treatment in this group of patients.

PB 953 | Dosing of rVIII-SingleChain Based on Clinical Bleeding Phenotypes Results in Low Bleeding Rates in Pediatric Patients Treated with Prophylaxis Two or Three Times Weekly

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Background: The safety and efficacy of rVIII-SingleChain was investigated in the AFFINITY program in two pivotal and one extension study. Studies were designed to reflect real-world practice; dose range and regimen were at the investigator's discretion based on bleeding phenotype and FVIII treatment used prior to enrollment. Dose and schedule could be adjusted at any time during the study.

Aims: This analysis evaluated initial and final dose assignment and corresponding bleeding rates in pediatric patients (< 12 years) receiving prophylaxis 2- or 3-times weekly.

Methods: Total and spontaneous annualized bleeding rates (ABR/AsBR) were determined for patients stratified by age group (0 to 6 and 6 to < 12 years) and either initial or final dose assignment. This analysis included pediatric patients assigned to prophylaxis 2- or 3-times weekly (84% of the subjects on prophylaxis therapy) in dosing brackets of 20 to < 30 IU/kg, 30 to < 40 IU/kg and 40 to ≤50 IU/kg.

Results: In the final dose assignment, 24, 19 and 24 patients were assigned a dose of 20 to < 30 IU/kg, 30 to < 40 IU/kg, and 40 to ≤50 IU/kg, respectively. ABR and AsBR were lower in those receiving ≥30 IU/kg than in those receiving < 30 IU/kg. Median ABR for the dosing brackets of 20 to < 30 IU/kg, 30 to < 40 IU/kg and 40 to ≤50 IU/kg was 5.61, 2.75 and 2.34, respectively; median AsBR was 2.10, 0 and 0, respectively. Similar findings were recorded for both 0 to < 6 year and 6 to < 12 year age groups.

Conclusions: Dosing of rVIII-SingleChain based on clinical bleeding phenotype results in low bleeding rates in pediatric patients treated with prophylaxis 2- or 3-times weekly. However, differences in ABR and AsBR suggest children receiving >30 IU/kg are more protected from traumatic bleeding than those receiving < 30 IU/kg. As pediatric patients are a very active population, a starting dose of 30-50 IU/kg 2- or 3-times weekly might be more appropriate.

PB 954 | Factor VIII (FVIII) Inhibitor Testing Using a Validated Chromogenic Bethesda Assay (CBA) in HAVEN 1 (BH29884), a Phase 3 Trial of Emicizumab in Persons with Hemophilia A (PwHA) with Inhibitors

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Background: FVIII inhibitors in PwHA are commonly measured using a mixing-clotting method (Bethesda assay), where residual FVIII activity is quantified by 1-stage FVIII activity test. Emicizumab (ACE910), a novel bispecific antibody, is under investigation for prophylactic treatment of PwHA. Emicizumab is not inactivated by heat treatment or FVIII inhibitors, and therefore interferes with standard Bethesda assays, leading to potential false negative results. However, the CBA (Miller, *JTH*, 2012) uses a chromogenic FVIII activity assay containing bovine components that is insensitive to emicizumab, allowing detection and quantification of FVIII inhibitors in PwHA receiving emicizumab.

Aims: Validate CBA for use in presence of emicizumab and measure FVIII inhibitor titer for PwHA with inhibitors enrolled in the HAVEN 1 phase III trial (NCT02622321).

Methods: The CBA was validated using commercially available FVIII inhibitor plasma. Values ≥0.6 chromogenic Bethesda Units (CBU) were considered positive, and titers >45 CBU were right-censored. HAVEN 1 FVIII inhibitor titers were measured in a central lab by CBA using citrated plasma collected at multiple time points. The study was approved by all local site ethics committees; informed consent/assent was obtained.

Results: CBA results from donor plasma (n=6) correlated with the supplier's labeled titer (r^2 = 0.98). No false-positive test results were seen, and addition of emicizumab had no effect. HAVEN 1 data are presented for PwHA who completed ≥7 and ≥24 weeks of emicizumab prophylaxis. Inhibitor titers ranged from 0-45 at each time point. Median titers at baseline, Wk7, and Wk25 were 11.9 (n=102), 8.0 (n=97) and 8.0 (n=50) CBU.

Conclusions: CBA is a robust method for determination of FVIII inhibitor titer, and is accurate even in the presence of emicizumab. Inhibitor titer was measured successfully for PwHA with inhibitors in HAVEN 1 who received weekly emicizumab prophylaxis, and there was a trend toward lower median titers over time.

PB 955 | Recombinant FVIII Product Type and the Risk of Inhibitor Development in Previously Treated Patients with Hemophilia A: Preliminary Results of a Systematic Review and Meta-analysis

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Background: Inhibitor development in previously treated patients (PTPs) with severe hemophilia A is a rare but severe complication of FVIII treatment. Evidence on product-related immunogenicity is increasing.

Aims: To perform a systematic review of the evidence on the relationship between inhibitor development and recombinant factor FVIII (rFVIII) products in PTPs with severe hemophilia A.

Methods: The following electronic databases were searched; PubMed, Embase, Web of Science, Cochrane database and CINAHL. Longitudinal studies reporting on de novo inhibitor formation in severe (baseline FVIII < 0.01 IU/ml) and moderately severe (baseline FVIII < 0.02 IU/ml) PTPs with hemophilia A (defined as having > 50 exposure days to FVIII at baseline) were included. A random intercept Poisson regression model was used calculate the pooled incidence rate ratio of inhibitor development (RR, 95%CI) according to product type. In addition, a sensitivity analysis was undertaken to assess the robustness of our findings. Eighteen studies had missing or incomplete information, corresponding authors have been asked to provide additional information.

Results: 26 cohorts were included. There were 36 inhibitor events over 17,049 person-years of observation. The incidence rate among PTPs was 2.79 per 1000 person-years (95%CI: 1.54-5.05). Compared with Advate, the pooled RR was 5.54 (95%CI: 0.46-66.60) for Kogenate/Helixate, 1.85 (95%CI: 0.45-7.60) for Kogenate FS/Helixate NexGen, 12.44 (95%CI: 2.69-57.62) for Refacto and 4.04 (95%CI: 0.89-18.41) for Refacto AF. Compared with full-length rFVIII, the pooled RR for B-domain-deleted rFVIII was 4.27 (95%CI: 1.43-12.75).

Conclusions: These preliminary results suggest that some rFVIII products are associated with increased immunogenicity as compared with Advate in severe and moderately severe PTPs with hemophilia A. The final results may change depending on the outcome of our information request to several study authors.

PB 956 | Preliminary Observations from a Prospective, Dose Escalating, Prophylaxis Study in Young Boys with Severe Hemophilia A in China: The China Hemophilia Individualized Prophylaxis Study (CHIPS)

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Background: In China, 90% of boys with severe hemophilia A (SHA) manifest significant arthropathy by age six years (Wu R et al Haemophilia, 2014). Short-term pilot studies demonstrated that low-dose prophylaxis (10-15 IU/kg) reduced joint bleeds by 80% but was ineffective in eliminating joint damage
(Wu R et al Haemophilia, 2013). Optimizing prophylaxis in China for boys with SHA is an urgent priority.

Aims: To investigate the efficacy of an individualized, dose escalating prophylaxis protocol in young boys with SHA.

Methods: This is a prospective, ethics approved clinical trial conducted at 2 major Hemophilia Treatment centers in China. Boys with SHA (baseline FVIII < 2%), ages 1-7 years, inhibitor negative, >50 Exposure

TABLE 1 Dose Escalation Criteria

Index Joint Assessment	Parameters	Frequency	Description	Score
Bleeding	Frequency per joint	Any time	≥ 3 bleeds in any single index joint	+ 2
		Consecutive 3 months	≥ 2 bleeds in any single index joint	+ 2
		Consecutive 3 months	1 index joint bleed	+ 1
Joint score by imaging	US based on most severe finding/location	Every 3 months	Changes of Grey-scale US Score = 3	+ 2
			Changes of Grey-scale US Score = 1 and changes of Color Doppler synovial vascularity Score ≥ 1; or changes of Grey-scale US Score = 2	+ 1
Joint Score by physical examination	HJHS score for any index joint	Every 3 months	Change of swelling score on HJHS from 0 to 2 or 1 to 3 (not considered to be related to an acute bleed)	+ 2
			Persistent swelling that is mild (score 1 on HJHS) or moderate (score 2 on HJHS)	+ 1
TOTAL SCORE FOR ESCALATION			Escalate to the next step of prophylaxis	≥ 2

TABLE 2 Baseline Characteristics of the Study Cohort

Characteristic	N	Median	Range	Best - Worst Score
Age	32	5.0	2.3 - 7.9	
CHO-KLAT* Parent Score	15	66.7	46.0 - 78.7	100 - 0
CHO-KLAT* Parent Socio-Economic Context (SEC) module score	15	65.6	33.3 - 84.4	100 - 0
HJHS*	11	6.0	0.0 - 11.0	0 - 124
*Preliminary Results (Ages ≥ 4 years)				

Days and a history of index joint (elbows, knees, ankles) bleeds were eligible. The design included 4 dose escalation steps; Step 1: 10-15 IU/kg x2/week; Step 2: 10-15 IU/Kg, x3/week; Step 3: 15-20 IU/Kg x3/week; and Step 4: 20-25 IU/Kg every other day. A priori escalation criteria (Table 1) was based on the frequency of index joint bleeds, scores from the Hemophilia Joint Health Score (HJHS) and Ultrasound (US) examinations of index joints.

Results: 32 boys were consented and enrolled. Table 2 provides baseline characteristics. Median follow-up was 4 months (range: 2 -5 months). During the first 3 months on study, 10 patients (31%) had their dose escalated: 7 due to bleeding alone and 3 due to a combination of bleeding and/or joint findings on the HJHS and/or US.

Conclusions: This is the first prospective, individualized, dose escalating prophylaxis study for boys with SHA in China with the goal of minimizing bleeding and joint damage. Findings from the HJHS and US examinations to guide dose escalation, in conjunction with index joint bleeds, is a unique feature of this clinical trial. These observations will be useful in the design of cost-effective prophylaxis regimens for boys with SHA in China.

The study is financially supported by Bayer HealthCare Co. Ltd., China.

PB 957 | Pilot Study to Determine the Frequency of Cerebral Microbleeds among Adult Patients with Hemophilia a or B

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Background: Cerebral microbleeds (CMBs) represent small, clinically silent hemorrhagic events. Current data suggest CMBs may portend future stroke, intracranial hemorrhage, and cognitive impairment. The prevalence of CMBs has not been evaluated in patients with hemophilia (PWH).

Aims: To compare the prevalence of CMBs between PWH and normal controls.

Methods: Adults with hemophilia A or B of any severity and controls without a prior diagnosis of a bleeding disorder were recruited. Subjects were excluded if chronically taking an antithrombotic agent other than low-dose aspirin (< 100mg). Clinical and demographic data were collected using a standardized form. All subjects underwent T2*MRI of the brain. Two neuroradiologists blinded to patient group reviewed the scans independently and determined whether CMBs were present. Differences in clinical characteristics were assessed by t-test (continuous variables) and either chi-square or Fisher's exact test (categorical variables). A two-sided p-value of < 0.05 was considered statistically significant. SPSS software (version 22.0) was used.

Results: We recruited 31 PWH and 32 controls. HCV infection was more prevalent among PWH and smoking was more common among controls. Other characteristics were similar between the two groups.

TABLE 1 Demographic and clinical characteristics of study population

	PWH (n=31)	Controls (n=32)	p-value
Age, median (range)	39 (20 - 81)	41 (21 - 69)	0.57
History of Hepatitis C infection, n (%)	19 (61.3)	0 (0)	<0.001
Smoking, n (%)	6 (19.4)	14 (43.8)	0.04
Hemophilia A, n (%)	22 (71)	N/A	N/A
Mild hemophilia, n (%)	7 (23)	N/A	N/A
Moderate hemophilia, n (%)	4 (13)	N/A	N/A
Severe hemophilia, n (%)	20 (65)	N/A	N/A
Use of prophylaxis, n (%)	11 (35)	N/A	N/A
Mean annual units infused	241145	N/A	N/A

Among the two neuroradiologists, Reviewer 1 detected 9 CMBs, 7 (22.6%) in PWH and 2 (6.3%) in controls (p=0.06). Reviewer 2 is completing his interpretations, after which consensus review will be undertaken. Among PWH, those with CMBs were older, used less factor annually, and had higher HCV infection rates compared to those without CMBs. All other characteristics were similar.

TABLE 2 CMB in PWH and associated clinical characteristics (Reviewer 1 only)

	PWH with CMBs (n=7)	PWH without CMBs (n=24)	p-value
Mean age, year (SD)	56 (17)	40 (14)	0.05
Hemophilia A, n (%)	5 (71)	17 (70)	0.97
Mild hemophilia, n (%)	2 (28.6)	5 (20.8)	0.64
Moderate hemophilia, n (%)	1 (14.3)	3 (12.5)	1.00
Severe hemophilia, n (%)	4 (57.1)	16 (66.7)	0.67
Use of prophylaxis, n (%)	2 (28.6)	9 (37.5)	0.66
Mean annual units infused, mean (SD)	83000 (181185)	287271 (415003)	0.04
History of HCV infection, n (%)	7 (100)	12 (50)	0.02
Smoking, n (%)	2 (28.6)	4 (16.7)	0.48

Conclusions: Data from this pilot study suggest that CMBs are common among PWH and may be more prevalent than in normal controls. Increased factor use may be protective. The impact of CMBs on neurocognitive function in PWH and whether prophylactic factor administration reduces the prevalence of CMBs requires further study.

PB 958 | Thrombo-Embolic Events Reported with the Use of Activated Prothrombin Complex Concentrate (APCC) in Congenital Haemophilia: A Cumulative Review on Four Decades

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Background: The use of bypassing agents has contributed to a better management of bleeding of persons with haemophilia (PWH) and inhibitors. APCC became commercially available 40 years ago in 1975. While bypassing therapy has been proven effective, it introduced a potentially increased risk of treatment-associated thrombo-embolic events (TEEs).

Aims: The small size of clinical trials and post-authorization studies in PWH with inhibitors limits the capability to ascertain risk factors for APCC-associated TEEs. This review provides an overview of all spontaneous and literature TEE cases reported with the use of APCC in congenital haemophilia, documented in the Company's global safety database (GSD).

Methods: The GSD was reviewed for all spontaneous and literature adverse events reports of APCC from 1975 to July 2016, addressing patient demographics, dosing regimens, confounding and risk factors deemed relevant for the development of TEEs in temporal association with APCC treatment.

Results: More than 7 billion units of APCC (beyond 2 million infusions) were distributed during the review period. 85 reports including one or more TEE events were received for PWH, aged 0-76 years (median age of 22).

TABLE 1 Details of reported TEEs in Patients with Congenital Haemophilia

	Number of cases	Median age (range)
All reported TEEs in Patients with Congenital Haemophilia	85	22 years (0-76)
Deep vein thrombosis and/or pulmonary embolism	18	11 years (1-22)
Myocardial infarction/ischemia	17	41 years (8-73)
Cerebrovascular accident	18	55 years (2.5-70)
DIC	18	49 years (0-71)
Other	14	30 years (3-57)

rFVIIa was reported to be received in temporal relationship with APCC in 32/85 (37.7%) of TEEs. From 01 February 2000 to 31 July 2016, 73 TEEs for all indications were received from spontaneous sources (excluding literature), resulting in a reporting rate of approximately 3.5 TEEs/10^5 infusions (3000 U per infusion).

Conclusions: The reporting rate of TEEs associated with APCC is comparable with published data and confirms its long-time overall safety profile. It has been noted that a relevant part of the TEEs occurred in the presence of additional/confounding risk factors such as underlying disease and concomitant medications. The review of all TEEs reported in temporal association with the use of APCC is a valuable resource for the understanding and perhaps the prevention.

PB 959 | Effects of Acute Moderate Physical Activity on Haemostatic Parameters in Severe Haemophilia A Subjects Using a Third Generation B-Domain Deleted Recombinant Factor VIII

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Background: Persons with haemophilia A (HA) are encouraged to perform routine physical activity to improve their quality of life.
Aims: To evaluate the effects of acute moderate exercise on thromboelastography parameters (TEG), von Willebrand factor levels (VWF:Ag) and FVIII pharmacokinetics (PK) in persons with severe HA.
Methods: Eighteen adults (mean age: 27.5 years) were enrolled. At the first study visit, blood samples were drawn prior to FVIII infusion and over the following 24h, while subjects remained sedentary. At the second visit, subjects performed 4 exercise sessions (2x15min stationary cycling and treadmill walking) over a period of 5h (65% of maximum heart rate +/- 10 bpm) and blood samples were taken at same previous time points. TEG, FVIII activity (FVIII:C) and VWF:Ag were compared between the two visits using paired statistical tests (Two-way ANOVA and t-test). The study was approved by the research ethics committee and all subjects provided written informed consent.
Results: FVIII:C and VWF:Ag were significantly higher immediately after the first exercise session compared to rest ($p < 0.05$ and $p < 0.001$, respectively). This difference rapidly disappeared for FVIII whereas it remained significant for VWF:Ag at least 3h after the last exercise session. FVIII half-life (median: 10h [7.33-16.5h] at rest; median: 10.66h [5.66-16.66h] with exercise) and TEG parameters did not differ significantly between rest and exercise. FVIII half-lives measured both at rest and following exercise strongly correlated with baseline VWF:Ag ($r=0.82$; $p < 0.0001$ and $r=0.74$; $p=0.0004$ respectively). The area under the curve (AUC) of VWF:Ag (0,24h) was 14.3% higher following exercise. VWF:Ag AUC (0, 24h) was associated with FVIII clearance ($p=0.036$) and FVIII half-life ($p < 0.0001$) independently of blood group and exercise.
Conclusions: Acute moderate exercise did not significantly alter the PK of a third generation B-domain deleted rFVIII following a standard prophylactic dose.

PB 960 | Clinical Characteristics of Patients with Hemophilia A and B: Preliminary Results of the Hemfil Cohort Study

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Background: Inhibitor development is the main complication of hemophilia A (HA) and B (HB). Risk factors for inhibitor development are still not completely understood.
Aims: To describe the clinical characteristics of patients included in the HEMFIL study, an ongoing multicenter prospective cohort study on risk factors for inhibitor development.
Methods: Previously untreated patients (PUPs) have been followed up to 75 exposure days (ED) or until inhibitor development. Clinical characteristics were collected using standardized forms. Number of events and respective percentages were calculated for the categorical variables and the median with interquartile range (IQR) for the continuous variables.
Results: Currently, 65 patients were included, 58 (89.2%) with HA and 7 (10.8%) with HB. A total of 53 patients (81.5%) have severe and 9 (13.8%) moderate hemophilia. The median age at inclusion was 10 months [IQR, 6.5-14.0], median weight 9.3 kg [IQR, 8.45-11.0]. Patients were predominantly white (55.4%), blood group O (43.1%) and born by caesarean section (67.7%) with median gestation age of 39.0 weeks [IQR, 38-40]. The median time to breastfeeding was 18 weeks [IQR, 8-25]. Most patients were diagnosed due to bleeding manifestations (75.4%) and 24.6% due to family history (no bleeding). Currently, 52.3%, 44.6%, and 3.1% of patients, respectively, receive prophylactic, on demand and no treatment. Family history of hemophilia was reported in 67.7%, of whom 3.1% also have family history of inhibitor. Allergies were reported by 9 patients (13.8%). A total of 35 patients (53.8%) have completed follow-up, of whom 18 (27.7%) with 75 ED without inhibitor and 17 (26.1%) developed inhibitor.
Conclusions: In this cohort, most of patients are white, have family history of hemophilia, were diagnosed due to bleeding and are on prophylaxis. The inclusion of more patients and a longer follow-up will allow an appropriate analysis between clinical characteristics and inhibitor development.

PB 961 | Target Joint Outcomes with Prophylaxis with rFIXFc in Adults and Adolescents with Hemophilia B: Updated Results from B-YOND

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Background: The B-YOND extension trial evaluates long-term safety and efficacy of rFIXFc in adults/adolescents with severe hemophilia B who completed the Phase 3 B-LONG study.
Aims: To present longitudinal data outcomes from subjects with target joints at BLONG entry through the 2nd interim data cut of B-YOND.
Methods: Subjects completing B-LONG enrolled in 1 of 4 treatment groups in B-YOND: weekly prophylaxis (WP; 20-100 IU/kg every 7

TABLE Summary of joint and target joint ABRs[a]

	Pre-study prophylaxis[b]		Pre-study episodic treatment		
	WP (n = 11)	MP (n = 6)	WP (n = 23)	IP (n = 10)	MP (n = 4)
Pre-study joint bleeding rate, median (IQR)	6.0 (2.0, 13.0)	7.5 (4.0, 20.0)	17.0 (9.0, 30.0)	23.0 (18.0, 33.0)	12.0 (10.5, 17.0)
Overall on-study joint ABR, median (IQR)	1.5 (0.0, 3.2)	1.2 (0.6, 4.8)	1.0 (0.5, 3.0)	3.4 (0.9, 5.6)	1.2 (0.3, 6.2)
Overall on-study target joint ABR, median (IQR)	0.4 (0.0, 3.2)	1.1 (0.5, 3.8)	0.5 (0.0, 1.1)	2.1 (0.9, 3.6)	0.0 (0.0, 3.9)

ABR, annualized bleeding rate; WP, weekly prophylaxis; MP, modified prophylaxis; IQR, interquartile range.
[a]Includes data from adult/adolescent subjects with target joint at entry into parent study who entered the efficacy period in B-LONG or B-YOND. n = number of subjects with non-missing data for both prestudy and on-study joint ABR. Subjects are included in each treatment regimen they participated in for the duration of time on that regimen and as such may appear in more than one treatment regimen in BYOND.
[b]One subject on pre-study prophylaxis was in the IP group and entered the efficacy period. This subject had a pre-study joint bleeding rate of 5.0, overall on-study joint ABR of 5.7, and overall on-study target joint ABR of 4.6.

days), individualised prophylaxis (IP; 100 IU/kg every 8-16 days), modified prophylaxis (MP; for subjects not achieving optimal dosing with IP or WP), or episodic treatment. Subjects with ≥1 target joint (major joint with ≥3 bleeding episodes in a 3-month period) at B-LONG entry were evaluated. A target joint resolved if there were ≤2 spontaneous bleeds in the target joint over 12 months. Outcomes were analysed over the cumulative duration of B-LONG through the second B-YOND interim data cut (11 Sep 2015).

Results: Of 117 B-LONG subjects with on-study data, 60 had a total of 166 target joints at baseline. These subjects received a cumulative median (IQR) of 3.4 (1.4-4.2) years of rFIXFc. In subjects with target joints at baseline, on-study overall and target joint annualised bleeding rates (ABRs) with rFIXFc prophylaxis were lower than bleeding rates with prestudy (pre-B-LONG) prophylaxis or episodic treatment (**Table**). 37.5% of WP, 8.3% of IP, and 33.3% of MP subjects had no target joint bleeding episodes during B-LONG/B-YOND, and 100% (37/37) of subjects with evaluable target joints at B-LONG baseline (≥12 months follow-up; no target joint surgery ≤12 months) had target joints resolved. In the WP and IP groups, median (IQR) average weekly prophylactic dose was 45.2 (37.3-55.8) IU/kg and 64.7 (46.7-82.3) IU/kg, respectively; median (IQR) dosing interval in the IP group was 10.3 (8.9-13.2) days.

Conclusions: Long-term rFIXFc prophylaxis resulted in low target joint ABRs and target joint resolution in all adults/adolescents with evaluable target joints at baseline in all dose groups.

PB 962 | Pressure Pain Thresholds Hint at Structural Findings in Knees of Patients with Haemophilia

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Background: Patients with haemophilia (PwH) suffer from an enhanced pain sensitivity, depending on the joint status. However, the main reason of the altered pain condition is often unknown.

Aims: The objective of this study was to determine, if a comprehensive assessment of altered pain condition is directly linked to changes of underlying anatomical structures in PwH.

Methods: Pressure pain thresholds (PPT) were assessed at 11 different points around the knees of 36 PwH (severe A=29, B=3; moderate A=3, B=1) and 36 healthy controls (C). PPT were used to generate four groups (pain sensitive (PS-) / insensitive (PI-) knees of PwH and C). Structural conditions of underlying anatomic structures were examined by ultrasound sonography (US). Metric data between the groups were analysed by Kruskal-Wallis tests, categorical data by Pearson Chi-Square-tests.

Results: PPT in PwH were statistically significant decreased at all landmarks when compared to C (p≤0.004). US findings assessed across the lateral joint space revealed that especially osteophytes are more pronounced when PPT are decreased (Tab. 1). The synovia tissue, assessed at the suprapatellar region of the knees, was also thickened in PS-PwH when compared to other groups (Tab. 1). In contrast, no differences were found between the groups regarding effusion, whether assessed at the distal edge of m. vastus lateralis (p=0.893), the lateral knee joint space (p=0.417) or the suprapatellar region (p=0.274).

TABLE 1 Selective structural differences between pain sensitive (PS) / pain insensitive (PI) knees of PwH / controls (C). Mean±SD

anatomical structure	PS-PwH	PI-PwH	PS-C	PI-C	p-value
osteophytes femoral (mm)	5.6±5.8	1.3±2.7	0.0±0.0	0.0±0.0	0.000
osteophytes tibial (mm)	2.2±2.9	1.0±2.3	0.0±0.0	0.0±0.0	0.002
synovia tissue (mm)	2.2±2.2	1.1±0.7	0.8±0.6	0.7±0.3	0.001

Conclusions: Significant differences of knee-surrounding structures exist between PwH and C. Particular degenerative changes, in terms of osteophytes and thickness of synovial tissue, are linked with an enhanced pain sensitivity in PwH. However, altered PPT which were not associated with structural findings may be an indicator for a complex modified pain perception of the affected joints. This leads to the conclusion that pain is caused not only by structural, but also by functional alterations in PwH.

PB 963 | Once-weekly Prophylaxis with GlycoPEGylated Recombinant Factor VIII (N8-GP) in Severe Haemophilia A: Safety and Efficacy Results from a Randomised Phase 3 Trial (pathfinder™2)

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Background: N8-GP is a glycoPEGylated recombinant FVIII (rFVIII) with an extended half-life developed for use in haemophilia A. N8-GP was well tolerated and efficacious in the main phase of pathfinder™2, a pivotal, ongoing, multinational, open-label phase 3 trial.[1]

Aims: The extension phase of pathfinder™2 investigated the long-term safety and efficacy of N8-GP prophylaxis (PPX) when administered as two separate dosing regimens to a subset of eligible patients.

Methods: For the extension phase, patients aged ≥12 years with ≤2 bleeds during the last 6 months of the main phase in pathfinder™2 on PPX were eligible to be randomised (1:2) to N8-GP PPX either at 50 IU/kg every fourth day (Q4D) or 75 IU/kg weekly (Q7D). Safety and efficacy endpoints were the incidence of FVIII inhibitors (≥0.6 Bethesda units) and the annualised bleeding rate (ABR), respectively.

Results: Fifty-five out of 143 (39%) patients who completed the main phase on PPX received 50 IU/kg Q4D (n=17) or 75 IU/kg Q7D (n=38). During the 24 week treatment period, there were 1539 exposure days in total. No FVIII inhibitors were detected. Median ABR was 0 for both cohorts. Estimated mean ABR was 1.77 and 3.57 for the 50 IU/kg Q4D and 75 IU/kg Q7D cohorts, respectively. In the 75 IU/kg Q7D cohort, 22 patients (58%) did not report any bleeds during the 24 weeks, comparable with the 50 IU/kg Q4D cohort (53%). Nine patients (23.7%) in the 75 IU/kg Q7D cohort reverted to their previous Q4D regimen in accordance with pre-defined criteria in the trial protocol (bleeds [n=8] and investigator's discretion [n=1]).

Conclusions: N8-GP dosed at 50 IU/kg Q4D or 75 IU/kg Q7D was well tolerated in this extension phase of pathfinder™2. More than 50% of patients in both dosing cohorts did not bleed during the treatment period, suggesting that once-weekly dosing is efficacious for a subset of patients with severe haemophilia A.

References:

1. Giangrande P *et al. Thromb Haemost* 2016;accepted.

PB 964 | End-of-trial Results from a Large Multinational Extension Trial (guardian™2) Using Turoctocog Alfa for Prophylaxis and Treatment of Bleeding in Patients with Severe Haemophilia A

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Background: Turoctocog alfa (NovoEight®), a recombinant factor VIII (rFVIII) product, has been approved by major regulatory authorities for the prophylaxis and treatment of bleeds in patients with haemophilia A. guardian™2 was a large, multinational, extension trial of turoctocog alfa in paediatric and adult haemophilia A patients from 19 countries who were previously enrolled in the pivotal guardian™1 and guardian™3 trials, or who had completed pharmacokinetic trials.

Aims: To assess the long-term safety and efficacy of turoctocog alfa for the prevention and treatment of bleeds.

Methods: Previously treated patients, aged 1–70 years, with severe haemophilia A (FVIII activity ≤1%) without inhibitors, received prophylactic therapy with turoctocog alfa (20–50 IU/kg once every second day, 20–60 IU/kg three times weekly, or 40–60 IU/kg once every third day or twice weekly), with additional treatment for bleeding episodes. Treatment success was defined as a patient-reported ‚excellent' or ‚good' haemostatic response.

Results: Data are included for more than 6 years from when the first patient was enrolled and a total of 753 patient-years of turoctocog alfa treatment (mean total number of doses: 164 per patient year for patients on prophylaxis; 55 per patient year for patients treated on-demand). No FVIII inhibitors (≥0.6 Bethesda Units) were detected and no safety issues were identified. The overall estimated annualised bleeding rate (ABR) (Poisson estimated mean) for patients on prophylaxis was 2.44 and the median ABR was 1.37 (Poisson estimated mean ABR for spontaneous bleeds was 1.34). The overall success rate for treatment of bleeds during the preventative regimen was 89.8%; 88.2% of all bleeds were successfully treated with 1–2 injections of turoctocog alfa.

Conclusions: The end-of-trial results of guardian™2 show data that support the long-term safety and efficacy of turoctocog alfa in the

prophylaxis and treatment of bleeds in patients with severe haemophilia A.

PB 965 | rVIII-SingleChain in Surgical Prophylaxis: Efficacy and Safety in 35 Surgeries

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Background: rVIII-SingleChain, a novel recombinant Factor VIII, was designed as a B-domain truncated construct with a covalent bond between heavy and light chains, resulting in high binding affinity to von Willebrand Factor.

Aims: To investigate the safety and efficacy of rVIII-SingleChain to control hemostasis in pediatric, adolescent and adult patients with severe hemophilia A undergoing surgery.

Methods: Studies in the AFFINITY program were approved by the relevant Ethics committee and national authorities and conducted according to GCP and the Declaration of Helsinki. In the surgical substudies, 28 patients underwent 35 procedures requiring general, spinal or regional anesthesia. Dosing was guided by WFH recommendations. rVIII-SingleChain was used either as a bolus or continuous infusion. Hemostatic efficacy of rVIII-SingleChain during surgery was rated by investigators.

Results: Surgical procedures performed were: abdominal hernia repair, ankle arthroplasty, ankle hardware removal, appendectomy, arthrodesis of the ankle joint, cholecystectomy, circumcision (9), debridement (2), elbow replacement, excision curettage and bone grafting, dental extraction (3), knee arthroscopy, knee replacement (7), knee spacer and immobilization, lengthening of achilles ligament (2), open reduction and internal fixation of ankle and port-a-cath removal. 27 surgeries were performed using bolus infusion while 8 surgeries used continuous infusion of rVIII-SingleChain. In 32 (91%) procedures efficacy was rated as excellent (defined as hemostasis not clinically significant different from normal) and in 3 (9%) surgeries efficacy was rated as good (defined as hemostasis normal or mildly abnormal in terms of quantity and/or quality eg, slight oozing). No related AEs or SAEs were observed during the surgery period.

Conclusions: rVIII-SingleChain provides very effective and safe control of hemostasis during a wide range of surgical procedures when dosed either by bolus or continuous infusion.

PB 966 | Platelet Aggregation and Clinical Phenotype in Pediatric Patients with Severe Hemophilia A (SHA)

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Background: There is phenotypic heterogeneity in SHA but the underlying biological cause is unknown. We recently examined platelet function in pediatric patients with SHA, reporting there is no intrinsic platelet aggregation defect (Rand et al, Haemophilia 2016,22(Suppl 4):97).

Aims: To investigate the role of platelet aggregation in the bleeding phenotype in SHA.

Methods: In 2 study sites, whole blood platelet aggregation stimulated by ADP, collagen, arachidonate and TRAP was measured in boys with SHA (factor (F)VIII≤1%) using a Multiplate® analyzer. Informed consent was obtained; the study was approved by the Research Ethics Boards. An ‚overall aggregation response' was calculated for each patient as the sum of responses to the 4 agonists. Bleeding severity was determined as age at 1st joint bleed and annual FVIII consumption (U/kg), averaged over 3yrs.

Results: 42 boys with SHA were enrolled from 2 pediatric hemophilia treatment centres, 20 from Site 1 (median age: 11yrs; range: 4-16) and 22 from Site 2 (12yrs; 6-16). The median age at 1st joint bleed was 3.3yrs (0.5-8.1) at Site 1 and 2.4yrs (0.3-8.1) at Site 2. At either site, there were no correlations between platelet aggregation stimulated by individual agonists or the ‚overall aggregation response' and age at 1st joint bleed. The median annual FVIII consumption was 3431 U/kg (1120-5410) at Site 1 and 5203 (1430-9474) at Site 2. Aggregation responses did not correlate with annual FVIII consumption at Site 1. However, at Site 2, there were significant, strong inverse correlations between annual FVIII consumption and both ADP-induced aggregation (r=-0.69; p=0.0004) and the ‚overall aggregation response' (r=-0.55; p=0.008).

Conclusions: These results suggest a role for augmented platelet aggregation, in particular to ADP, as a determinant of a milder clinical phenotype in SHA that warrants further investigation in a larger, multicentre study; the small number of patients examined in the present study may play a role in the differences between the 2 study sites.

PB 967 | Characterisation of the Inhibitor Status of Patients with Haemophilia A in Australia

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Background: Inhibitor formation is a significant complication of haemophilia treatment and leads to increased bleed frequency and reduced quality of life.

Aims: To evaluate the incidence of inhibitor formation in Australian patients with haemophilia A (HA), and the treatment strategies utilised in patients with inhibitors, using information from a National registry.

Methods: Data was obtained from the Australian Bleeding Disorder Registry (ABDR) on disease severity, age, inhibitor results, and tolerisation therapy. Patients with a positive inhibitor test result were sub-classified based on whether the inhibitor was persistent, had spontaneously resolved, or previous tolerisation had resulted in eradication.

Results: 1514 patients had undergone testing for the presence of an inhibitor, with 242 (16.0%) having repeat positive tests. The incidence was higher in patients with severe HA (174/651, 26.7%) than in non-severe HA (68/855, 7.95%), p< 0.001. Treatment history and recent tests results were available for 135 patients (100 severe HA, 35 non-severe). 47 (32 severe, 15 non-severe) had persistent inhibitors. Patients with severe HA with a current inhibitor were more likely to be on tolerisation therapy (20/32, 62.5%) than those with non-severe disease (0/15). In patients in whom the inhibitor was no longer present, patients with severe HA were more likely to have previously undergone tolerisation therapy than those with non-severe HA (78% versus 45%; p= 0.001) with spontaneous remission more common in the latter group. Of the 62 patients with successful eradication, mean tolerisation time was 582 days
(SD 521).

Conclusions: The incidence of inhibitor formation in Australian patients with HA is similar to previously reported rates, and remains a significant management issue. Tolerisation appeared to be successful in the majority of patients with severe HA in whom it had been attempted. The optimal management of patients with non-severe HA requires further study.

PB 968 | Long-term Quality-of-Life Outcomes with rFIXFc in Adults with Hemophilia B: Results from B-LONG and B-YOND

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Background: Long-term quality-of-life (QOL) effects of rFIXFc are being evaluated in the Phase 3 B-LONG clinical trial and the ongoing B-YOND extension study of adults with severe hemophilia B.

Aims: To evaluate longitudinal QOL data in hemophilia B subjects from B-LONG study entry through the second interim data cut of B-YOND at 24-month follow-up.

Methods: Subjects were evaluated using the Haem-A-QOL patient-reported outcome from B-LONG baseline to B-YOND baseline, through the second B-YOND interim data cut (11 Sept 2015) at B-YOND month 24. Last observation carried forward was used to impute missing data. Within group t-tests compared mean change from baseline on Haem-A-QOL total score and sub-domain scores.

Results: Out of 67 B-LONG prophylaxis subjects (age ≥17) with baseline Haem-A-QOL score, 44 had total change scores from baseline to B-YOND month 24 (mean age 31.3, SD 11.9). 24 patients received prophylaxis prior to trial enrollment, 18 on-demand regimen, and 2 with missing data on prestudy regimen. Patients were from North America (27%), Europe (32%), and other continents (41%), and included 68% White, 9% Asian, 9% African-American, and 14% other. Half (52%) had target joints bleeding at baseline and the median prestudy annualized bleeding rate was 10 (IQR 2, 20). QOL improved from B-LONG baseline to B-YOND baseline (median follow-up 12.2 months, IQR 11.8-13.3) with a mean change of 5.7 (p< 0.01). The QOL improvement was maintained over 24 months of follow-up (p< 0.01). An examination of the sub-domain scores over time suggested that the most pronounced improvements were in sports and leisure, followed by physical health, self-view, and feeling sub-domains.

Conclusions: Subjects treated with rFIXFc reported improvements in overall QOL during B-LONG, and these improvements were maintained for 24 months of B-YOND follow-up. The biggest QOL gains reflected engagement in sports and leisure pursuits, physical health, self-view, and feeling Haem-A-QOL sub-domains.

PB 969 | Lower Risk of Factor VIII Inhibitors among Severe Hemophilia A Patients with Indigenous Background from Brazil

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Background: Inhibitor of factor (F) VIII involves a complex multifactorial process with several genetic and non-genetic risk factors.

Aims: To assess the influence of genetic factors in the development of FVIII inhibitors in severe HA (sHA) patients from Brazil.

Methods: 422 sHA patients from the five geographic regions in Brazil, with different ethnic background, were enrolled. For ethnicity, we considered the last 3 generations. Inhibitor >0.6BU on at least two occasions by modified-Bethesda assay were considered positive. DNA samples were used for *F8* genotyping and to determine the 12 single nucleotide polymorphisms (SNPs) in immune regulatory genes (*IL10*, *IL5*, *INFG*, *TGFB1*, *TNFA*, *CTLA4* and *HMOX1)* using TaqMan®SNP Genotyping kit.

Results: Presence or history of inhibitor was confirmed in 97/422 (23%), and 82 (19.4%) were high-responding inhibitors (≥5BU). 39/132 (29.5%) of African descents sHA patients had

inhibitor detected, with a two-fold higher risk of inhibitor compared to Caucasians ($p< 0.01$; OR=2.18; 95%CI 1.36-3.50). Interestingly, among the non-black group, patients with indigenous background had significantly lower occurrence of inhibitor 1/38 (2.6%), compared to Caucasians ($p< 0.01$; OR=0.11; 95%CI 0.01-0.89). In addition, *F8* nonsense and frameshift mutations showed higher risk (OR=2.54, 95%CI, 1.17-5.52 and OR=3.08, 95%CI, 1.03-9.22, respectively) compared to *F8* intron 22 inversion (INV22). SNPs in immune regulatory genes indicated increased susceptibility to inhibitor in patients with the -857CT *TNFA* genotype. However, patients with -819TT and -592AA in *IL10* gene and „CG/CG" haplotype in *TNFA* gene have lower risk of inhibitors. However, multivariate analysis showed that none of the significantly genetic risks explained the difference observed in the ethnic groups.

Conclusions: This study contributes to genetic risk factors for inhibitor development. For the first time, we identified an ethnic group with lower risk of inhibitor, despite the same access and type of treatment they received.

PB 970 | Surgery with Turoctocog Alfa: Efficacy and Safety in Bleeding Prevention During Surgery in Patients with Severe Haemophilia a - Results from the Guardian™ Trials

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Background: Novo Nordisk has developed turoctocog alfa (NovoEight®), a recombinant factor VIII (rFVIII) for the prophylaxis and treatment of bleeds in haemophilia A patients. During the pivotal trial in adult/adolescent previously treated patients (PTPs) with severe haemophilia A (guardian™1), patients needing surgery participated in a subtrial to document safety/efficacy of turoctocog alfa in surgical bleeding prevention. Paediatric PTPs in guardian™3 underwent minor surgery as needed. Once the initial trials completed, patients could continue with turoctocog alfa in the guardian™2 extension trial (with surgical subtrial).

Aims: To assess efficacy/safety of turoctocog alfa in the prevention of surgical bleeding during the guardian™ trials.

Methods: Eligible patients required major or minor surgery during the guardian™ trials. Haemostatic efficacy during/after surgery was rated on a 4-point scale (excellent/good/moderate/none) by the Investigator and/or Surgeon.

TABLE Details and outcomes of major surgical procedures in the guardian™ trials using turoctocog alfa for prevention of excessive surgical bleeding

Surgery indication/duration of surgery (h:mm)/Patient age (years)	Duration of surgery (h:mm)	Patient age (years)	Haemostatic response during surgery	Haemostatic response after surgery
• Arthroscopy of left ankle	0:40	24		
• Evacuation of damaged tissue with metallosis; evacuation of broken fragment of the right elbow implant and replacement with new	1:30	47		
• Evacuation of infected joint prosthesis and evacuation of necrotic tissue	2:18	47		
• Revision of right elbow joint endoprosthesis	1:50	47	Excellent	Excellent
• Left knee surgery	1:45	47		
• Closed reduction of fractured nasal bones	1:00	23		
• Appendectomy	0:35	8		
• Implantation endoprosthesis totalis genus 1 sin synoviectomia subtotalis	2:40	25		
• Implantation endoprosthesis totalis genus 1 dex	3:27	25		
• Panproctocolectomy; ileoanal pouch	3:23	21	Excellent	Good
• Left hip arthroprosthesis, reduction finger fracture	3:25	55	Good	Excellent
• Decompressive craniectomy and evacuation of clot	1:30 3:45	27 37		
• Total knee and total hip replacement	1:30	28		
• Right knee arthroprosthesis				
• Revision replacement of the acetabular part and head of the right hip endoprosthesis	1:35 2:05	39 56	Good	Good
• Resection of radial head, preparation of *nervus ulnaris*	2:00 1:50	47 41		
• Laparoscopic cholecystectomy				
• Knee replacement and elbow radial head excision				

Results: In total, 18 major and 66 minor surgeries were included in the analysis results. In all the major and 64 minor surgeries, haemostatic efficacy during/after surgery was rated as either excellent or good in all cases. For the other 2 minor surgeries, 1 had no clinical evaluation and another endoscopy procedure was rated as ‚none'. Details and outcome of individual major surgeries performed are presented in the Table. No FVIII inhibitors (≥0.6 Bethesda Units) were detected. No thromboembolic events were reported and no hypersensitivity/allergic reactions to turoctocog alfa were observed. Results on adverse events, safety laboratory parameters and other safety-related examinations did not indicate clinically significant changes as a result of turoctocog alfa administration.

Conclusions: Prevention of excessive surgical bleeding is an important aspect of haemophilia treatment. The results support that turoctocog alfa has a favourable safety/efficacy profile when used across a wide range of surgical procedures.

PB 971 | Improved Pharmacokinetics of BAY 81-8973 versus Sucrose-formulated Recombinant Factor VIII (rFVIII-FS): Noncompartmental and Population PK Assessment Based on a Single-dose, Randomized, Crossover, Pharmacokinetics Study in Patients with Severe Hemophilia A

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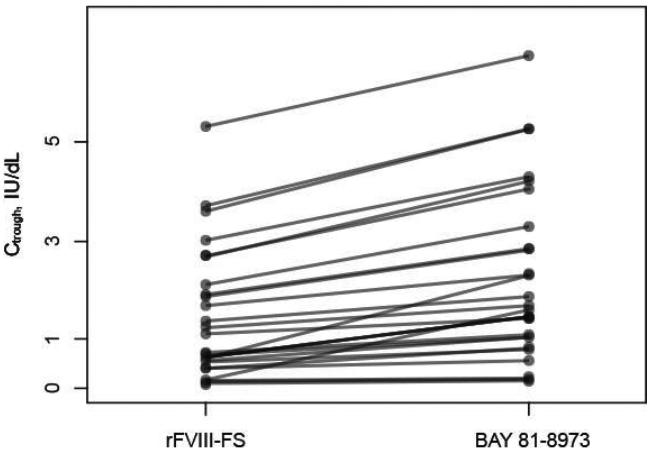
Background: BAY 81-8973 is a full-length, unmodified, recombinant human factor VIII (FVIII) with the same primary amino acid sequence as sucrose-formulated recombinant FVIII (rFVIII-FS) produced using newer manufacturing methods.

Aims: To compare the pharmacokinetic (PK) profile of BAY 81-8973 versus rFVIII-FS

Methods: PK samples were collected from 26 male patients aged 12-65 years with severe hemophilia A to compare PK of BAY 81-8973 to rFVIII-FS. Patients were randomized to receive a 50-IU/kg single dose of BAY 81-8973 or rFVIII FS followed by the other treatment after a washout period of ≥3 days. Population PK (popPK) models were developed based on chromogenic assay data for both products and simulations were conducted to estimate trough levels after administration of the products and to project dose and/or time to achieve threshold levels of 1, 3, 5, 10 IU/dL FVIII expected to provide bleeding protection. A similar study previously showed a superior PK profile of BAY 81-8973 versus antihemophilic factor (recombinant) plasma/albumin-free method (Shah et al, *Clin Pharmacokinet*. 2016:Epub).



A solid line indicates that a switch from rFVIII-FS to BAY 81-8973 results in an increase in trough levels for this patient.
C_{trough}=trough level; PK=pharmacokinetic; rFVIII-FS=recombinant factor VIII formulated with sucrose.

FIGURE 1 Estimated PK trough levels at steady state following a dose of 30 IU/kg BAY 81-8973 or rFVIII-FS 3x/week

Results: Mean PK parameters for BAY 81-8973 were higher for area under the curve and half-life and lower for clearance **(Table 1)**, indicating a more favorable PK profile versus rFVIII-FS. Estimations based on popPK models showed higher trough levels for all patients for BAY 81-8973 at steady state following a dose of 30 IU/kg 3x/week **(Figure 1)**. Simulations showed that median time to 1 IU/dL was ~16% (~10 h) longer for BAY 81-8973 versus rFVIII-FS over 25-50 IU/kg doses. The same FVIII threshold level could be achieved with a lower dose of BAY 81-8973.

Conclusions: BAY 81-8973 showed an improved PK profile versus rFVIII-FS based on noncompartmental analysis and popPK. For BAY 81-8973, higher trough levels can be achieved with similar doses and frequencies which can lead to better protection against breakthrough bleeds. Longer time to threshold levels for BAY 81-8973 can allow for less frequent dosing.

PB 972 | Towards the Development of a Core Set of Outcome Measures for Standardized Assessment of Outcomes in Persons with Hemophilia

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Background: There is a lack of systematic use of well-defined outcome measures (OM) in care for persons with hemophilia (PWH). New therapeutics and an emphasis on outcomes in clinical care have resulted in unprecedented activity in OM research. Over the last decade, many tools have been developed, nearly covering all domains of the International Classification of Functioning, Disability and Health (ICF) model. There is an urgent need to define the important OM to be used in the various clinical contexts.

TABLE 1 PK Parameters Calculated Using Noncompartmental Methods*

Parameters	BAY 81-8973 (N=26) Geometric Mean (%CV)	rFVIII-FS (N=26) Geometric Mean (%CV)	Least Squares Mean Ratio (95% CI)	P Value
Cmax, IU/dL	130.1 (23.0)	136.2 (23.8)	0.96 (0.85-1.08)	0.45
AUC, IU·h/dL	1889.2 (36.1)	1583.9 (39.9)	1.19 (1.09-1.30)	0.0003
t1/2, h	13.8 (28.0)	12.0 (28.2)	1.15 (1.06-1.24)	0.0016
MRTiv, h	19.3 (26.8)	16.5 (27.4)	1.17 (1.09-1.25)	<0.0001
CL, dL/h/kg	0.026 (36.1)	0.032 (39.9)	0.84 (0.77-0.91)	0.0003

*Data based on the chromogenic assay.
AUC=area under the curve from time 0 to infinity; CL=clearance; Cmax=maximum concentration; CV=coefficient of variation; MRTiv=mean residence time; PK=pharmacokinetic; rFVIII-FS=recombinant factor VIII formulated with sucrose; t1/2=half-life.

Aims: The aim of this work is to define a core set of OM that should be considered for the assessment of PWH in research and practice, within the ICF framework.

Methods: As a follow-up to a meeting in 2014, we initiated a 5 step process to define a core set, guided by the findings of systematic reviews of tools for joint health scores, activity/participation, and health related quality of life. Step 1 was to develop a list of potential OM by conducting a centre-based survey of a multidisciplinary group of international experts in hemophilia care and PWH (n=55). The survey was for item-generation and classification into the 6 ICF domains. In step 2, individual respondents (n=74) voted on each item. Next, in a 2 day consensus meeting (n=49) the following were discussed: the results from the surveys, systematic reviews, and information from experts in each ICF domain. Two rounds of voting and discussion by participants (steps 3 & 4) occurred for each item from the surveys. In step 5, participants (n=48) were surveyed and voted for their top 5 items from a list of those with >50% agreement identified in steps 3 and 4.

Results: Table 1 shows the response rate for each step in the process.

TABLE 1 Response rate per step in the process

Step in the Process	Response Rate (%)
Step 1: Item Generation, hemophilia treatment centre (HTC) based	55/76 HTCS (72.4)
Step 2: Initial voting, individual based	74/106 individuals (69.8)
Steps 3 & 4: Consensus conference attendees	49/52 individuals (94.2)
Step 5: Final voting, post-meeting, individual based	48/52 individuals (92.3)

Table 2 shows the top 5 OM that emerged from this process.

TABLE 2 Proposed outcome measure core sets

Core set for pediatric patients (% yes to core set)	Core set for adult patients (% yes to core set)
A measure of treatment satisfaction (92.7)	Total bleeding events (88.1)
Hemophilia Joint Health Score (HJHS) (83.3)	EuroQol five dimensions (EQ-5D) (85.4)
A measure of access to treatment (82.5)	A measure of treatment adherence (82.1)
A measure of treatment adherence (72.5)	Hemophilia Joint Health Score (HJHS) (79.1)
Generic performance based physical function (72.1)	Number & location of bleeds per unit time (78.6)

Conclusions: This process generated a core set of OM which should be considered for assessment of outcomes in PWH. This information now requires refinement to define optimal core sets for use in different clinical/research contexts.

Funding provided by Novo Nordisk Health Care AG.

PB 973 | Low-dose Factor VIII Infusion in Chinese Adult Haemophilia A Patients: Pharmacokinetics Evidence that Daily Infusion Results in Higher Trough Level than with Every-other-day Infusion with Similar Factor VIII Consumption

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Background: Pharmacokinetics (PK) modeling suggests improvement of trough levels are achieved by using more frequent infusion strategy. However, no clinical study data exists to confirm or quantify improvement in trough level, particularly for low-dose prophylaxis in patients with haemophilia A

Aims: To provide evidence that low dose daily (ED) prophylaxis can increase trough levels without increasing FVIII consumption compared to every-other-day (EOD) infusion

Methods: A cross-over study on 5 daily vs 10 IU Kg^{-1} EOD FVIII infusions, each for 14 days was approved and conducted at the PUMCH. On the ED schedule, trough (immediate prior to infusion), and peak FVIII:C levels (30 mins after infusion) were measured on days 1 to 5; and trough levels alone on days 7,9,11,13; for the EOD schedule, troughs and peaks on days 1, 3, 5, 7; troughs alone on days 2, 4, 6, 9, 11, 13.

Results: Six patients were enrolled. At the 24-hour post-infusion time point, the median trough levels of FVIII:C was higher in the EOD group (6.9, IQR 6.23-9.03 IU dL^{-1}) compared to the ED group (5.6, IQR 4.0-6.6 IU dL^{-1}) given the higher dose (10 vs. 5 IU kg^{-1}) and thus higher peak achieved in the EOD group [29.3 (IQR 23.9-31.5) vs. 17.5 (IQR 14.3-19.13) IU dL^{-1}]. However, by 48 hours, both regimens are at a trough time point and the trough FVIII:C levels in ED group (due to another additional daily infusion) was nearly 2 (range 1.8-2.3) times higher than that in EOD group [5.6 (IQR 4.0- 6.6) vs 2.5 (IQR, 2.01-3.65)]. When steady state trough levels (defined as after 5 consecutive infusions) were achieved in each infusion group (days 6-13 for ED, days 11,13 for EOD), the improvement in trough level remained ~2 times higher in the ED group compared to EOD [5.8 (IQR 4.05-6.8) vs 2.7 (IQR 2.08-4.65)].

Conclusions: Our PK study shows low-dose Factor VIII daily infusion results in higher trough level than with EOD infusion with similar factor VIII consumption in Chinese adult haemophilia A patients.

PB 974 | Association of Factor VIII and Factor IX Mutations, HLA Class II, Tumor Necrosis Factor-Alpha and Interleukin-10 on Inhibitor Development among Thai Hemophilia A and B

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Background: Mutations on factor VIII and factor IX genes as well as polymorphisms of the immune response genes of human leukocyte antigen (HLA)-*DRB1*15*, tumor necrosis factor-alpha (TNF-alpha)-*308A* and interleukin-10 (IL-10)-*1082G* alleles have been suggested to be contributing determinants of inhibitor risk.
Aims: To study the association of factor VIII and factor IX mutations, HLA-*DRB1*15*, TNF-alpha-*308A* and IL-10-*1082G* alleles on the inhibitor development among Thai hemophilia A and B patients.
Methods: A total of 116 hemophilia A patients from 100 families and 42 hemophilia B patients from 36 families were enrolled.
Results: Hemophilia A patients were divided in two groups: 55 patients without inhibitor and 45 patients with inhibitor (25 high titer ≥5 Bethesda unit/mL, 20 low titer). Factor VIII mutations were identified in 97 of 100 families. Twelve were novel. Forty-one of 81 patients (50.6%) with inversion intron 22, large deletion and nonsense mutation developed significantly higher inhibitor than those with missense mutation (2/16=12.5%). No significant difference was found between the frequencies of HLA-*DBR1*15*, TNF-*a*-308A and IL-10-*1082G* among hemophiliacs with and without inhibitor. For hemophilia B patients, factor IX mutations were identified in all subjects. Seven were novel. Unfortunately, one severe hemophilia B patient with nonsense mutation developed inhibitor. He was heterozygote for GA at TNF-alpha and GA for IL-10 genes while another similar hemophilia B patient without inhibitor was homozygote for the wild type of GG for TNF-alpha and AA for IL-10 genes.
Conclusions: For Thai hemophilia A patients with inhibitor, factor VIII mutations showed a significant contribution while the HLA-*DRB1*15* gene had some contribution. However, *-308A* at the TNF-alpha gene and *-1082G* at the IL-10 gene were of lesser impact. On the contrary, TNF-alpha-*308A* and IL-10-*1082G* alleles might be associated with inhibitor development in one Thai severe hemophilia B patient.

PB 975 | Participation of Clinical Psychologist in a Non-hemophilia Treatment Center

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Background: An innovative therapeutic management of hemophilia has been implemented at many hospitals in Japan. However, psychosocial support for hemophilia patients and their families is limited because there are few hemophilia treatment centers which can provide multidisciplinary care and support in Japan. Our hospital is a non-hemophilia treatment center and only medical doctors have seen patients with hemophilia. In 2013, one clinical psychologist joined the adult hemophilia care service. Since then the clinical psychologist has contributed to hemophilia health care, and resolved diverse medical and psychosocial issues.
Aims: This study reports on the assessment of these issues.
Methods: We evaluated 13 patients with a median age of 35 (range; 21-69). Ten patients had severe hemophilia and one of them had inhibitors. All patients with severe hemophilia were on regular prophylaxis. A clinical psychologist had interviews with patients and/or families independently of doctor's medical examination, and measured mood and emotion state by POMS2 (Profile of Mood States Second Edition).
Results: The main issues in young patients were self-infusion and transition from pediatric to adult services. In older patients, the major concerns were joint problems including decision of surgical management. Their POMS2 showed that "confusion - bewilderment" became greater scores and „vigor - activity" became lower scores. We thought that these results reflected the relevant mood such as anxiety. In addition, we found that the positive mood state of „vigor - activity" and the negative mode state of „friendliness" in the patients who have serious problems on joints and who have inhibitors.
Conclusions: The clinical psychologist had a large impact on hemophilia health care and the health-related quality of life in hemophilia patients was greatly improved. Addressing the psychological problems in hemophilia is of great value and a clinical psychologist plays an important role even in a non-hemophilia treatment center.

PB 976 | Continuous Infusion of Recombinant Factor IX Fc Fusion Protein during Major Surgery

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Background: While extended-half-life-recombinant factor IX (EHL-rFIX) is becoming popular among Hemophilia B patients as prophylaxis, hemostatic management of major surgery using such agents is not established.
Aims: To evaluate the efficacy and safety of continuous infusion (CI) of recombinant factor IX Fc fusion protein (rFIXFc), the first EHL-rFIX, in major surgery.
Methods: Tissue-factor-triggered thrombin generation assay was performed by means of calibrated automated thrombogram (Thrombinoscope BV). Other data was gathered from clinical records. Informed consent was obtained from patient, which was approved by the local ethics committee.
Results: Case: The patient is an 81 years old man with mild Hemophilia B. He developed cystic tumor in his pancreas head and required surgery.

Laboratory data: Hct 29.7%, Plt 222×10^9/L, APTT 48.1sec (normal control 30.8sec), Factor IX activity (FIX:C) 14IU/dL. PT, Fibrinogen and other coagulation factors were all within normal limits.

Clinical course: After rFIXFc bolus infusion (95.2IU/kg), CI was started at 3.81IU/hr/kg. Additional 4000IU of rFIXFc was administered to make up for the loss by bleeding (1039mL including ascites) during the surgery. As bleeding was easily controlled after the surgery, we gradually reduced rFIXFc and stopped CI at post-operative day (POD) 15. There were no bleeding or thrombotic complications throughout the course.

Hemostatic analysis: Actual clearance of rFIXFc during the CI was higher than those obtained from Pharmacokinetic (PK) test (table.1). It was similar to those of rFIX (previous data). After the surgery, thrombin generation was better than those of normal control plasma (table.2). This might be due to increased procoagulant factors and decreased anticoagulant factors (data not shown).

Conclusions: We successfully managed major surgery under CI of rFIXFc. The advantage of rFIXFc reducing factors consumption was limited during CI. That seems to be because the clearance during CI strongly depend on the early-phase of distribution.

TABLE 1 Comparison of calculated and actual clearances of rFIXFc and rFIX. *Suzuki N, et al. Haemophilia 2015

	rFIXFc PK (before surgery)	rFIXFc CI (POD7)	rFIX (previous data*)
Clearance(mL/hr/kg)			
Calculated from distribution half-life	2.51		4.0-5.1
Calculated from terminal half-life	0.66		1.7-2.8
Calculated from AUC	2.04		3.3-4.3
Actual clearance during CI		4.10	4.2-5.6

TABLE 2 Course of thrombin generation

			rFIXFc CI	
	Normal control plasma	Baseline	3.81IU/hr/kg (POD7)	1.9IU/hr/kg (POD11)
FIX:C (IU/dL)	91	14	107	85
Thrombin generation assay				
Lagtime (min)	6.19	6.64	5.86	6.86
Peak thrombin (nmol/μL)	85.02	56.22	160.82	132.58
Endogenous Thrombin Potential (nmol min/L)	1118	1383	1378	1295

PB 977 | United States Post-marketing Safety (PMS) Study of rpFVIII in Patients with Acquired Hemophilia A: Preliminary Enrollment Data

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Background: Acquired hemophilia A (AHA) is a rare autoimmune disorder characterized by development of neutralizing autoantibodies to circulating factor VIII (FVIII). Obizur is a recombinant, B-domain deleted, porcine-sequence FVIII (rpFVIII) with low cross-reactivity to anti-human-FVIII antibodies for the treatment of AHA.

Aims: Collection of data to assess safety and safety-related factors, utilization and effectiveness of rpFVIII in real-world clinical practice was committed by Shire with the United States (US) Food and Drug Administration.

Methods: This is a multi-center, non-controlled, open-label, non-interventional PMS surveillance study conducted in the US on AHA patients treated with rpFVIII. Prospective and retrospective data will be collected from 40 patients. Statistical analyses will be conducted and include specifically, but not exclusively, descriptive statistics.

Results: A preliminary, unplanned data read-out was carried out on Dec 15, 2016 on 7 subjects (4 males and 3 females, 10 bleeds) with AHA recruited in 5 centers in the US: median age 73 years (range 59-75 years). Demographics details are listed in table 1.

TABLE 1 Demographic details

AGE (years)	Mean	Median	Range
Overall	70.4	73.0	59-75
Males	67.8	68.5	59-75
Females	74.0	74.0	73-75
WEIGHT (kilograms)	Mean	Median	Range
Overall	97.0	85.1	69-159.3
Males	110.4	99.6	83-159.3
Females	79.3	83.7	69-85.1

In 1 subject, an underlying malignancy was reported. Bleed characteristics are described in table 2.

Five of 7 patients were reported to have been treated with other hemostatic drugs before being administered rpFVIII, while 2 subjects were treated with rpFVIII as first option once diagnosed for the specific bleeding event. The median loading dose was 100.2 IU/kg (range 50-203, mean of 122.3 IU/kg).

Conclusions: rpFVIII represents an innovative treatment for AHA subjects and this study, along with the PMS study just started in Europe, will provide real world clinical data on its safety and efficacy profile.

TABLE 2 Bleed characteristics

Patient details (Sex, age, body weight)	Description / severity of initial bleeding event	Location of initial bleeding event	Specific anatomical location of initial bleeding event
Male, 64 yrs, 104.3 kg	Traumatic, severe	Skin	_
Male, 59 yrs, 159.3 kg	Traumatic, severe	Deep (musculoskeletal, retroperitoneal)	Retroperitoneal bleed and hematoma on thigh
Female, 74 yrs, 69 kg	Traumatic, severe	Deep (musculoskeletal, retroperitoneal)	Left pectoralis major and minor muscles
Male, 75 yrs, 94.9 kg	Spontaneous, not severe	Mucosa	Hematuria
Male, 73 yrs, 83 kg	Spontaneous, severe	Deep (musculoskeletal, retroperitoneal)	Neck
Female, 73 yrs, 83.7 kg	Spontaneous, not severe	Other	Right lower extremity from toes to gluteal region, right upper arm
Female, 75 yrs, 85.1 kg	Spontaneous, severe	Deep (musculoskeletal, retroperitoneal)	Left forearm-flexor digitorum profundus muscle and flexor pollicis brevis muscle

Data from real world use of rpFVIII might help with further assessment of dosing regimen and guidance regarding appropriate dosing in order to personalize the treatment for each individual condition.

PB 978 | Initial Safety Results from a Prospective Post-marketing Surveillance Study Using rFVIIIFc in the Real World Setting in Japanese Hemophilia A Patients

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Background: Recombinant factor VIII Fc fusion protein (rFVIIIFc), manufactured in a human cell line, was developed to extend the half-life of FVIII. Safety and efficacy of rFVIIIFc in previously treated adults, adolescents, and children with hemophilia A have been demonstrated in the Phase 3 A-LONG and Kids A-LONG studies. rFVIIIFc has been marketed in Japan since March 2015.

Aims: To report initial safety data in subjects in the real world setting from the rFVIIIFc post-marketing surveillance study (PMS) that was initiated as a regulatory requirement in Japan.

Methods: This prospective, multicenter, observational study evaluated safety and efficacy of rFVIIIFc in hemophilia A patients of any age and any disease severity. Data are collected every 6 months using the electronic data-capture system.

Results: Of 123 patients enrolled at 52 sites, 83 signed informed consent for publication and 70 received rFVIIIFc as of 31 Oct 2016. Mean age was 28 years (range: < 1-65). 12 patients were < 6 years; 6 were 6-< 12 years; 11 were 12-< 18 years; and 38 were ≥18 years of age (age not provided for 16 patients). At study entry, 60 patients had >100 exposure days (EDs), 6 patients had 4-99 EDs, and 3 patients had 0-3 EDs. Of the 3 previously-untreated patients, 2 were aged < 1 year and 1 was 2 years. ED data were unavailable for 14 patients. A total of 49 patients had severe (< 1%) hemophilia. Among them, 32 had prophylactic dosing frequency data for prestudy non-rFVIIIFc products and on-study rFVIIIFc. In the patients with severe hemophilia who were dosed ≥3 times per week prestudy, prophylactic dosing frequency was reduced to < 3 times per week (Table 1) with rFVIIIFc. 19 patients have completed 12 months on-study. As of 31 Oct 2016, no inhibitor development, serious allergic reactions, or serious vascular thrombotic events were reported in 70 patients who received rFVIIIFc.

Conclusions: These data support a favorable safety profile for an extended half-life product, rFVIIIFc, in the real world in Japanese hemophilia A patients.

TABLE 1 Dosing Frequency of Eloctate* by Hemophilia Severity**

On study prophylaxis regimen	Severe (<1%) n=32	Moderate (1-5%) n=11	Mild (5-40%) n=6
Every 3 days	8 (25%)	2 (18%)	0
Twice weekly	15 (47%)	4 (36%)	1 (17%)
Every 5 days	2 (6%)	0	0
Weekly	7 (22%)	3 (27%)	5 (83%)
Others	0	2 (18%)	0

*At onset of the study. **Hemophilia severity was not available for 5 patients: 4 were treated twice weekly and 1 was weekly.

PB 979 | Factor Utilization and Costs in Patients with Hemophilia B Using Standard and Extended Half-life Recombinant Factor IX Replacement Products: Real-world Analysis in Japan

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Background: Management of hemophilia B requires replacement therapy with factor IX (FIX) coagulation products. Extended half-life

TABLE 1 Analysis of FIX Concentrate IU Utilization Before and After Switching From an SHL to EHL Product (MDV database) - MEDIAN VALUES

	Months Pre-switch				SWITCH	Months Post-switch			
Median Units	10-12 (n=7)	7-9 (n=7)	4-6 (n=8)	1-3 (n=10)		1-3 (n=10)	4-6 (n=6)	7-9 (n=4)	10-12 (n=4)
IU Utilization	40,000	30,000	38,000	31,000		25,500	20,500	27,500	28,500
Factor Costs	¥ 4,122,720	¥ 3,191,610	¥ 4,018,724	¥ 3,296,830		¥ 5,281,332	¥ 4,246,219	¥ 5,690,430	¥ 5,885,545
Total Health care costs*	¥ 4,154,735	¥ 3,240,767	¥ 4,636,173	¥ 3,339,487		¥ 8,801,094	¥ 4,656,339	¥ 5,855,292	¥ 5,946,696

* Total health care costs were calculated for the patients who have FIX treated record. Data presented for the SHL product are specifically for nonacog alfa, and data presented for the EHL product are specifically for eftrenonacog alfa. EHL, extended half-life; SHL, standard half-life.

(EHL) recombinant FIX products are now available along with the existing standard half-life (SHL) FIX replacement products.

Aims: Examine factor utilization and costs for patients in Japan who switched from a SHL to an EHL product in a real world setting.

Methods: Japanese real world data provided by Medical Data Vision Co., Ltd (MDV) was used to compute FIX utilization (number of IUs dispensed) and direct product costs (¥) and total health care costs (¥) among patients with claims data for ≥3 mo before and after switching from an SHL to EHL product (April 2010-October 2016). MDV is the most populated real-world database collected from medical and pharmacy claims data in hospitals, regardless of insurance, for >10 million inpatients and outpatients in Japan.

Patients filled ≥2 FIX prescriptions or 1 FIX prescription with ≥84-day supply. Medians for cost and IUs were used to accommodate for the skewness of data distribution.

Results: Ten patients switched from an SHL (Nonacog alfa) to EHL (eftrenonacog alfa) product and had ≥3 mo of data before and after the switch. Factor replacement costs were uniformly higher after the switch from SHL to EHL in each of the corresponding 3-month time periods examined before versus after the switch. Median quarterly costs were ¥4,122,720, ¥3,191,610, ¥4,018,724, ¥3,296,830 (SHL) and ¥5,281,332, ¥4,246,219, ¥5,690,430, ¥5,885,545 (EHL) while median quarterly metric IUs were 40,000, 30,000, 38,000, 31,000 (SHL) and 25,500, 20,500, 27,500, 28,500 (EHL) in the 12-10 (n=7), 9-7 (n=7), 6-4 (n=8), 3-1 (n=10) and 1-3 (n=10), 4-6 (n=6), 7-9 (n=4), 10-12 (n=4) months pre- and post-switch, respectively (Table 1).

Conclusions: This analysis shows that switching from SHL to EHL product was associated with increased and more variable FIX replacement costs. Further analyses in larger patient populations should be explored.

PB 980 | Long Term Hemostatic Efficacy of Once a Week Administration with a Combination Medicine of Plasma Derived Factor Viia and Factor X for a Hemophilia B Patient with Inhibitor

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Background: Case - The patient is a man in his thirties. The closer examinations for repetitive bleedings revealed to diagnose as severe type of hemophilia B at 1-year-old. Since then he had been receiving episodic treatments for his bleedings with several kinds of FIX products, however those treatments were interrupted by allergic/anaphylactic reactions. When he was nineteen, an antibody to FIX initially developed and further reached to 62 BU with a nephrotic syndrome three years later after inhibitor developed. Clinical course: A large amount of rFVIIa has been used for his hemostatic control because of his high frequency of bleeding, therefore once a week administration with Byclot® (Kaketsuken, Japan) as a new bypass agent developed in Japan, which contains plasma derived FVIIa combined with plasma derived FX (pdFVIIa/FX) has been introduced in his routine life.

Aims: Bypass agents are useful and indispensable for hemostatic control for prophylaxis as well as acute bleedings in hemophilic patients with inhibitor. We evaluated the long term hemostatic efficacy in a hemophilia B patient with inhibitor by once a week administration with Byclot®.

Methods: Both the frequency of bleedings and the infusion times were compared at each end of scale between on-demand with rFVIIa and once a week administration with Byclot®.

Results: Mean monthly bleeding episodes and infusion times during their observational periods were 3.8 and 19.3 in on-demand with rFVIIa, and 1.2 and 3.9 in once a week administration with Byclot®, respectively. No thrombotic adverse events were observed in two years with Byclot®.

Conclusions: Both bleeding episodes and infusion times decreased by 68.4% and 79.8% respectively in Byclot®. The ABR also reduced from 45 to 14.5 after introducing once a week administration with Byclot®. Our experiences suggested that once a week administration with Byclot® might be a new option as a regular treatment in hemophilia patients with inhibitor.

PB 981 | Economic Impact of Recombinant FVIII (rFVIII) vs Plasma-derived FVIII with von Willebrand Factor (pdFVIII/VWF) and Low-dose vs High-dose Immune Tolerance Induction in Previously Untreated Hemophilia A Patients (PUPs) in Germany

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Background: Inhibitor development to FVIII therapy results in increased complications and economic burden. The SIPPET study found higher inhibitor rates in previously untreated patients (PUPs) treated with conventional rFVIII than with pdFVIII/VWF.

Aims: To quantify the potential economic impact of treating PUPs with pdFVIII/VWF vs rFVIII and eradicating inhibitors with low-dose (LD) vs high-dose (HD) immune tolerance induction (ITI).

Methods: An Excel-based clinical and economic model was run from a German healthcare payer perspective over a 5-year period. One-year-old PUPs initiated prophylactic or on-demand therapy with rFVIII or pdFVIII/VWF. High-titer inhibitor development rates were obtained from the SIPPET study (28.4% for rFVIII and 18.6% for pdFVIII/VWF). PUPs developing inhibitors received LD or HD ITI and bypass agents. Patients successfully tolerized with ITI returned to FVIII treatment, while unsuccessful patients received bypass-agent prophylaxis. Treatment regimens, ITI outcomes, and rates of serious bleeds were based on the literature and expert opinion. All cost inputs were identical between the 2 arms, except for the costs of the 2 antihemophilic agents. Treatment costs were calculated monthly based on patient weight while total costs were over 5 years.

Results: Total 5-year per-patient treatment costs for pdFVIII/VWF were €448,356 for LD and €612,481 for HD ITI while rFVIII resulted in €691,639 for LD and €1,090,694 for HD ITI, saving over €243,000 for LD and €478,000 for high-dose ITI. FVIII costs for patients without high-titer inhibitors were 26% lower for pdFVIII/VWF than for rFVIII. Costs for patients with high-titer inhibitors receiving low-dose ITI were 44% lower for pdFVIII/VWF than rFVIII, and costs for patients with high-titer inhibitors receiving high-dose ITI were 52% lower for pdFVIII/VWF than rFVIII.

Conclusions: Treatment of hemophilia A PUPs with pdFVIII/VWF has the potential to result in significant cost-savings compared to rFVIII, particularly when high-dose ITI is utilized.

PB 982 | Low Dose Long Acting CFC (Eloctate, Biogen) Replacement during Bilateral Total Knee Replacement Surgery (TKA) for Hemophilia A - Feasible and Extremely Cost Effective Approach

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Background: Knee arthropathy is crippling situation affecting QOL for PWH. TKA is performed using Total 2000iu/kg of PD or Rec CFC. Literature recommends 1000-1200iu/kg of Total Eloctate for TKA.

Aims: Aim for Feasible, safe & Cost Effective CFC replacement policy for TKA

1) Use lower dose Eloctate during TKA

2) Perform Bilateral TKA in same sitting.

Methods: Data:

1) Total Patients: 6

2) Age: 44 years (28-51years)

3) Pre OP dose: 50iu/kg

4) Pre OP FVIIIc Level: 104 iu/dl (86-126iu/dl)

5) Post OP Eloctate doses: 40-50iu/kg once daily till day 5, then 30iu/kg once daily till Day15.

6) FVIIIc assay:Twice daily for first 3days, Once daily till day7, Alternate day till day 14.

7) Trough FVIIIc level > 60iu/dl for 5 days

8) LMWH Thrombo-prophylaxis & CPM was started from Day 2 post OP.

Results: Total 10 TKA performed on 5 patients where all underwent Bilateral TKA in same sitting.One patient underwent Pseudo tumor excision and TKA in the same sitting,which required extra 50iu/kg dose of Eloctate+ 8PCV+8 FFP + 10 Cryoprecipitate intra OP because of massive bleeding during excision of Pseudo Tumor;following which there was no further bleeding till the day of discharge. No excessive bleeding recorded among rest 5 patients in Peri & Post Op period & did not require any blood products or extra doses of Eloctate till discharge.All patients mobilization was started on day 2. All patients received LMWH prophylaxis with 0.4ml Enoxiparin sc once daily till discharge.No wound or prosthesis infection was noted.

Toatal Eloctate used: Median 550 iu/kg.

Conclusions:

1) Low dose Eloctate replacement is safe and cost effective for TKA in PWH

2) Bilateral TKA in same sitting is feasible and increases cost effectiveness in PWH

3) Thrombo-prophylaxis with LMWH can be done safely in PWH

4) Bleeding events did not increase with low dose Eloctate & LMWH

5) Our protocol offers a cost effective and safe option in resource constraint countries.

PB 983 | Improving Care in Haemophilia: A Qualitative Study

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Background: The availability of coagulation factor replacement therapy in industrialized countries has dramatically increased survival of patients with haemophilia. In order to improve patient-centered care

for people with haemophilia, it is important to understand the patients' perspectives of their outcomes and treatments.

Aims: The purpose of this study was to understand the perceived impact of hemophilia and its treatment on patients' quality of life and their health care needs, including information provision and shared decision-making.

Methods: Semi-structured interviews were conducted with 13 patients with different severities of haemophilia A or B at the Adult Haemophilia Program of British Columbia, Canada. Interviews were transcribed verbatim and analysed qualitatively. A second phase of data collection is being planned.

Results: Preliminary results indicate that haemophilia impacts daily activities as well as decisions about education and employment. Care needs do not only pertain to treatment of haemophilia and its associated co-morbidities HIV and hepatitis C, but also to interactions with non-haemophilia care providers. Finally, the clinic's approach to patient engagement through visualization of bleed history and factor use, and information sharing about future treatment options were perceived helpful in addressing additional patient needs.

Conclusions: In addition to addressing clinical needs in haemophilia treatment, there is an opportunity for clinicians to improve patient-centered care, for example by sharing clinical information in a variety of ways, including graphic representations, to facilitate shared decision-making. The results will inform ongoing quality of care and quality of life studies in patients with haemophilia in British Columbia, Canada and in the Dutch Haemophilia in the Netherlands (HiN) study.

PB 984 | Improving the Survival for Life-threatening Hemorrhage with Hemophilia Patient in One Hemophilia Treatment Center for 34 Years

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Background: In life threatening hemorrhage such as brain and abdomen, several important factors are affect for improving the survival. One tenth (223) of hemophilia patients in Korea lived in Daegu city and Kyungpook province and have treated in our regional treatment center.

Aims: We reviewed the result of life threatening hemorrhage and our unique care of hemophilia patients for 34 years.

Methods: Korea Hemophilia Foundation was established in 1991. After that all factor concentrates were free to all hemophilia patients. Home treatment are available for rapid administration of factor concentrate of full required amount. Rapid transportation to emergency room are available for immediate operation. Hot line of mobile phone between patient and doctor for 24 hours are available for emergency care. Monthly group education has done. Prophylactic treatment was started to all who had a life threatening hemorrhage history in our hospital since 1996. But HIRA permitted officially since 2011.

And then recovery rate test was done for the optimal blood level for life threatening hemorrhage patient. Continuous infusion with every 2 to 4 hours reconstitution dilution fluid has been done for preserve in vitro factor activity to all surgery cases.

Results: Thirty five events were intracranial hemorrhage in 17, general surgery in 9 and orthopedic surgery in 9. Age distribution was 0-32 yr (mean; 24.8 yr). Severity was severe (16), moderate (7) and mild (5). Time interval between first symptom and arrival at ER were 15 min to 10 days (mean; 1.7days). We confirmed in vivo factor activity within permissible level in all patients. All recovered from hemorrhage or surgery and are healthy, but one had limping gate and one had mild neurologic sequela for more than 10 years follow-up period.

Conclusions: Education, financial support, home and prophylactic treatment, hot-line, individual pharmacokinetics with effective blood level and fresh concentrate during continuous infusion are important factors to improve the survival of surgery case.

PB 985 | Single Centre Experience of Obstetric Management of Women with Inherited Bleeding Disorders (IBD) in a Multidisciplinary Joint Women's Bleeding Disorders / Obstetric Clinic

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Background: The inception of a Multidisciplinary Bleeding Disorders/Obstetric clinic has led to optimal management of pregnant women with IBD as they are known to be at increased risk of bleeding complications in both mother and fetus.

Aims: To report on outcomes of 142 pregnancies in 97 consecutive women with IBD who were seen in the last 5 years in our Centre, which is a tertiary referral centre.

Methods: Retrospective analysis was conducted going through obstetric and hospital records of patients who were seen in the last 5 years with special attention to bleeding complications, clotting factor use, maternal and fetal outcomes. Details of any previous pregnancies were included for women who presented during this period.

Results: Results are presented in the table below. The types of IBD included carriers of haemophilia A and B, VWD, FXI deficiency, platelet function defects and rare bleeding disorders. The last group included fibrinogen disorders, FVII deficiency, FV deficiency, FX deficiency, familial macro-thrombocytopenia and unclassified bleeding disorders. Antepartum haemorrhage (APH) was seen in 5 pregnancies and of these, 3 cases required clotting factor replacement (CFR) - one Haemophilia A carrier woman, one patient with type 2 VWD and one patient with type 3 VWD. Of note, these two patients with VWD had a subsequent pregnancy each and were managed with upfront CFR (three times a week) during these without any bleeding complications.

TABLE 1 Bleeding Complications by Diagnostic Category

Diagnosis	No. of patients	No. of pregnancies	No. of pregnancies requiring Blood Products	No. of pregnancies with Antepartum haemorrhage	No of pregnancies with Postpartum haemorrhage
Haemophilia A	27	36	3	2	2
Haemophilia B	7	11	3	0	1
VWD Type 1	18	26	2	0	3
VWD Type 2	6	10	7	1	0
VWD Type 3	2	6	6	1	3
F XI deficiency	12	18	12	0	2
Platelet function defect	12	13	2	1	1
Rare Bleeding Disorders	13	22	6	0	7

In 6 pregnancies, late amniocentecis was undertaken to inform delivery plans. Significant post-partum haemorrhage was seen in 19 of 142 pregnancies with blood transfusion being required in 5 deliveries.
Conclusions: Overall, the maternal and foetal outcomes were excellent in this high risk patient population. The rate of maternal complications was 3.5% for APH and 19% for PPH. No fetal complications were noted. Obstetric management of women with IBD can be optimised using a multidisciplinary team including haematologists, obstetricians and anaesthetists.

PB 986 | At Home PK to Tailor Prophylaxis

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Background: PK tailored prophylaxis seems to be the best way of treatment to individualize replacement haemophilia therapy and improve cost/effectiveness. Unfortunately, single dose PK is a quite demanding procedure both for patients, especially children and their parents. Patients and/or parents are generally disappointed for losing four to five consecutive days of school or work.
Aims: To facilitate the compliance of patients to undergo PK, we designed together with Kedrion (Castelvecchio Pascoli, Lucca, Italy) a home PK program NuPreviq service.
Methods: This service has been offered to seven severe haemophilia A patients under treatment with Simoctocog alfa. The number of blood samples has been limited to six: A) at HC: baseline sample, just before infusion and 1st post-infusion hour. B) at patient's home: at 7th-9th, 24th, 48th, 72nd post-infusion hour. All blood samples, performed by a professional nurse, were submitted to high speed centrifugation just after their collection. Four aliquots (0.5 ml each) of platelet poor plasma were frozen immediately in dry ice and then shipped to a central laboratory for FVIII:C assay.
Results: y)FVIII PK was performed by means of One-compartment (1CP) and Two-Compartment (2CP) method (Accovion GmbH, Eschborn, Germany). PK parameters were in the following range: IVR 0.69-2.02 IU/dL/IU/kg; Half-life 14.17-24.51 hrs; Volume of distribution 46.30-131.40 mL/kg. The parameters from the model best fitting the patient's data (1CP in 3 and 2CP in 4 patients) were used to build an array of doses and every 12 hrs intervals according to 1, 3, 4 or 5 IU/dL troughs, as asked by the treater. The range of estimated doses was 18.30-81.70 IU/kg and the interval between bolus was 48-120 hrs.
Conclusions: The accessibility of this home PK service improved the compliance of patients with this procedure providing their treaters with simulated dosing schemes, according to different troughs.

PB 987 | Bayesian Approach to Individualize Prophylaxis of Simoctocog Alfa in Haemophilia

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Background: Prophylaxis with Factor VIII (FVIII) can reduce annual bleeding events in severe haemophilia A patients. However, inter-individual pharmacokinetic (PK) variability of FVIII is large and PK-tailored personalized prophylaxis may help to adjust FVIII activity at the level desired.
Aims: The aims of this study were to develop a Bayesian estimator of simoctocog alfa using a PK population approach and to evaluate its capacity to estimate individual pharmacokinetic parameters using sparse blood samples.
Methods: Data from 3 clinical trials with simoctocog alfa in 86 adults and 61 children/adolescents were compiled. A total of 1114 samples were collected and FVIII was measured centrally. The data analysis workflow consisted of three steps i) to develop a PK population model using non-linear mixed effect models; ii) to develop a Bayesian estimator from the previous estimates; iii) to evaluate the predictive value of Bayesian approach to estimate individual parameters using a limited sampling strategy. FVIII was then measured locally and the values were used centrally to run the predictive model.
Results: Individual PK parameters were estimated with only 2 or 3 blood samples per individual after simoctocog alfa injection, given the prior distribution of the molecule in the population. The results were provided to the haemophilia treaters and their impact on therapeutic adjustment was evaluated.

Conclusions: Pharmacokinetics of FVIII has mostly been empirical so far, with a combination of outcome measures such as clinical bleeding phenotype (ABR) and determination of trough factor VIII activity. The use of individual pharmacokinetics has likely been restricted by the need to collect several samples following FVIII injection. Prophylactic schedule can be adjusted from only a few determinations of drug plasma levels, by means of a population approach and Bayesian analysis. This approach was promoted at the country level in order to help clinicians individualizing treatment.

PB 988 | Sports/Recreational Activity-specific Risks and Drivers of Risk in People with Hemophilia: Results of the Activity-intensity-Risk (AIR) Consensus Meeting of US Physical Therapists

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Background: Limited evidence supports assessment of activity-associated risks for people with hemophilia (PWH), and general cautions provided in consumer materials generically describe types of risk.

Aims: To assess activity-specific risks in PWH and define inherent and modifiable drivers of increased risk based on consensus of physical therapy (PT) experts.

Methods: Peer-nominated PTs in the US hemophilia treatment center (HTC) network were surveyed on specific risks for injury (6 at-risk joints and bruising, muscle, and head injuries) associated with ~100 sports and other physically intensive activities, including specific positions (eg, baseball pitcher, catcher, field positions). Drivers of risk were identified from free text comments and explored at a consensus meeting. Drivers were categorized as inherent (I), modifiable (M), activity-driven (A), and patient-driven (P).

Results: Of 32 invited PTs, 17 responded to the survey with median (mean) 26.5 (22.4) years as a PT and 15.5 (16.8) years at an HTC; 8 participated in the full-day consensus meeting. Most respondents indicated ≥1 specific risk for each activity, with specific risks identified being largely consistent with free text comments (sample data in **Table** and **Figure**). Key drivers of risk that were identified included progression from recreational participation to year-round competitive play, overtraining, competitive level, tournaments, and improper body mechanics. Inherent risks identified included impact with surface/ball/equipment, players, or falls. Modifiable risks included tricks/stunts and use of safety equipment when not required.

Conclusions: Consensus of experienced US PTs provides insights into activity-specific risks for PWH and drivers of risk that contribute to the range of risk identified by the AIR project. HTC discussions with patients/families concerning participation in activities should include specific consideration of sustainability of participation and patient-driven modifiable risks.

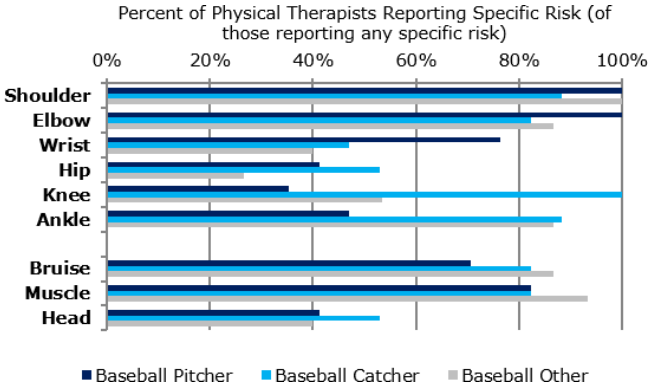

FIGURE 1 Percent of Physical Therapists Reporting Specific Risks for Injury Associated With Baseball (Hardball) Positions (N=17)

TABLE 1 Percent of Physical Therapists Reporting Specific Risks for Injury on Survey and Drivers of Risk Assessed via Consensus Meeting

Activity (number of respondents scoring each activity)	Elbow	Knee	Ankle	Muscle	Head	Overuse/ Over-train	Repetitive Motion	Competitive	Impact - Surface or Ball
Bicycling (n=12)	42%	92%	58%	100%	92%	MP	IP	IA	
Baseball (hardball) - pitcher (n=17)	100%	35%	47%	82%	41%	MP	IA	MP	IA
Baseball (hardball) - catcher (n=17)	82%	100%	88%	82%	53%	IA	IA	MP	IA
Basketball (n=17)	47%	88%	100%	82%	29%	MP	MP	MP	IA
Football (tackle) (n=17)	82%	100%	100%	88%	100%	MA	IA	IA	IA
Ice hockey (n=17)	65%	82%	71%	82%	100%	MP	IA	MP	IA
Soccer - goalie (n=16)	56%	88%	94%	94%	94%	MP		IA	IA
Skateboarding (n=17)	65%	76%	88%	71%	100%		IA		MA
Swimming (n=16)	25%	44%	31%	75%	0%	MP	MP	IA	MA

PB 989 | Preferences of Hemophilic Patients and their Parents on the Characteristics of Replacement Treatment Used in Prophylaxis

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Background: New modified coagulation factors concentrates (e.g. extended half life) are coming into the market, generating expectancies and concerns on which treatment is preferable for patients with hemophilia (PwH).

Aims: Evaluating preferences of PwH and their parents towards the characteristics of prophylactic treatment with different coagulation factor concentrates.

Methods: A discrete choice experiment was conducted. Patients with severe hemophilia without inhibitors aged ≥13 years and parents of those aged ≤17 years were enrolled. Possible options of prophylactic treatment were described with 7 characteristics previously selected for their relevance, including 2-3 possible levels each (Table 1). A fractional factorial combination of every characteristic levels generated 16 scenarios including each a pair of possible treatments. The participants were asked to choose one option for each scenario (Figure 1). The relative importance assigned to each characteristic over the others was estimated with a conditional logistic regression model.

Results: 66 patients (59% haemophilia A) and 32 parents participated. Risk of infections and of inhibitor development were the most important for patients (29% and 17%, respectively) and parents (27% and 33%). The third more important characteristic for patients was frequency of injections (17%), corresponding to the fourth more important for the parents (11%), while the third more important characteristic for parents was time of preparation (8%), followed by additional costs. As regards "Frequency of administration", while parents did not assign a statistically significant preference to any level specified, patients preferred significantly more using a product requiring "1 infusion every 15 days" rather than a higher infusion frequency.

Conclusions: Decisions on treatments must take into account patients clinical needs; the results of this study reveal different preferences between adult PwH and parents of pediatric patients.

TABLE 1 selected characteristics and levels to describe the treatment options

	CHARACTERISTICS	LEVELS
1	Frequency of administration	- 2 infusions per week - 1 infusion per week - 1 infusion every 15 days
2	Risk of infections transmitted by the product	-Increased -Reduced
3	Risk of inhibitors development after treatment	-Increased -Reduced
4	Time necessary to pain relief	- 1 hour - 4 hours
5	Number of injections necessary to solve the symptoms of bleeding	- One - More than one
6	Time of preparation	- 5 minutes - 20 minutes
7	Additional cost	- None (The healtcare taxes remain the same) - The healtcare taxes are doubled (130€ more to be paid monthly on gross income of 1.700€) - The healtcare taxes are tripled (260€ more to be paid monthly on gross income of 1.700€)

PB 990 | Evaluation of Unmet Medical Needs of Hemophilia Patients in Bavaria

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Background: Hemophilia care in Germany has achieved a high level. Generally, most hemophilia patients are able to lead a largely normal life. The "Bluter Betreuung Bayern e.V." (BBB), which was originally founded to help HIV-infected hemophilia patients, aims to improve patient support.

Aims: The aim of this study was to evaluate unmet medical needs of hemophilia patients in Bavaria from a patient perspective.

Methods: A questionnaire of 45 items has been designed by BBB representatives. Questions comprised three parts: 1. Demographics 2. General health, substitution therapy, worries in relation to hemophilia, satisfaction with medical care. 3. Wishes related to extra service and information offers, suggestions how to improve hemophilia care. The survey was sent to 290 hemophilia patients and/or their parents in Bavaria in November 2015.

Results: The response rate was 51.4%. For demographics see Table 1.

TABLE 1 Demographics of 146 patients with hemophilia

Age groups [years], number of patients (n)	<15 (66)	15 - 24 (30)	25 - 44 (26)	>44 (24)
Severity of hemophilia n (%)				
Severe	44 (66.7)	20 (66.7)	21 (80.8)	23 (95.8)
Moderate	6 (9.0)	1 (3.3)	1 (3.8)	0 (0)
Mild	13 (19.7)	8 (26.7)	3 (11.5)	0 (0)
Prophylaxis in patients with severe hemophilia n (%)				
"Always"	43 (97.7)	16 (80.0)	14 (66.7)	14 (60.9)
"Often"	0 (0)	3 (15.0)	6 (28.6)	7 (30.4)
"Sometimes"	1 (2.3)	1 (5.0)	1 (4.8)	1 (4.35)
"Seldom" or "Never"	0 (0)	0 (0)	0 (0)	1 (4.35)

Substitution therapy was mostly uncomplicated. More than 80% up to 100% of patients in all age groups reported that the injections were "never" or "seldom" problematic and "never" or "seldom" painful. Satisfaction with medical care was high. "Chronic pain due to hemophilia" increases in patients >24 years old. Patients in the age group 25-44 years worried least regarding future health, safety and availability of factor products, patients aged >44 years most. Overall, 80-100% of patients from all age groups were interested to be informed on the current state of science, 60-72% on topics with regard to social legislation, and 49-74% wished additional information offers from the treating physician.

Conclusions: The survey confirmed the high level of German health care for patients with hemophilia. Most patients wished more information by their treating physicians. Worries of elderly hemophilia patients and chronic pain should be addressed. Offers of the BBB for psychosocial support in addition to the medical care seem to be helpful and needed in all age groups.

This study was sponsored by the Rudolf-Marx-Foundation.

PB 991 | The Occurence of Inhibitors in PUPs Treated with Octocog α in Polish Haemophilia Care Centers

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Background: The most challenging complication of haemophilia replacement therapy (HRT) is the occurrence of neutralizing anti-factor VIII alloantibodies (inhibitors - INH) in up to 30% of previously untreated patients (PUPs) with severe haemophilia A (HA), typically during the first 50 days of exposure (EDs) to therapeutic factor VIII (FVIII) infusion. Recently published data showed higher incidence of inhibitors in patients treated with recombinant FVIII than those treated with plasma-derived FVIII (44.5 vs 26.8% respectively).

Aims: Analysis of the INH incidence and efficacy of ITI in PUPs treated with full lengh recombinant FVIII octocog[α in Poland between 2011-2016.

Methods: From 2011 to 2016 in all Polish Haemophilia Care Centers 88 PUPs with severe haemophila A were receiving replacement therapy with octocog α for 2 - 75 months (median 34.6). HRT was initiated[at the age of 0 to 34,5 months (median 10.7). Only 6/88 boys (6.7%) were treated on-demand. Prophylaxis was started at the median age 12.6 months (2.3 - 39,5).

Results: INH was diagnosed in 14/88 (15.9%) cases after 3 - 489 EDs (median 20). Most of them (11/14) were high responders with the peak inhibitor titer (PIT) 5,4 - 716.8 (median 20.1) BU/ml. All of 3 low responders had PIT 2.8 BU/ml. Treatment-related risk factors for inhibitor development were presented in table 1.

Conclusions:

The incidence of inhibitors in PUPs treated with octocog α is lover then reported in patients receiving other forms recombinant factor VIII.

PB 992 | The Impact of a Comprehensive Hemophilia Management Programme in Vulnerable Population

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Background: This project is an observational study which describes the results of a comprehensive management program designed for prophylaxis in hemophilic patients.

TABLE 1 Treatment related risk factors in patients with inhibitors (P - prophylaxis; OD - on demand Me - median

	Peak inhibitor titer BU/ml range / Me	Age at 1st dose of FVIII [months] range / Me	Age at start of prophylaxis [months] range / Me	Number of EDs range / Me	Type of treatment	Surgery	High doses of FVIII due to bleeding
Low responders n = 3	2.8	0 - 11.9 Me = 11.7	11.9 - 22.8 Me = 12.6	6 - 28 Me = 10	P - 3	1	0
High responders n = 11	5,4 - 716.8 Me = 20,1	0 - 32.9 Me = 11.2	0 - 12.2 Me = 11.5	3 - 489 Me = 15	P - 6 OD - 5	4	3 (CNS, GI, massive to scrotum

Aims: Following the gold standard for the treatment of hemophilia, the aim of this study was to assess the impact of a comprehensive prophylactic programme in vulnerable population. The programme guaranteed ambulatory and home care by qualified interdisciplinary professionals. The number of spontaneous bleeding episodes, waiting times for treatment, the number of hospital admissions and the amount of clotting factor used at home to control bleeding, were used to determine the effectiveness of the programme compared to the regular on-demand treatment.

Methods: A sample of 28 patients was included in the program at 11 Integral Solutions Hemophilic centers over a period of 2 years. Retrospective data were obtained through a survey administered at recruitment and participants were followed up while their inclusion in the program. Patients were treated three times a week. The frequency and doses were adjusted according to weight, phenotype and PK.

Results: The mean follow-up time was 1,04 years. On average, each patient received 25,6 UI/Kg per dose (range 16,66 - 35,7 UI/Kg). The average spontaneous bleeding person per year was of 4.71 before their inclusion in the program compared to 0.96 with the program. This represents a reduction of 79.6% in the spontaneous bleeding. While in the program, only a 3% of additional clotting factor was required to control bleeding at home. The average waiting time for treatment within the program was less than 1 hour in 82% of the sample and 1-3 hours in 18%, with no hospital admissions per spontaneous bleeding.

Conclusions: The comprehensive prophylactic program, through the development of individualized protocols to administer the clotting factor at home, increased adherence to treatment and significantly reduced the number of spontaneous bleedings and hospital admissions in patients with hemophilia from vulnerable populations.

PB 993 | The Impact of Long-term Secondary Prophylaxis on Health-related Quality of Life in Adolescents and Adults with Severe Haemophilia A treated with rFVIII-FS

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Background: Benefits of secondary prophylaxis on long-term outcomes in adolescents and adults with severe haemophilia A are scarce. In the context of the Italian POTTER Study (Prophylaxis vs. On-demand Therapy Through Economic Report) the impact of secondary prophylaxis has been evaluated not only on clinical outcomes, but also on the patient perspective.

Aims: To determine the impact of secondary prophylaxis on HRQoL in haemophilia A patients.

Methods: 58 patients from 11 Italian HTC were enrolled and stratified into 2 age subgroups (12-25 and 26-55 years). 53 [27 prophylaxis (P), 26 on demand (OD)] were evaluated with a median follow-up period of 5.4 years. HRQoL was evaluated by generic instruments (SF-36, EQ-5D) and haemophilia-specific instruments (adolescents: Haemo-QoL; adults: Haemo-QoL-A. Data were collected at baseline and at follow-up visits.

Results: According to treatment arms, adolescents had an average age of (P: 16.63 ± 3.76 years, OD: 18.08 ± 5.75 years) and adults of (P: 31.08 ± 3.93 years; OD: 36.87 ± 7.53). Patients on prophylaxis had significantly less joint bleeds compared to on-demand patients (adolescents ABR: 1.97 vs. 16.80; adults: 2.46 vs. 16.71; p< .0043). Differences between on-demand and prophylaxis patients at baseline were significant in the domains ‚physical functioning' (p< .025), ‚role physical' (p< .026), ‚social functioning' (p< .025), ‚role emotional' (p< .032) of the SF-36 and the EQ-VAS (p< .01); moreover in the domains ‚physical functioning' (p< .02), ‚role functioning' (p< .026), ‚worry' (p< .004), ‚consequence of bleeding' (p< .017) and the Total Score (p< .040) of the Haemo-QoL-A, being similar at follow-up visits. Number of bleeds had an impact on patients' HRQoL.

Conclusions: The POTTER study is the first long-term prospective, controlled trial documenting long-term benefits of late secondary prophylaxis in adolescents and adults with severe haemophilia A. Beside clinical benefits patients reported significant improvements also in their HRQoL.

PB 994 | FEIBA Global Outcome Study (FEIBA GO) First Data Read-Out: Real-world Bleeding Frequency in Patients with Inhibitors on Prophylaxis with APCC

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Background: The FEIBA GO study was designed to capture long-term outcomes on effectiveness, safety and quality of life in subjects treated with APCC in routine clinical practice.

Aims: The primary objective is to describe the hemostatic effectiveness of APCC in different settings (prophylaxis and on demand, including patients on immune-tolerance induction); most relevant secondary objectives are: joint functionality outcomes, safety, health-related quality of life (HR-QoL), daily activity level, acute and chronic pain associated with haemophilia, health resources used.

TABLE 1 Patients analysed in the data read-out

Patients analysed in the data read-out	18	
Median Annualised Bleeding Rate (ABR)	3.7	
Subjects with ABR "0"		16.7%
Subjects with ABR "<2"		22.3%
Subjects with ABR "<3"		39.0%
Median Annualised Joint Bleeding Rate (AJBR)	1.6	
Subjects with AJBR "0"		38.9%
Subjects with AJBR "<2"		55.6%
Subjects with AJBR "<3"		77.8%

Methods: It is a prospective, non-interventional, observational multicenter cohort study in patients with hemophilia A or B and high-responding inhibitors treated with APCC prior the decision to enroll in the study. Target for enrollment is 100 subjects. Treatment regimens are at the discretion of the attending physicians according to routine clinical practice, either in prophylaxis or on demand, including immune-tolerance induction. The observation period per subject will be 4 years.

Results: An initial, unplanned data read-out was carried out on Sept 15, 2016 on 28 subjects with severe haemophilia A and inhibitors (median titer at screening 10 BU, min-max 1-2,410), recruited in 14 haemophilia centres in 8 countries: median age 23 years (range 3-71). 21 of them were on prophylaxis and data were available for 18 and showed in Table 1.

Conclusions: These preliminary findings show that prophylaxis with APCC in inhibitor patients can be effective, as it demonstrably prevents joint bleeding in a proportion of subjects similar to that reported in non-inhibitor patients on replacement prophylaxis. This study will further enhance the knowledge of long-term prophylaxis in the setting of real world data by assessing effectiveness, HR-QoL and safety of APCC in this rare patient population. Data read-out information will be consolidated and expanded on for the other study endpoints in an interim analysis.

PB 996 | Prevalence of Adult-onset Medical Comorbidities and Active Intervention via Web-based Monitoring in Korean Hemophliacs

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Background: With easier access to the coagulation concentrates hemophiliacs the life expectancy of hemophilic patients is approaching to the general population and the prevalence of age-related medical comorbidities is expected to rise. We have followed 136 patients with hemophilia A and B, and monitored closely for their medical comorbidities.

Aims: In this 136 hemophilic cohort we analyzed their age-related comorbidities for overall prevalence, and applied web-based monitoring of their BPs, blood sugars for tighter control.

Methods: Comorbidities monitored were hypertension, obesity, hyperlipidemia, diabetes, and cardiovascular disorder. The medical interventions were web-based monitoring of self-checked BP and blood sugar level of at least once a week, entering data online by the pt and monitored at the clinic by the nurse and physician, and monthly clinic visit for medical intervention.

Results: Of 136 patients, 112 were hemophilia A (82%) and 24 hemophilia B (18%). Their median age was 33 years with range from 3-70 years, 46 (37%) were over age 40. Their average follow-up was 5 years and their average annual factor usage was 5,000units/kg/year/pt. Of the medical comorbidities, hypertension was observed in 15 of 136 (11%) hemophilic pts, obesity in 31 (23%), hyperlipidemia in 22 (16%), diabetes in 12 (9%). Medical comorbidities tended to be higher in older group over 40 years. Web-based monitoring were done in 5 of 15 HBP pts and 3 of 12 DM pts, and compared to non-Web monitor participating pts, the overall control were excellent.

Conclusions: With improved life expectancy in hemophiliacs, the prevalence of medical comorbidities in older age is expected to rise. Therefore, in addition to the management of the hemostatic problems, careful monitoring and therapeutic intervention of their medical conditions are warranted. Active intervention of these age-related comorbidities via web-based monitoring provides excellent care to these pts ridden with life-long struggle with hemophilia-related conditions.

PB 997 | Prevalence of Inhibitors in Tunisian Hemophiliac B

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Background: Inhibitor development is the major complication encountered in hemophilia. Hemophiliac B patients developed factor IX (FIX) inhibitors with a low prevalence (1.5-3%) compared to hemophiliac A patients.

Aims: Our aim is to establish the prevalence of inhibitors in Tunisian hemophiliac B followed in AOHTC.

Methods: Data were collected from the registry from 2010 to 2016.

Results: Our AOHTC followed 46 patients with hemophilia B. 3 of them developed inhibitors. Diagnosis was performed during routinely screening with Bethesda method. The patients have respectively 8, 9 and 16 old age, with a sever phenotype. The molecular study was done for one patient and the gene defect is due to the nonsense mutation p.Glu72X. Replacement therapy with factor 9 is efficient in 2 patients despite absence of biological response.

Conclusions: Since the prevalence of inhibitors development in Tunisian hemophilia B patients is of 6.5%, it seems to be higher than the reported one in the literature. This may be due to a regular routine diagnosis, a genetic predisposition, the effectiveness of substitution or the extra-plasma distribution of F9. An immunological diagnostic should be studied. Further studies have to be done in order to explain this data.

PB 998 | Pharmacokinetics and Annual Bleeding Rate (ABR) during Prophylaxis with a Full-length Recombinant FVIII: One Center Experience

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Background: It is suggested that knowledge of factor half-life, in addition to observation of the bleeding pattern, may help tailor prophylaxis to individual patients. In clinical practice, performing a Pharmacokinetic (PK) study requieres significant commitment of time. The Bayesian estimation method uses a population PK model based on FVIII levels from a large population of patients. Using this strategy, factor half-life may be calculated from two or three time points.

Aims: To analyze PK (with 2 samples used for determination), schedules, ABR and annual joint bleeding rate (AJBR) in patients on prophylaxis with a full-length recombinant FVIII (FLrFVIII) (octocog alfa).

Methods: Files of patients with severe hemophilia A on prophylaxis with a FLrFVIII were evaluated retrospectively, considering the year 2015. Pharmacokinetics and data were analized using a specific tool program (myPKFiT) and Epi Info respectively.

Results: 36 patients were included. Age 3-29 years (mean 12.69). 80% of children (≤12 years) were on primary prophylaxis; 72.2% of the rest of the population (>12 years) were on secondary prophylaxis. Most patients (91.6%) were following a three times a week schedule dosis. In children mean dose was 30.3 IU/kg; in the older group it was 24.3 IU/kg. Mean FVIII half-life was 11.4 hours vs 13.05 respectively (P< 0.0001). Mean FVIII trough levels (48 hours post infusion) was 2.58 %. In children ABR and AJBR were 2.25 and 0.75. In adolescents and adults ABR and AJBR were 1.68 and 0.87. Twenty five patients (69.4%) had cero hemarthrosis. 30% of patients had FVIII trough levels < 1%. In this group, the ABR was 1.63.

Conclusions: Children have lower FVIII half-life and use higher dosis. The ABR and AJBR are low as expected. Although some patients present FVIII trough levels < 1%, the ABR remains very low.

PB 999 | A Unique Case Involving Delivery of Bolus rFVIIa via an Innovative Syringe Pump to a Patient with Haemophilia and Inhibitors Undergoing a Kidney Transplant

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Background: Haemophilia patients with inhibitors undergoing surgery under haemostatic cover with recombinant Factor VIIa (rFVIIa) require regular bolus doses, peri-operatively and for a variable number of days afterwards, determined by the type and location of surgery. This places considerable demand on nursing time as it necessitates the timely delivery of doses to ensure optimal haemostasis management. We investigated the use of an infusion pump to deliver timed and accurate rFVIIa bolus doses in a patient undergoing renal transplantation surgery.

Aims: To evaluate the efficacy and safety of using the B-Braun Perfusor® Space syringe pump to deliver frequent regular bolus doses of rFVIIa to a patient with haemophilia and inhibitors undergoing kidney transplant surgery.

Methods: The patient had complex medical issues, including haemophilia with inhibitors, renal disease and HIV positive status. The transplant surgery was electively planned and involved detailed discussions within a multidisciplinary team, to ensure the haematology team were confident of managing haemostasis, and that both the renal transplant team and the patient had full trust in the chosen delivery method for rFVIIa.

Results: The patient was administered rFVIIa via a PICC line. The pump was programmed to deliver an initial rFVIIa bolus dose of 120 mcg/kg, with subsequent doses of 90 mcg/kg every 2 hours for the first 48 hours, then 3-hourly doses for 24 hours, and finally 4-hourly for 64 hours. The pump was then discontinued and the patient was discharged one day later.

Conclusions: The kidney transplant was successful, and the patient made a full recovery. Haemostatically, the procedure went smoothly, with normal blood loss and no complications, bleeding episodes or line infections. This method of rFVIIa administration appears safe and effective, allowing centres to deliver haemostatic cover with great precision in complex surgical cases.

PB 1062 | Abnormal Plasma Clot Formation and Stability Distinguish Bleeding Risk in Patients with Severe or Partial Factor XI Deficiency

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Background: Factor XI (FXI) deficiency is a rare, autosomal disorder. Many patients have minimal or no bleeding, whereas others have severe bleeding usually associated with trauma or surgery. Bleeding risk cannot be predicted from plasma FXI antigen or activity levels. A pilot study suggested plasma clotting assays can distinguish bleeding risk in patients with severe FXI deficiency (Zucker et al. 2014 *J Thromb Haem*).

Aims: Validate the ability of plasma clotting, structure, and stability assays and use of contact pathway inhibitor (corn trypsin inhibitor, CTI) for predicting bleeding risk in a larger, independent cohort that includes patients with severe and partial FXI deficiency.

Methods: Research was approved by the medical ethics committee. Blood was collected in the absence or presence of CTI from patients with severe (*N*=14) or partial (*N*=57) FXI deficiency and healthy controls (*N*=49). Patients were divided into non-bleeders (NB, *N*=48) and bleeders (B, *N*=23) based on bleeding after tonsillectomy and/or dental extraction before diagnosis of FXI deficiency. Platelet-poor plasmas were prepared by centrifugation. Clotting was triggered with dilute tissue factor, $CaCl_2$, and phospholipids in the absence/presence of tissue plasminogen activator (tPA). Clot formation and fibrinolysis were assessed by turbidity. Fibrin structure was visualized by confocal microscopy.

Results: FXI:C levels, PT, and APTT did not distinguish between NB and B. In the presence of CTI, B had reduced clot formation rates and lower turbidity change compared to both controls and NB, in both the absence and presence of tPA (P< 0.005). B also had lower fibrin network density than controls or NB. In the absence of CTI, only clot formation rate significantly distinguished B from NB.

Conclusions: Contact pathway inhibition with CTI exposes clotting and clot stability deficits that are highly-associated with bleeding risk in patients with severe and partial FXI deficiency. These assays may have clinical utility for predicting bleeding risk.

PB 1063 | Anti FIXa/FX Bispecific Antibody (Emicizumab) Enhances Plasma Procoagulant Activity In Hemophilia B in the Presence of Very Low Level of Factor IX

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Background: Emicizumab is a bispecific antibody that promotes coagulation by bridging FIXa and FX. Minimal plasma level of FIX enough for functional effect of emicizumab remains unknown.

Aims: To elucidate the efficacy of emicizumab under a minimal amount of FIX.

Methods: A dynamic clotting test, thromboelastometry (ROTEM) using blood of severe hemophilia B (HB) patients under prophylactic therapy was performed. Clotting time (CT) and clot formation time (CFT) were evaluated with *ex vivo* addition of emicizumab (50 µg/mL). % fractional shortening of CT+CFT values ($\%FS^{CT+CFT}$) were calculated as [(1 - presence/absence) x 100]. $\%FS^{APTT}$ were also measured in selected patients' plasma.

Results: In 17 HB patients (median FIX:C 1.9 IU/dL, < 0.2~16.5), CT+CFT was shortened in the presence of emicizumab (median $\%FS^{CT+CFT}$ 18%) with large variations (no response~60%). Poor response group ($\%FS^{CT+CFT}$< 10%, n=7) had undetectable FIX activity (FIX:C < 0.2 IU/dL, n=3) or dysfunctional molecule (FIX:Ag/FIX:C ratio >2.0, n=4). $\%FS^{APTT}$ were evaluated in plasmas available from 10 HB patients including 4 poor response patients. In 9 patients (FIX:C 0.9-6.4 IU/dL) median $\%FS^{APTT}$ was 39% (35~45). One patient with FIX:C < 0.2 IU/dL showed no response. To determine the minimal amount of FIX necessary for emicizumab effects, commercial FIX-deficient plasma was spiked with rFIX (0, 0.01, 0.1, 1, 10 IU/dL), resulting in FIX dose-dependent effect of emicizumab ($\%FS^{APTT}$ 7, 11, 19, 26, 30%, respectively). $\%FS^{APTT}$ in FIX-deficient plasma (7%) was reversed to no response after adding anti-FIX antibody, suggesting that very low amount of FIX activity (< 1.0 IU/dL) existed in this FIX-deficient plasma. APTT in the presence of emicizumab with FIX:C of 0.1, 1, 10 IU/dL were equal to FIX:C of 0.6, 11, 114 IU/dL, respectively.

Conclusions: Minimal amounts of plasma FIX (on the order of 0.01 IU/dL) allowed emicizumab to function. Since most of severe HB patients have very low level of FIX, the results imply the possible utility of emicizumab in HB.

PB 1064 | Abrogating Fibrinolysis Does Not Markedly Affect the Bleeding Phenotype or Response to rFVIII Therapy in Mice with Haemophilia A

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Background: Additional knowledge on the role of fibrinolysis and antifibrinolytic intervention in haemophilia is desirable. For example, tranexamic acid (TXA) is indicated for treatment of topical bleeds, especially in the oral cavity, in haemophilia A and B. Its role and potential value as a systemic agent in haemophilia is, however, less well understood.

Aims: To investigate the role of fibrinolysis in haemophilia A, mice lacking both plasminogen (Plg) and FVIII were characterized for bleeding phenotype and response to recombinant coagulation factor VIII (rFVIII) therapy. Moreover, the effect of intravenously administered TXA on bleeding phenotype and response to rFVIII therapy was studied.

Methods: Experiments were conducted in F8-KO and F8-KO/Plg-KO mice using the sensitive tail vein transection (TVT) bleeding model under highly standardized conditions. Test compounds rFVIII (Advate) and TXA (500 mg/kg) were administered intravenously. All animal studies were approved by the Novo Nordisk Ethical Review Council and Cincinnati Children's Hospital Medical Centers Institutional Animal Care and Use Committee.

Results: Total Plg deficiency did not alter the apparent bleeding phenotype of F8-KO/Plg-KO mice when compared to F8-KO mice in the TVT bleeding model. Similarly, intravenously administered TXA did not significantly affect the bleeding phenotype or response to rFVIII therapy in F8-KO mice.

Conclusions: Neither congenital knockout of Plg nor intravenously administered TXA was observed to influence the bleeding phenotype or response to rFVIII in F8-KO mice as assessed by tail vein transection. Thus, the data failed to demonstrate a significant effect of systemic inhibition of the fibrinolytic system in haemophilia A mice.

PB 1065 | Vascular Remodeling in Hemophilic Joint Bleeding is Associated with Vascular Leak: A Possible Mechanism for Re-bleeding

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Background: Vascular remodeling is associated with hemophilic joint bleeding. Preliminary evidence points to leakiness of the remodeling vessels, which may contribute to frequent re-bleeding in affected joints.

Aims: To study the vascular integrity of remodeling synovial vessels in FVIII-deficient mice after hemarthrosis, and to characterize the associated inflammatory and tissue-reparative pathways.

Methods: Hemarthrosis was induced in FVIII-deficient mice by subpatellar knee puncture. Vascularity was assessed 2 weeks post-bleed by histology, including staining of α-smooth muscle actin (αSMA), and by musculoskeletal ultrasound with Power Doppler to detect microvascular blood flow. Markers of inflammatory and tissue-reparative macrophage M1 and M2 responses, respectively, were analyzed in synovial tissue 1-10 weeks post-bleed by real-time PCR.

Results: Hemarthrosis caused a 2.0-fold increase in vessel number ($p=0.006$) and a 2.9-fold increase in synovial microperfusion ($p<0.0001$). αSMA staining increased 4.0-fold ($p=0.001$) and remained elevated (2.4-fold, $p=0.04$) when corrected for vessel number, indicating thicker, remodeling vessels. A new method was established to measure the permeability of synovial vessels, whereby extravasated Evans Blue dye was quantified in the knee joint by near-infrared fluorescence. Vascular permeability increased 1.4-fold ($p=0.01$) 2 weeks post-bleed compared to uninjured contralateral joints, and returned to near-baseline levels after 4 weeks. Permeability correlated with the extent of microperfusion ($r=0.6579$, $p=0.02$). A transient inflammatory M1 macrophage response in the synovium was superseded by a pronounced elevation of M2 markers 2-4 weeks post-bleed.

Conclusions: The vascular integrity of remodeling synovial vessels associated with hemarthrosis in FVIII-deficient mice is significantly compromised. Vascular changes may be driven in part by a prolonged polarization of macrophages towards the tissue-reparative M2 phenotype, and are likely to promote re-bleeding.

PB 1066 | Development of a Pharmacokinetic-Pharmacodynamic (PK-PD) Model of Fitusiran, an Investigational RNAi Therapeutic Targeting Antithrombin for the Treatment of Hemophilia in Patients with and without Inhibitors

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Background: Fitusiran is a subcutaneously (SC) administered investigational RNAi therapeutic targeting antithrombin (AT) to promote hemostasis in patients with hemophilia.

Aims: To predict liver exposures in hemophilia patients and describe the population PK-PD relationship between fitusiran liver exposure, AT activity and thrombin generation (TG).

Methods: AT and TG data from 41 patients with hemophilia A and B with and without inhibitors from the phase 1 study of fitusiran were used in the development of a population PK-PD model. Observed fitusiran liver concentrations in rats were described by a 2-compartment PK model with 1st order absorption. Rat liver PK parameters were scaled allometrically to predict liver PK in humans. AT activity and TG were modeled using an indirect response model where: 1) fitusiran liver RISC PK (effect compartment) suppressed synthesis of AT thus decreasing AT activity; 2) decrease in AT activity decreased the inhibitory effect on TG, thus increasing TG. The population PK-PD model was subsequently used to simulate expected AT activity and TG response across a wide dose range in patients with hemophilia.

Results: The half-life of fitusiran in human liver was predicted to be ~20 days. Model predicted steady-state median AT lowering at 50mg and 80mg QM dose were 82% and 87%, respectively. Baseline TG (bTG) was identified as a significant covariate for maximum TG: higher baseline TG yielded greater absolute value of maximal TG. Steady-state median TG (at bTG=15nM) was predicted to be 65nM and 71nM, at QM doses of 50 and 80mg, respectively. Difference between peak and trough AT and TG responses was minimal throughout the dosing interval at these regimens. Dose-response relationship for AT and TG showed an asymptote at QM doses >80mg.

Conclusions: The PK-PD model described the time course, extent, and variability in AT and TG responses in patients with hemophilia. This PK-PD model is being used to guide dosing regimens in clinical trials.

PB 1067 | Electrostatic Analysis of Hemophilia B Causative Mutations

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Background: Hemophilia B (HB) is a bleeding disorder caused by mutations in the Factor IX (FIX) gene. There are about 1,095 different HB mutations described, with a prevalence of missense mutations. The role of how mutations interfere on FIX function is not clearly

TABLE 1 Mutations included in the study classified according to domain and clusterization by eletrostatic distances

Domain	Cluster[1]	N° of mutated models	Amino acid changes[2] / cluster	Distance[3] from the wild structure	Observations:
GLA	1	4	p.Phe55Cys, p.Phe55Ile, p.Phe55Ser, p.Cys64Tyr	0.0	[1] Clusterization was obtained using the webPIPSA tool, based on a pair-to-pair comparison.
	2	1	p.Cys64Arg	0.3	[2] Sequence changes at the protein level following the Nomenclature for Description of Genetic Variations approved by the Human Genome Variation Society.
	3	2	p.Arg75Gln, p.Arg75Pro	0.6	[3] Distances were calculated with webPIPSA by a pair-to-pair comparison and matrix generation (Da,b=√2-2Sla,b).
EGF1 and EGF2	1	5	p.Cys134Phe, p.Gln143Pro, p.Cys170Phe, p.Cys170Ser, p.Cys170Tyr	0.0	
	2	3	p.Asp95Gly, p.Asp95Tyr, p.Asp95Val	1.5	
	3	11	p.Cys134Arg, p.Gln143Arg, p.Gln143Lys, p.Cys157Arg, p.Cys170Arg, p. Cys134Ser, p.Gln143Glu, p.Val153Ala, p.Val153Met, p.Cys157Ser, p.Cys157Tyr	1.5	
Serine Protease	1	16	p.Val374Ile, p.Thr342Ala, p.Val374Phe, p.Gln413Ala, p.Gly236Val, p.Ile390Asn, p.Gly236Cys, pGly432Ala, p.Gly432Ser, p.Ala366Gly, p.Thr386Ile, p.Ala366Pro, p.Ile390Phe, p.Thr342Met, p.Gly432Val, p.Ile390Ser	0.0	
	2	5	p.Gly432Asp, p.Gly413Glu, p.Gly236Asp, p.Lys387Glu, p.Lys387Asn	0.8	
	3	9	p.Ala366Val, p.Ala366Asp, p.Hys302Arg, p.Ile390Thr, p.Thr342Arg, p.Thr342Lys, p.Gly413Arg, p.Th386Lys, p.Thr386Arg	0.8	

determined; different aspects could be involved, as structural modifications, loss of stability and electrostatic changes.

Aims: To investigate FIX structural and electrostatic effects of previously detected hemophilia B causative missense mutations.

Methods: A total of 54 different HB missense mutations among activated FIX domains were included in the study, 18 of them detected in southern Brazil individuals and the other 36 recovered in the factor ix database. For each mutation, a 3D homology model was generated and the electrostatic potential was calculated. A physicochemical evaluation was conducted through the calculation of the electrostatic distances between models.

Results: The models were compared and clustered according to electrostatic distances, as illustrated in Table 1.

The electrostatic potential distances evaluation revealed that 31 out of 54 mutations were considered as different, compared to the normal model. The electrostatic distances of these 31 mutations compared to normal varied: 0.3 and 0.6 for GLA domain (3/31); 1.5 for the EGF1 and EGF2 domains (14/31); and 0.8 for the serine protease (14/31) domains. Cluster 1 of every domain shows no distance from normal models. Representative models in terms of domains and clusters are presented in Figure 1.

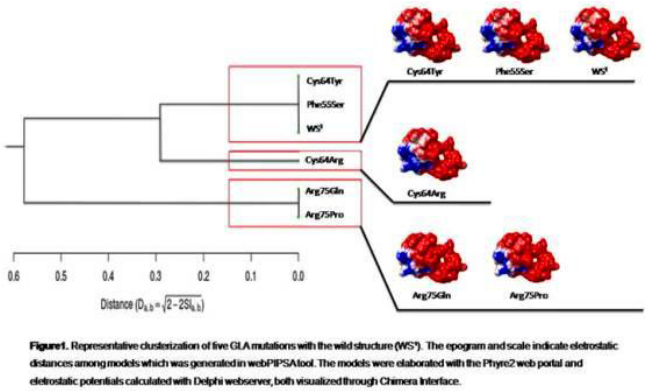

Figure1. Representative clusterization of five GLA mutations with the wild structure (WS¹). The epogram and scale indicate eletrostatic distances among models which was generated in webPIPSAtool. The models were elaborated with the Phyre2 web portal and eletrostatic potentials calculated with Delphi webserver, both visualized through Chimera interface.

FIGURE 1 Representative clusterization of five GLA mutations with the wild structure.

Conclusions: Differences in electrostatic potential apparently influence 31 of the 54 mutations. Five other mutations with normal electrostatic parameters lead to cysteine bridges disruption. The position of the mutations matter, especially considering regions that suffer post-translational modifications or regions involved in stability (disulfide bonds, for example)of the protein; however the mutant

eletrostatic modifications can actively interfere in Factor IX functions, causing HB.

PB 1068 | Identification of Regulatory B Cells in Severe Hemophilia A Patients

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Background: The occurrence of an immune response blunting the therapeutic efficacy of exogenous factor VIIII (FVIII) is the most relevant adverse event in hemophilia A (HA) treatment. The reason why only a portion of the patients develop neutralizing antibodies - "inhibitors" - to FVIII has, however, been unclear. A regulatory subset of B (Breg) cells has recently been identified that contributes to the maintenance of immunological tolerance in humans and mice. Breg cells can suppress pro-inflammatory cytokine production by dendritic cells and can convert effector $CD4^+$ T cells into $FoxP3^+CD4^+$ regulatory T (Treg) cells. Although Treg cells have been credited with an ability to oppose inhibitor development, no studies have yet investigated any possible contribution of Breg cells to the effect.
Aims: Investigating the ability to induce Breg cells in severe hemophilia A patients with inhibitors.
Methods: In this pilot study, we enrolled 5 severe hemophilia A patients with a history of long-standing, high-titer inhibitors (>5 BU/mL) and 5 age-matched healthy controls. All subjects gave written informed consent in accordance with the Declaration of Helsinki. PBMCs were isolated and cultured for 72 hours in presence or absence of CpG type C oligonucleotide (ODN) to activate Toll-like receptor 9 (TLR9). Cells were harvested and stained with fluorochrome-conjugated antibodies anti-CD19, anti-CD24, anti-CD38, anti-CD138 and analyzed.
Results: Unlike PBMCs from healthy donors, that significantly increased the frequency of $CD24^+CD38^{hi}$ Breg cells at 72 h (untreated: mean = 3.2%, s.d. = 1.1; CpG ODN: mean = 16.5%, s.d. = 3.4; $P< 0.01$), cells from hemophilic patients with inhibitors failed to induce Breg cells when exposed to CpG-C ODN (untreated: mean = 2.8%, s.d. 1.3; CpG ODN: mean = 3.4%, s.d. = 1.4; NS).
Conclusions: Data argue for the differential occurrence of Breg cells in inhibitor positive patients relative to healthy controls after in vitro stimulation with a TLR9 ligand.

PB 1069 | Prophylactic Treatment with Turoctocog Alfa Pegol (N8-GP), a GlycoPEGylated Recombinant Factor VIII, Completely Prevents Development of Arthropathy in a F8 - Knockout Mouse Model of Induced Knee Bleeding

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Background: Turoctocog alfa pegol (N8-GP) is a novel glycoPEGylated recombinant B-domain truncated factor VIII (FVIII) product developed for treatment of haemophilia A patients. The extended half-life of N8-GP permits less frequent injections compared with standard FVIII products. N8-GP is currently in clinical development and shown to be safe and effective for prophylaxis and treatment of bleeds in previously treated patients with haemophilia A, where haemophilic arthropathy is the main cause of morbidity.
Aims: To investigate if N8-GP can prevent development of arthropathy following induced knee bleeding in an animal model of haemophilia.
Methods: On days 0 and 7, F8-knockout (F8-KO) mice were anesthetized and received an i.v. injection of 500 IU/kg N8-GP (n=9) or saline (n=10) followed by needle puncture through the infrapatellar ligament to induce a knee bleed. Joint diameter was measured at baseline and on day 8, the day after the second induced knee bleed. On day 14, mice were euthanized and knees were scanned by micro-computed tomography (μCT) followed by histopathological evaluation.
Results: In saline treated mice, induced knee bleeding caused marked swelling measured as increased joint diameter, severe synovitis, and substantial hemosiderin deposition in the joint. Prophylactic treatment with N8-GP prevented hemosiderin deposition and significantly diminished joint swelling ($p< 0.0001$) and development of synovitis ($p< 0.0001$). Bone pathology was assessed by μCT and showed that saline treated F8-KO mice developed significant arthropathy with bone erosions, formation of periosteal bone, and loss of trabecular bone. Prophylactic treatment with N8-GP completely protected against bone pathology.
Conclusions: In a mouse model of haemophilic arthropathy prophylactic treatment with N8-GP diminishes synovitis and completely prevents pathological bone remodelling following induced knee bleeding.

PB 1070 | Two Large *F8* Deletions Possibly Related to the Mechanism of Microhomology-mediated Break-induced Replication (MMBIR) as a Cause of Severe Haemophilia a and Inhibitors

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Background: Large *F8* deletion (LD) genotyping in haemophilia A (HA) (8-15% of severe cases) is important because it associates with the highest risk to develop inhibitors against FVIII replacement therapy.
Aims: Characterisation of the molecular event in two *F8* LDs, detected by consistent absence of exons 7-12 and 5-7 PCR analysis in patients with severe HA (FVIII:C< 1IU/dL) and inhibitor titters of 260 and 13,6BU/mL from family 1 (F#1) and 2 (F#2), respectively.

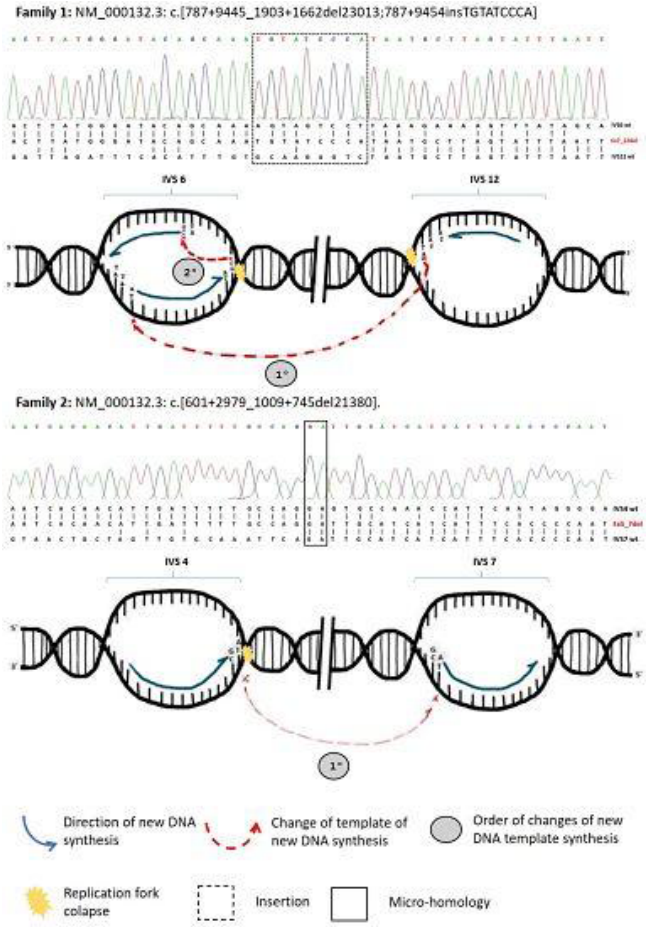

FIGURE 1

Methods: To chase LD breakpoints, PCR tagging schemes were designed by specific bipartition analysis on DNA samples from hemizygous probands. Long range-PCR (lrPCR) amplifications were performed with primers of the nearest 5´- and 3´-positive PCR tags. F#1´s primary lrPCR yielded a specific signal of 7.8kb and F#2´s, of 5.1kb. Restriction analysis of lrPCR products allowed designing new LD-specific amplifications. F#1 (IVS6newup+IVS12newlo) yielded a product of 1.45 kb and F#2 (H617_Delup+H700_Dello), of 1.67kb. Sanger sequencing spanning the breakpoints permitted full characterisation of both events.

Results: F#1 showed a LD of 23kb (ChrX: 154,952,228-154,975,240) NM_000132.3: c.[787+9445_1903+1662del23013;787+9454insTGTATCCCA] and F#2, a LD of 21.4kb (ChrX: 154,968,575-154,989,956) c.[601+2979_1009+745del21380]. F#1 event showed an insertion of 9bp at the recombination site (TGTATCCCA) which is also found at inverted orientation 5bp upstream; and F#2 showed a microhomology of 2bp at the breakpoint junction (Fig. 1). Bioinformatic scanning of sequences surrounding the LD junctions revealed motifs associated with DNA instability (i.e., topoisomerase I consensus cleavage sites, DNA polymerase alpha pause site core sequence, LINE L1 and LTR).

Conclusions: The microhomology between 5´ and 3´ ends, the insertion/synthesis of new material at the breakpoint from vicinal sequence templates and the insertion of nucleotides at the junctions observed in both LD events are consistent with the mechanism of MMBIR (Fig. 1).

PB 1071 | Measurement of Basal Levels of TF, FVII, FVIIa, Free and Total TFPI and FVIIa.AT Complex Levels in Patients with Haemophilia A and B

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Background: Phenotypic heterogeneity in haemophilia is a recognised phenomenon. There is also a suggestion that haemophilia B is less severe than A. This is a growing area of interest with the emphasis on individualised care and development of novel therapies targeting regulatory components. The components contributing to the initiation of haemostasis may play a role in moderation of bleeding phenotype.

Aims: This was a study of patients with moderate and severe haemophilia A and B measuring levels of TF, FVII, FVIIa, free and total TFPI, FVIIa.AT complex in the absence of overt bleeding.

Methods: Ethical approval and informed consent were obtained via the KD Coagulation Research Plasma Bank. TF levels were measured with the chromogenic ACTICHROME® TF assay (Sekisui Diagnostics, Invitech, UK); FVII by one-stage PT assay; FVIIa using a STACLOT® FVIIa-rTF clotting assay; Free & total TFPI levels, FVIIa.AT complex by ELISA (ASSERACHROM®, Diagnostica Stago, UK). Local normal ranges were created. Statistical analysis was conducted using non-parametric tests for comparison between diseases.

Results: 51 patients with haemophilia A (6 moderate) and 13 with haemophilia B (2 moderate) were recruited to the study. See Table 1 for

TABLE 1 Results of laboratory assays. Normal range (using 20 volunteers) ± 2SD (mean). Patients 10th, 90th percentile (median). ND=not detected

Assay	All patients	Haemophilia A	Haemophilia B
TF (ND - 2.39 pM; mean 1.04)	0.34 - 0.64 (0.45)	0.34 - 0.64 (0.45)	0.34 - 0.64 (0.45)
FVII (45 - 180 IU/dL)	55 - 119 (84.8)	66 - 120 (87.9)	42.4 - 96.5 (56.4)
FVIIa (10.60 - 62.12 mIU/dL; mean 36.36)	9.2 - 67.3 (34.1)	23.9 - 67.8 (41.5)	3.2 - 18.6 (7.5)
Free TFPI (2.8 - 13.8 ng/ml; mean 8.3)	7.6 - 21.3 (10.2)	7.5 - 18.3 (10.2)	8.1 - 22.2 (9.4)
Total TFPI (31.7 - 88.8 ng/ml; mean 60.2)	48.9 - 82.0 (62.7)	49.3 - 79.5 (63.0)	48.3 - 87.7 (61.3)
FVIIa.AT complex (41.7 - 243.0 pM; mean 142.3)	50.8 - 202.5 (122.7)	97.4 - 220.8 (132.4)	32.3 - 84.0 (47.9)

results. TF levels were equivalent between groups but significantly lower than normal ($p < 0.0005$). All patients with haemophilia B had significantly lower levels of FVII than A ($p = 0.001$). This was also true for FVIIa ($p < 0.0005$). Free & total TFPI levels were equivalent. The levels of FVIIa.AT complex were significantly lower in patients with haemophilia B ($p < 0.0005$).

Conclusions: This was a study investigating the components of the initiation and regulation of haemostasis in haemophilia A and B. All patients had low levels of TF. The results highlight differences between haemophilia A and B with significantly lower levels of FVII, FVIIa and FVIIa.AT complex. TFPI levels are equivalent. Further work is required to understand the underlying mechanisms and potential role in phenotypic heterogeneity.

PB 1072 | *F8*-deficiency in Mice is Associated with Increased von Willebrand Factor (VWF) Content in the Hepatic Endothelium and with Increased VWF Plasma Levels

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Background: The anti-hemophilic coagulation factor FVIII is synthesized in the liver sinusoidal endothelium and co-stored together with von Willebrand Factor (VWF) in Weibel-Palade bodies of endothelial cells. While VWF is recognized as the carrier molecule of FVIII in plasma, stabilizing FVIII activity by preventing proteolytic degradation, the possible impact of *F8*-deficiency on VWF plasma levels is unresolved.

Aims: To pinpoint if *F8*-deficiency in mice is associated with altered VWF plasma levels.

Methods: The $F8^{-/-}$ mouse model is a useful tool to study hemophilia A, avoiding the confounding variables deriving from the heterogeneity of the disease origin. Therefore, we used a hemophilia A mouse model with disrupted exon 16 of the *F8* gene, which we back-crossed onto a pure C57BL/6J genetic background. In addition to the hemostatic, bleeding, and coagulation characteristics, we analyzed littermates from heterozygous breedings for their hepatic endothelial VWF content, VWF plasma levels, and VWF multimer size.

Results: The expected bleeding phenotype of *F8*-deficiency was noted, that was related to reduced thrombin-induced thrombin generation in platelet-rich plasma. Strikingly, immunohistochemistry analysis of the liver of $F8^{-/-}$ mice revealed increased VWF content in the liver endothelium. Increased hepatic endothelial VWF staining was accompanied by signs of increased hepatic inflammation, as indicated by increased CD45 and TNF-α mRNA levels. VWF plasma levels were significantly elevated in $F8^{-/-}$ mice compared with their co-housed WT littermate controls, but plasmatic ADAMTS13 concentrations and VWF multimer patterns were unchanged.

Conclusions: Collectively, our results demonstrate that *F8*-deficiency in mice is associated with increased VWF content in the hepatic endothelium and with increased VWF plasma levels.

PB 1073 | Feasibility of Prospective Transcriptome Profiling in Previously Untreated Patients with Severe Hemophilia A: NuProtect Gene Expression Satellite Study

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Background: Inhibitor formation occurs in a third of severe hemophilia A (HA) patients treated with Factor VIII (FVIII) concentrate. This is dependent on genetic and environment factors. There is poor understanding of the immunological mechanisms underlying inhibitor formation and immune tolerance induction (ITI). Evaluation of dynamic changes in gene expression (transcriptome) may identify biomarkers of inhibitor risk and provide novel therapeutic targets.

Aims: To demonstrate feasibility of RNA retrieval from previously untreated patients (PUP) with severe HA to analyze transcriptome changes at first exposure to FVIII and during ITI.

Methods: Blood samples for transcriptome analysis were obtained in an international phase 3 study (NuProtect, ClinicalTrials.gov Identifier: NCT01712438) of a recombinant human cell line FVIII concentrate (hcl-rFVIII, Nuwiq®). Whole blood samples were collected using a bespoke low volume PAXgene (1mL) RNA tube prior to and following first exposure to hcl-rFVIII (up to 20ED or inhibitor) and/or during ITI.

Results: 80 patients from 25 study sites have been recruited to date providing 375 samples (322 extracted). Median age at first FVIII exposure was 13 months (1-146). Median number of samples per patients was 5 (1-18). 16 patients (high titer=9, low titer=7) were known to have an inhibitor within this satellite study, representing a subset of the total recruited to the NuProtect PUP study. The median time to inhibitor formation was 9ED (4-25). Mean RNA yield (SD) was 11.8±8.7μg with all having >1μg (84%, 270/322, >5μg). Mean RNA purity (260/280) was 2.2±0.5. Mean RNA integrity (RIN) was 8.5±1.2 with 92% (296/322) having a RIN≥7 and only 5 samples (5/322, 1.5%) had sub-optimal RIN (≤5) for downstream analysis.

Conclusions: A low volume RNA tube provides an acceptable solution for transcriptome analysis in pediatric studies demonstrating high quality/quantity RNA. This transcriptome library will be one of the largest prospective datasets at first exposure to a protein therapeutic.

PB 1074 | The Bone Disease in Haemophilia: A Possible Role of Coagulation Factors?

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Background: Until now, the pathogenesis of bone loss associated with severe hemophilia A (HA) remains still unknown; however it could be related to different risk factors, including decreased physical activity, viral infections, bleeding and inflammation. The reduction of Bone mineral density (BMD) seems due to perturbations of the Receptor Activator of Nuclear factor-B RANK Ligand (RANKL) and Osteoprotegerin (OPG) pathways. Moreover, bone cells express protease-activated receptors (PARs) and FVIII deficiency could alter BMD also by decreasing thrombin production.

Aims: This study aimed at investigating the role played by each coagulation factor (FVIII, VWF and thrombin) on osteoclast differentiation and function.

Methods: In vitro assays assessed the effects of the different coagulation factors (VWF/FVIII complex, human rVWF, human full length rFVIII, rFVIII B-deleted and thrombin) on 1) osteoclastogenesis (M-CSF/RANKL-generated osteoclasts) and 2) bone resorption activity (Cathepsin-K and MMP-9 expression).

Results: VWF appears to play a relevant inhibitory effect, showing by itself about 45% reduction of osteoclastogenesis comparable to OPG (the physiologic inhibitor), and even more if is complexed with FVIII (53% inhibition). Thrombin seems to counteract osteoclast differentiation with a variable effect (30-50% inhibition). In contrast, no significant result was obtained by gene expression studies, excluding a role in bone resorption activity.

Conclusions: These findings showed that the biology of osteoclasts is negatively influenced by VWF/FVIII complex. Likewise, thrombin interaction with PARs induces a significant inhibition of osteoclast activity. These results may have important implications in the clinical practice of hemophilia patients to better orient the choice of the FVIII concentrate to prevent bleeding and at the same bone disease in severe HA patients.

PB 1075 | Determinants of Thrombin Generation in Haemophilia A and B

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Background: The thrombin generation assay (TGA) is increasingly used in haemophilia. Thrombin generation (TG) is affected by various coagulation factors and inhibitors, known as determinants. In healthy subjects, fibrinogen and factor XII have been identified as positive determinants of TG, antithrombin and free TFPI being negative determinants (Dielis A, JTH 2008). Would the determinants influencing TG be different in haemophilic patients?

Aims: To identify the determinant factors of TG in haemophilia A and B patients.

Methods: Plasma levels of coagulation factors and inhibitors, as well as TG, were measured in 40 haemophilia A and 32 haemophilia B patients. The effect of the levels of coagulation factors and inhibitors on ETP and peak were analysed using stepwise multi-linear regression models.

Results: In haemophilic A patients, factor VIII was a positive determinant of ETP and peak, whereas TFPI and factor V were negative determinants.

In haemophilic B patients, factor IX was a positive determinant of ETP, whereas antithrombin, protein S, and free TFPI were negative determinants. Factor VII was a positive determinant of peak, whereas free TFPI and factor X were negative determinants.

Overall, TFPI was the major determinant of peak and ETP in haemophilic patients.

Conclusions: Positive and negative determinant factors of TG are not the same in healthy individuals and haemophilic patients. TFPI seems to greatly influence TG in haemophilic patients.

PB 1076 | Unusual Genomic Rearrangement Combined with Inv22 and Wild-type X-chromosome in Severe Hemophilia A Patients

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Background: Various genetic defects of coagulation factor VIII gene (*F8*) cause hemophilia A (HA). Intron 22 inversion (Inv22) found in a half of severe HA patients results from a recombination between intra- and extra-genic intron 22 homologous copies.

Aims: In this study, we investigated the brother cases of severe HA with complicated X-chromosomal rearrangement.

Methods: We analyzed gDNA from male probands (7 y/o and 3 y/o, severe HA, FVIII:C < 1%), their mother (39 y/o, FVIII:C = 39.4%), maternal grandfather (67 y/o, FVIII:C = 171.2%), and grandmother (64 y/o, FVIII:C = 147.8%). *F8* Inv22 was analyzed by long-PCR and inverse shifthing-PCR (IS-PCR). Quantitative genome mapping was performed by quantitative-PCR (qPCR). Genomic breakpoint search was performed by inverse-PCR, and direct DNA sequencing. This study

was approved by the ethics Committee of the Nagoya University Graduate School of Medicine.

Results: Long- and IS-PCRs of probands showed a mixed pattern of wild-type (WT) and Inv22 type 2. Mother gDNA also exhibited the same long-PCR pattern of probands. However, maternal grandfather and grandmother displayed a WT genome pattern. Quantitative genome mapping indicated that *F8* from exon 24 to intron 25 were duplicated as compared to WT gDNA. *CLIC2* from exon 1 to intron 3 was also duplicated. From these results, we hypothesized that Inv22 X-chromosome (X-chr) was combined with translocated WT X-chr as a breakpoint between *CLIC2* on Inv22 X-chr and *F8* on WT X-chr. Inverse-PCR and direct DNA sequencing revealed that, in patients, sense-oriented *CLIC2* intron 3 connected to antisense-oriented *F8* intron25 via 3 bases microhomology.

Conclusions: The patients have an unusual rearranged Inv22 X-chr combined with a WT telomeric side of X-chr. We propose a rearrangement mechanism repairing the telomere side of a double-strand broken Inv22 X-chr by microhomology-mediated break-induced replication with WT X-chr.

PB 1077 | Biochemical and Biophysical Characteristics of Commercially Available FVIII Products

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Background: Recombinant and plasma-derived (pd) coagulation factor concentrates available for haemophilia A treatment differ in purity and structure, which might affect their efficacy and immunogenicity. Recently, the SIPPET trial showed that previously-untreated-patients have higher incidence of inhibitor development if treated with recombinant FVIII products. This phenomenon could arise from several features of recombinant products, such as the absence of VWF and albumin, different glycosylation profile or their molecular aggregation.

Aims: This study investigated the purity grade, FX activating ability and aggregation status of three rec-products (Advate [Baxalta], Refacto AF [Pfizer] and Kogenate [Bayer]), compared with four commercially available pd-FVIII concentrates (Haemoctin [Biotest] , Fanhdi [Grifols], EmoclotÒ [Kedrion], and Beriate [CSL Behring]).

Methods: Rec- and pd-factors were studied with FPLC-chromatography, (immuno)-electrophoresis, dynamic light scattering (DLS) and steady-state enzyme kinetics.

Results: DLS data showed that no molecular aggregation was present in the three rec-FVIII products that showed a constant Z-average value (~70 nm) over a range of concentrations similar or even higher than that obtained after their reconstitution (100-250 IU/ml). A similar result was obtained for the pd-concentrates. The presence of VWF content in the pd-FVIII products slightly accelerates the thrombin-induced kinetics of FVIII activation and FVIIIa inactivation.

Conclusions: These results show that, although the molecular dimension, glycosylation profile and presence of different stabilizers are different, the most commonly used recombinant products do not undergo molecular aggregation at pharmaceutical concentrations obtained after their reconstitution. Thus, the hypothesized molecular aggregation of recombinant products cannot be invoked as a plausible cause of their increased propensity to induce formation of FVIII inhibitors.

PB 1078 | Global Assays of Hemostasis among Severe Hemophilia A Boys Detects Variation

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Background: Severe hemophilia A patients vary in FVIII treatment requirements. Optimal treatment limits antigen exposure and expense, and is currently guided by clinical observations (e.g. bleeding frequency). It would also be useful to employ predictive laboratory assessments, but heterogeneity among severe hemophilia A patients is poorly understood.

Aims: To use global hemostatic assays to measure variation in baseline hemostatic function after factor washout in a cohort of hemophilia A boys receiving a range of rFVIII replacement regimens.

Methods: We used thromboelastography (TEG; whole blood) and calibrated automated thrombography (CAT; plasma with and without platelets) with experimental variations including use of the contact activation pathway inhibitor corn trypsin inhibitor and differing concentrations and preparations of tissue factor. Attempts were made to correlate the results of these assays with each other and with clinical parameters to gain insights into their potential utility in the prediction of factor requirements.

Results: We observed that contact pathway inhibition is required for accurate assessment of variation in baseline hemostatic function using TEG and CAT. Both assays detected substantial heterogeneity within our cohort when performed with low tissue factor concentrations, and inter-individual variation was sufficiently large that efforts to find relationships with clinical traits such as factor requirements, were statistically not achievable. Different tissue factor preparations had an influence, with results of TEG and CAT assays performed with Innovin showing a significant correlation. The results of TEG and CAT assays converged towards normal ranges with increasing levels of tissue factor activation, but several severe hemophilia A samples did not reach normal levels of thrombin generation.

Conclusions: Our results indicate that CAT is the most promising assay for further studies of hemostatic function variation in severe hemophilia A patients.

PB 1079 | A Bispecific Antibody Lacks Measurability in Routine Coagulation Assays and Comparability to Factor VIII

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Background: For optimal management of hemophilia A patients receiving ACE910 non-factor therapy, accurate monitoring is needed. However the best testing methodology is not known.

Aims: To examine the effect of assay type on hemostatic activity monitoring of a sequence identical analogue to ACE910 (SIA) and to assess FVIII activity equivalency.

Methods: SIA was analyzed in 4 commercial chromogenic assay kits; rFVIII served as comparator. Clotting times were measured by APTT using 5 differently composed trigger reagents followed by clot waveform analysis. In TGA, SIA was tested using extrinsic and intrinsic trigger conditions. As needed, concentrations ranged from 2-1200 NM.

Results: Lack of cross-reactivity of SIA to bovine factors rendered only the Biophen FVIII:C test suitable for analysis. As the dose response of SIA and FVIII was not collateral, the chromogenic assay is not a reliable test for FVIII equivalence assessment. APTT was highly sensitive to SIA which resulted in substantial reduction of clotting time at low and potentially non-therapeutic concentrations. Different APTT reagents yielded a FVIII equivalence range of 18-69% (20 nM SIA) and >200% at a presumed clinical concentration of 600 nM. SIA (600nM) only partially restored TG in hemophilia A patient plasmas, resulting in FVIII equivalents of 4-8% (intrinsic) and 16-36% (extrinsic) based on peak thrombin. Assessment of other TG parameters resulted in FVIII equivalency ranging from 0 to >100%.

Conclusions: Analysis of SIA and derivation of FVIII equivalence therefore is challenged by its novel mechanism of action/regulation. FVIII equivalence of SIA cannot be determined using standard FVIII protocols and is highly influenced by assay type, analytical conditions, and parameters used for calculation. Thus, new methods and a product specific standard are required for better prediction of the hemostatic effect of non-factor therapies.

PB 1080 | Efficacy of Anti-TFPI Antibody PF-06741086 Compared to rFactor VIIa in Mouse Hemophilia A Bleeding Models

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Background: Patients with hemophilia use replacement factor as prophylaxis to prevent bleeds. Some patients develop inhibitory antibodies and become refractory to treatment. Hemophilia patients with inhibitors are currently managed with rFVIIa or aPCC bypass therapy. An alternative approach is to augment the extrinsic tissue factor (TF) pathway by inhibiting Tissue Factor Pathway inhibitor (TFPI). TFPI modulates the initiation of coagulation by directly binding and inhibiting the TF/FVIIa/FXa complex. PF-06741086 is a fully human monoclonal antibody engineered to inhibit TFPI activity.

Aims: Here, we compare the hemostatic effect of PF-06741086 to rFVIIa in mouse bleeding models.

Methods: Hemophilia A (Hem A) mice received a single intravenous dose of PF06741086 (1, 2 or 6 mg/kg), vehicle, or rFVIIa (2, 5, or 10 mg/kg) dosed 30 minutes or 5 minutes before a 3 mm tail clip, respectively. Blood was collected for 10 minutes and quantified against a standard curve of hemoglobin. In a second model, cremaster microcirculation in Hem A mice was observed using intravital microscopy (IVM).

Results: PF-06741086 restored hemostasis at 1 mg/kg (49%), 2 mg/kg (63%), and 6 mg/kg (78%) compared to vehicle treated mice. In rFVIIa treated mice, the highest tested dose of 10 mg/kg reduced bleeding by 55%, with a lower reduction at 5 mg/kg (33%), and no effect at 2 mg/kg. In a laser injury model using IVM, the effect of PF-06741086 (6 mg/kg) was similar to 5 mg/kg of rFVIIa in enhancing platelet clot and fibrin formation. A lower dose of rFVIIa corrected clot formation in the laser injury model compared to a tail clip. Assay sensitivity or vessel size in injury model could explain the difference in efficacy between tail clip and IVM. Data on co-treatment with PF-06741086 and rFVIIa in animal models of hemophilia with will also be presented.

Conclusions: Prophylactic administration of PF-06741086 in hemophilic mice neutralizes TFPI to enhance the extrinsic pathway and bypass deficiencies in the intrinsic pathway of coagulation.

PB 1081 | X Chromosome Inactivation and Plasma Level of Factor VIII and IX in Carriers of Hemophilia A and B

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Background: Hemophilia A and B are X-linked haemorrhagic disorders caused by mutations in *F8* and *F9* genes. Due to recessive inheritance, males are affected, while female carriers, with a wide range of FVIII or FIX levels, are mostly asymptomatic. Bleeding tendency in carriers is extremely variable and rare, associated with low clotting factor levels. This could be ascribed to homozygous *F8* or *F9* mutations or compound heterozygous, numerical or structural X chromosome abnormality, or to skewed X chromosome inactivation (XCI).

XCI is a normal epigenetic phenomenon that occurs early in the embryogenesis when, randomly, one of two X-chromosomes, in females, is transcriptionally silenced.

Aims: To determine whether the low activity of FVIII or FIX (below 20 IU/dL) in hemophilia carriers could be related to XCI.

TABLE 1 Data of carrier females

Case code	Disease	FVIII:C* [50-147 IU/dL]	FIX:C* [70-120 IU/dL]	Family history	XCI skewed	F8/F9 mutation	F8 and F9 variant database
E292	HB	ND	10	No	Yes	c.252+5G>A p.C335W	Yes
E1424			1	No		p.N48Y	No
E1236			13	Yes			Yes
E1389	HA	12	ND	Yes	XP active Yes	ND	NA
E1139		5		No	Yes	p.R2169H Inv22int	Yes
E158		12		No	Yes	Large del Large del	§
E386		16		No	Yes	p.A2227T p.R2182H	Yes
E1640		5		No	XP active Yes	p.I1374Tfs*49	Yes
E1690		14		Yes	Yes		No
E614		10		No			Yes
E253		3		Yes			Yes

*one-stage assay; ND: not determined; NA: not available; XP: X paternal chromosome;
§ Antonarakis SE et al, Blood 1995;86:2206-12

Methods: Molecular characterization was performed using PCR, Sanger sequencing, and MLPA analysis. X inactivation status was studied by methylation-sensitive endonuclease digestion, coupled with PCR analysis of a polymorphic repeat of the human androgen receptor (HUMARA) gene.

Results: Eight carriers of hemophilia A and three carriers of hemophilia B exhibited low plasma levels of FVIII:C (range from 3 to 16 IU/dL) and FIX:C (range from 1 to 13 IU/dL). All carriers showed joint bleeding and hematoma after trauma or surgery, and menorrhagia. A specific mutation was found in 10 hemophilia carriers in heterozygous state, as reported in table 1, not justifying alone their low activity levels.

HUMARA results showed unfavourable X chromosome inactivation in all carriers. The ratio of inactive X chromosome to active was dramatically skewed (≥80:20). In two cases of familial hemophilia, only the paternal allele was the active one.

Conclusions: In our group of carriers with FVIII:C and FIX:C level below 20 IU/dL, XCI seems to be an appreciable determinant of clotting factor residual level, even if phenotypic expression is multifaceted sustaining further investigation.

PB 1082 | Turoctocog AlfaPegol (N8-GP) Is Cleared from Subcutaneous Injection Sites without Local Degradation

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Background: N8-GP is a glycoPEGylated recombinant factor VIII (FVIII) with high bioavailability after subcutaneous (SC) administration in animal models and may provide a convenient SC therapy for patients with haemophilia A.

Aims: To analyse injection site distribution and absorption/elimination of N8-GP after SC administration in mice.

Methods: F8 gene-knockout mice received a high single (1850 IU/kg) or multiple (3 x 1850 IU/kg, 48 h apart) SC dose(s) of N8-GP or fluorescently labelled N8-GP VivoTag® 680XL. Temporal disappearance from the injection site was quantified with *in vivo* optical imaging. Blood samples were collected for FVIII antigen and activity assays. Micro-distribution of N8-GP at injection sites was studied with immunohistochemistry for FVIII. Any degradation of labelled N8-GP at injection sites was analysed by western blotting for FVIII. The Danish Animal Experiments Council approved all *in vivo* procedures.

Results: SC N8-GP displayed a prolonged presence of FVIII antigen and activity in plasma compared to the expected PK profile of FVIII. *In vivo* imaging suggested a gradual disappearance from injection sites. The fluorescence signal from labelled N8-GP was largely undetectable 48 h post injection and only a faint signal was detected at 72 h. Western blotting and immunohistochemistry demonstrated that a large fraction of the SC N8-GP depot persisted up to 6 h after injection, with a marked reduction at 24 h and minimal N8-GP detected at 72 h. Three injections of N8-GP at the same site did not lead to detectable accumulation. No degraded N8-GP was observed. The immunohistochemical reactivity for N8-GP was mostly interstitial and, to a lesser degree, cell associated. Double immunofluorescence identified a large proportion of these cells as CD11b-positive myeloid cells.

Conclusions: The disappearance pattern of N8-GP from injection sites in mice is supportive of further development of SC N8-GP as a convenient prophylaxis option for patients with haemophilia A.

PB 1083 | Thrombin Generation Profiling in Amish Factor IX Carriers and Controls: Plasma Composition and Bleeding Phenotype

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Background: Hemophilia B carriers differ in their factor (F)IX activity levels even in the same pedigree. Although FIX activity serves as a surrogate marker for potential bleeding tendency, understanding

the balance between procoagulant and anticoagulant proteins that regulates thrombin generation may improve assessments of bleeding potential.

Aims: Compare and contrast an Amish cohort of FIX Carriers and Controls with respect to thrombin generation and bleeding scores (BS).

Methods: Samples were collected from 170 Amish subjects, 68 Controls and 102 FIX Carriers, all with c.1025 C>T; p.Thr342Met genetic mutation. Thrombin generation in response to 5 pM tissue factor for each individual was calculated by a mathematical model incorporating levels of procoagulants: FII, V, VII, VIII, IX, X, and the anticoagulants: antithrombin, tissue factor pathway inhibitor and protein C. Thrombin generation parameters included time to clot, maximum rate (MaxR), maximum level (MaxL) and area under the curve. BS was assigned based on the Tosetto scoring system using an in-house developed bleeding questionnaire.

Results: Carriers were significantly younger in age, lower in functional activity of FIX, FVII and in MaxL of thrombin generation as compared to Controls (Table). Anticoagulants were not significantly different between groups. Across the entire cohort, MaxL and MaxR correlated with FIX activity (all r>0.7). FIX activity alone did not correlate with BS (r=0.02). BS was low overall and there was no difference between Carriers and Controls 1.3(0,8) vs. 1.4(0,9). However, when individuals were grouped based on BS (0, 1-2, 3+), there was a trend correlating higher BS with lower MaxL in Carriers, conversely Controls with lower BS trended with lower MaxL.

TABLE 1 Comparison of Carriers and Controls

	Carriers, N = 102	Controls, N = 68	
	Mean ± SD	Mean ± SD	p-value
Age, in years	21 ± 17	35 ± 20	< 0.0001
FIX activity, %	64 ± 27	115 ± 28	< 0.0001
FVII activity, %	108 ± 25	121 ± 33	0.003
Thrombin MaxL, nM	338 ± 116	412 ± 100	< 0.0001

Conclusions: The combination of suppressed procoagulants FIX and FVII in Carriers despite comparable anticoagulants relative to Controls, yielded lower levels of thrombin and trended with higher BS. Further studies are warranted.

PB 1084 | The Global Annual Bleed Rate in Haemophilia: A 2015 Estimate

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Background: Current global metrics to describe haemophilia burden reflect prevalence and access to care (e.g., IU/capita and proportion of persons with haemophila receiving prophylaxis). Additional metrics reflecting patient outcomes would improve our understanding of the burden of bleeding in haemophilia at the population level.

Aims: The primary objective was to estimate the number of bleeding episodes experienced by the known haemophilia population worldwide and highlight opportunities to collect data to track bleeding outcomes globally.

Methods: A literature-based model was developed to estimate country-specific haemophilia prevalence, broken down by presence of inhibitors, severity, age and prophylaxis use for each country. The primary data source is the World Federation of Haemophilia Annual Global Survey (AGS) (2012-2015). The mean ABRs associated with each severity level, age group, and treatment modality were obtained through a literature search. These were incorporated to estimate the total number of bleeds that occur annually in persons with haemophilia A and B in every country. One-way sensitivity analyses were performed to understand which model input variables contribute the most to the global bleed estimate.

Results: Among the known haemophilia population (n = 189,494), an estimated 2,509,817 bleeds occur yearly. In the projected haemophilia population (n = 658,418), the global ABR may be as high as 12,147,263. Given these results, a bleeding episode is estimated to occur every 3 to 12 seconds.

Conclusions: This project is the first attempt to estimate the total annual number of bleeds for people with haemophilia across the world. The results provide insight into the magnitude of the burden of bleeding to further strengthen our understanding of haemophilia today.

PB 1085 | Mortality of Hemophilia in Brazil: First Report

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Background: Brazil has the fourth largest population of hemophilia patients in the world, accounting for 11,857 individuals registered in 2015 (World Federation of Hemophilia, 2015). However, mortality rates over the years in this population are unknown.

Aims: To analyse the hemophilia mortality ratio in Brazil from 1997 to 2014, using the Brazilian National Mortality Information System (SIM) as data source.

Methods: Death of patients with hemophilia were identified according to the 10th International Classification of Diseases (ICD-10). Standardized mortality ratios (SMR) were calculated to estimate the rate of overall death of patients with hemophilia relative to that of the Brazilian general male population. Data were adjusted for age

and calendar period. Statistical analyses were performed using Stata (STATA Corp LP, USA).

Results: From January 1997 to December 2014, 821 (94%) patients with hemophilia A (HA) and 53 (6%) patients with hemophilia B (HB) were identified. Mortality of patients with HA was 22% higher when compared with the general male population (SMR 1.22, CI 1.13-1.30). SMR decreased over the years, with exception of the period between 1997-1998, which might have suffered influence of underreported deaths. Hemorrhage was the most frequent cause of death related to hemophilia, described in 268 patients (30.7%). Of these, 142 (53%) presented intracranial haemorrhage. The frequency of HIV related to hemophilia decreased from 1997 (26.5%) to 2014 (12.2%). Otherwise, deaths related with cardiovascular disease increased from 11% to 24% in the same period. A total of 130 (14.9%) deaths were related with hepatitis infection, of whom 110 (84.6%) also presented other liver diseases.

Conclusions: HA patients have a higher risk of death in comparison with general male population. Intracranial hemorrhage is still an important cause of death in Brazilian patients with hemophilia. Death due to cardiovascular disease has increased over the years, following the same tendency observed in the developed world.

PB 1086 | Alternative Payment Models in Hemophilia

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Background: Intravenous injection with replacement factors VIII and IX is the mainstay of treatment for hemophilia. After decades of similar products, novel therapies with diverse characteristics (eg, long-acting products) have recently been developed. Current payment for replacement factor is based on price-per-unit, but new payment models are needed that acknowledge patient benefit and provider/payer expectations for evidence-based, cost-effective healthcare. For products with similar properties, price-per-unit procurement is reasonable, but entry of diverse therapies into the market drives a need for payment models that support innovation and optimize patient care, accounting for all relevant aspects.

Aims: To develop alternative payment models for hemophilia that foster improvement of patient care and drive innovation.

Methods: An international, multidisciplinary panel of HEOR and hemophilia experts convened to develop concepts for payment models in hemophilia, taking into account strategies to support innovation, while optimizing patient care, and entry of long-acting replacement factor and non-replacement products.

Results: Two payment model concepts were developed: a pay-per-patient model that uses fixed payment based on expected healthcare utilization of an average patient per year, and a pay-per-outcome model where payment is based on achieved outcomes. The pay-per-patient model is expected to lead to physicians and patients selecting treatments that satisfy individual patient needs, while controlling the total cost for the provider/payer. Payment is independent of achieving treatment goals, but influenced by treatment guidelines and local policy. In the pay-per-outcome model, achievement of predefined outcomes is rewarded. This model encourages innovation that improves outcomes better than available products today.

Conclusions: Development of new payment models in hemophilia, such as pay-per-patient and pay-per-outcome models, can improve quality of care, drive innovation, and ensure access to new products.

PB 1087 | Rapid and Cost-effective FVIII Intron Inversion 22 Screening of the Central South African Haemophilia A Population

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Background: An inversion of intron 22 (Inv22) of the FVIII gene is reported in roughly 45% of all severe haemophilia A cases. Until recently, only the Southern Blot assay was available in a single laboratory in Johannesburg, South Africa for Inv22 detection. Therefore, the Inv22 status of a large part of the South African haemophilia A population is mostly unknown.

Aims: The aim of our study was to use our newly developed rapid and cost effective gel-based reverse transcription PCR (RT-PCR) assay to detect the Inv22 mutation in our local haemophilia A population.

Methods: We recruited 59 (n=59) volunteers who attended haematology clinics in Bloemfontein and Kimberley in central South Africa. We recruited 33 (n=33) males with severe haemophilia A, 1 (n=1) male with mild haemophilia A, 20 (n=20) female putative carriers, and 5 healthy controls. Volunteers gave informed consent prior to participation. Venous blood was stabilized on Trizol reagent. RNA was extracted and cDNA synthesis was performed. A conventional PCR was performed according to our newly developed published method and the PCR product was visualized on a 2% agarose gel.

Results: We detected the Inv22 mutation in 10 (n=10) out of the 33 male volunteers with severe haemophilia A, which accounts for roughly 30%. Inv22 was detected in 5 (n=5) of the 20 putative carriers, which is 20%. The Inv22 mutation was detected in neither the male with mild haemophilia A nor any of the controls. All volunteers without the Inv22 mutation (n=44) were positive for the wild-type.

Conclusions: We have successfully implemented the newly developed rapid and cost-effective Inv22 screening assay in our laboratory. The presence of the Inv22 mutation in the central South African severe haemophilia A population is lower than is reported globally (30% vs 45%). However, this may be attributed to the relative low sample numbers. Conversely, we believe that this new relatively uncomplicated method will make more wide-spread Inv22 testing in South Africa a reality.

PB 1088 | Patient Satisfaction of Home Delivery Program in Haemophilia through an Association of Patients

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Background: Most of the clotting factor (CF) dispensations to patients with haemophilia in Valencian Community are centralized in only one hospital, generating frequent visits to hospital, and requiring long travel distances. Since 2011, the Outpatient Pharmaceutical Care Unit (OPCU) established collaboration with the *Hemophilia Patients Association of the Valencian Community* (ASHECOVA) to develop together a home delivery program, allowing patients an easier and convenient access to their treatments.

Aims: To evaluate the satisfaction level of patients included in the home delivery program.

Methods: A questionnaire with 26 questions was used to explore organizational aspects, education and communication between patients and professionals, the use of apps, as well as the satisfaction level of certain aspects that concerned the delivery of medication. The satisfaction was also measured using a previously validated survey (Likert scale), with open answers and 10 possible closed answers
(1 disagree and 10 strongly agree). A specific Google® form was developed for this study.

Results: Thirty-eight of 44 asked patients, answered the survey (men, 38.8 mean age). Benefits mostly reported by 95% of patients were less frequent visits to hospital, reduced time and costs spent in transportation. Ninety-five percent of individuals believed that the program improves treatment adherence and 92% would be interested in using an app for register the CF administration. Frequency and horary of delivery were adequate for 100%. All patients would recommend this system to other patients and overall satisfaction extent was 9.7 (DE: 0.5).

Conclusions: Home delivery program guarantees a proper follow-up of treatments, obtaining full satisfaction evaluation from patients. This program improves patient´s convenience and overall score was very satisfactory. In view of the results, we will implement an app for recording CF administrations.

PB 1089 | Contribution to the Molecular Study of Hemophilia B in an Algerian Population

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Background: Hemophilia B (HB) is a hereditary X- linked recessive bleeding disorder of coagulation caused by mutation in factor IX (FIX) gene that result in FIX deficiency. Based on the plasma levels of FIX, HB is classified as severe (<1%), moderate (1-5%) and mild (>5-30%) and the severity of the disease is in close correlation with the type and position of the mutation in FIX gene.

Aims: The objective of this study was to identify the mutations that produce different forms of HB disease among Algerian patients, to characterise mutations of the FIX gene and to develop our knowledge about the molecular basis of this disease.

Methods: 9 patients with severe HB, were enrolled in this study. All patients were male. About 5-10 ml of peripheral blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA), and DNA was extracted by the salting out method. As most of the exons in the HB gene are relatively short, direct sequencing of a complete exon is possible once it has been amplified from genomic DNA with the polymerase chain reaction (PCR) technique , so, all exons of the FIX gene were amplified by PCR. The HB mutation was identified by automated sequence analysis using the Capillary electrophoresis method.

Results: 2 FIX gene mutations were detected, a point mutations (2 missense mutations). The first was c.323G>A (Cys 108 Tyr) located in the epidermal growth factor1 (EGF1) domain ; is coded by exon 4. The second revealed missense mutation 881G>A (p.Arg 294 Gln) within the protease domain, the greater part (codons 280-451) is coded by the largest exon in the FIX gene (exon 8).

Conclusions: This study confirms the correlation between the type of mutation and severity form. we showed in this study, the importance of molecular analysis for the characterization of HB in patients. HB is the result of a genetic error. Identifying this error is very important for patients because it helps to confirm the diagnosis and predict complications of treatment.

PB 1090 | A Hand-held Electronic Patient Diary for Haemophilia Home Care-pilot Study in Poland

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Background: Permanent administration of the missing factor in certain days in order to prevent bleedings is only a prophylactic treatment. To enable the doctors to monitor their patients' treatments, the diaries were introduced, in which those very patients record and write down all the undertaken courses of action. Nevertheless, owing to the fact

that patients see their physicians 1-2 times a year, those courses may turn out to be insufficient.

Aims: The aim of that pilot survey was to assess whether the electronic device can replace a diary and if so, whether will it improve patient care through monitoring both the haemorrhages and the consumption and reserves of the factor VIII. 30 patients from 6 different centres in Poland participated in this survey from 01.12.15 to 31.05.16. The mean age of patients in the study was 2-7 years (range 4.85) All of them were prophylactically given Advate (Baxter) three times a week at a dose of 35-40 IU/kg^{-1}

Methods: -

Results: In six months' time the bleedings were observed in 16 cases, while the other 14 patients didn't undergo any bleeds. Three out of those 16 cases experienced haemarthrosis, two -haemorrhages into soft tissues and the rest eleven - both haemarthrosis and haemorrhages into soft tissues. In ten cases patients that were given the factor VIII on Monday, Wednesday and Friday went through a haemorrhage on Sunday.

A problem with noting blood transfusions down in a diary occurred in three cases. Four patients required increasing the dose due to the reoccurrence of the haemorrhages.

Conclusions: The study shows that the electronic documentation system is feasible for hemophilia patients and provides the physician with the opportunity to monitor patients more closely. However, not all patients seem to be qualified for using that electronic tool, and the tool must run dependably without major errors for ensuring reliability and acceptability.

PB 1091 | Induction of FVIII Tolerance by Gene Transfer of IL2 and Factor VIII Plasmids in Hemophilia A Mice

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Background: Hemophilia A (HemA) patients lack functional FVIII protein, which results in an inability for the blood to clot when injury occurs. The current treatment for HemA patients is FVIII protein replacement, but this treatment often results in anti-FVIII immune responses, neutralizing the clotting effect.

Aims: Regulatory T cells (Tregs) help balance T effector cells (Teffs) through their suppressive function during an immune response. Tregs possess a high affinity epitope of the IL2 receptor. With a low dose of IL2, the expansion of Treg population exceeds the proliferation of Teffs, leading to a significant increase in activation and number of Tregs and Treg/Teff ratio. The increased suppressive activity can induce tolerance in HemA mice treated with FVIII plasmid gene therapy.

Methods: HemA mice were hydrodynamically injected with either 1 μg, 2 μg or 5 μg IL2 plasmid and 50 μg FVIII plasmid sequentially 2 weeks apart. Both plasmids were driven by a liver-specific promoter (hAAT-HCR). The following assays were performed at various time points: cell staining, APTT, Bethesda, ELISA.

Results: Flow cytometry analysis showed a dramatic increase in Treg population and activation in all three groups, demonstrating that a small amount of IL2 plasmid transferred into liver cells can significantly increase the Tregs. HemA mice injected with 5 μg IL2 plasmid showed a large increase in Teffs and no measurable FVIII levels, possibly due to the inadvertent activation of cytotoxic T lymphocytes. Mice injected with 2 μg IL2 plasmid had less Teff activation than the 5 μg group, and expressed stable levels of FVIII through Week 19 post FVIII plasmid injection. Mice injected with 1 μg IL2 plasmid expressed higher FVIII levels and are monitored for long term tolerance.

Conclusions: Combined gene transfer of FVIII and IL2 plasmids can produce therapeutic FVIII and simultaneously prevent inhibitory antibody formation, thus providing a potentially effective treatment of hemophilia A.

PB 1092 | Therapeutic Expression of Factor VIII Following Ultrasound-mediated Gene Delivery of High-expressing FVIII Variant Plasmids and Immunomodulation in Hemophilia A Mice

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Background: Ultrasound (US)-mediated gene delivery (UMGD) of a liver-specific (LC)-FVIII plasmid produced averagely ~15% of FVIII expression on day 1 in hemophilia A mice. However, anti-FVIII immune responses ensued following gene transfer significantly decreased FVIII levels.

Aims: In order to achieve persistent FVIII gene expression for therapeutic treatment, several improvements are pursued:

(1) Construct a high-expressing FVIII variant cassette.

(2) Reduce UMGD pressure by increasing pulse durations.

(3) Employ immunomodulation to suppress anti-FVIII immune responses.

Methods: A novel FVIII variant with 10 aa substitutions in A1 domain (F8X10, from Weidong Xiao) is incorporated to generate LC-F8X10. UMGD is carried out using a semi-focused transducer (H158) at acoustic pressure 0.4-2.7 MPa and pulse duration 0.018-0.4 ms. Mice were treated with immunomodulation using either dexamethozone for 5 days to reduce innate and adaptive responses and IL2/IL2mAb complexes for 4 days to induce regulatory T cell expansion or dexamethasone for 10 days and IL2/IL2mAb complexes for 7 days.

Results: LC-F8X10 generates more than 10 fold higher FVIII expression than LC-F8/N6 plasmid. We observe less liver damage following UMGD of LC-F8X10 at 1.0Mpa and 0.4 ms pulse duration evaluated by transaminase levels and histological examination. All groups of mice yield 25- 100% of FVIII on day 1-7. In mice without immunomodulation, FVIII levels drop to undetectable at day 14 and afterwards with

the formation of high-titer antibodies (>50 BU). In mice with shorter immunomodulation, the formation of low titer antibodies (~1 BU) is delayed at day 21 and 28 however the titers increase later. In mice with longer immunomodulation, no inhibitory antibody is observed during experimental duration and the FVIII expression remains at therapeutic levels (10-50%).

Conclusions: UMGD of high-expressing FVIII variant plasmids together with immunomodulation can achieve long-term, safe and effective treatment of hemophilia A.

PB 1094 | Integration Site Analysis in Mice Demonstrates Excellent Biosafety Profile of a Recombinant (R) FVIII Adeno-associated Virus (AAV8) Gene Therapy Product

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Background: A safety concern in gene therapy using rAAV is residual integration of the viral genome into the host genome. While rAAV vectors are thought to integrate at non-homologous locations without creating hot spots, Nault et al. (2015) identified chromosomal insertions of wild-type AAV2 that may have activated proto-oncogenes in human hepatocellular carcinoma (HCC). Integration site (IS) analysis of newly developed rAAV vectors is thus warranted to assess AAV gene therapy product biosafety.

Aims: To analyze IS in mice to determine the integration profile, characteristics, and clonal dominance of Shire's rAAV8 investigational product to treat hemophilia A (SHP654).

Methods: FVIII ko mice were treated with SHP654 at doses of 4E+12 and 2E+13 vg/kg. Livers were harvested 1 and 4 months thereafter and processed for DNA isolation. Vector copy number (VCN) was determined by vector specific qPCR. Non-restrictive (nr) and standard linear amplification-mediated (LAM) PCR was used to identify genomic sequences flanking the integrated AAV vector DNA. (nr) LAM-PCR amplicons were sequenced after sample preparation on a MiSeq instrument. Data were processed by (semi-) automated bioinformatic data mining.

Results: Liver transduction levels were ~13.7 and 16.8 for lower vector doses at 1 and 4 months, and ~106.5 and 115.8 for higher doses. Frequency of rearranged fragments correlated with the vector dosage, and ITR breakage occurred homogenously between each vector group. Sequencing of (nr)LAM-PCR products revealed 227 and 187 unique exactly mappable IS in all mice at 1 and 4 months. The frequency of common IS was low at both time points and only few identical IS were detected in repetitive (nr)LAM-PCR amplicons after dosing of SHP654.

Conclusions: IS analysis of SHP654 showed measurable, yet minimal integration (< 0.01%) with no indication of side effects, as neither clonal outgrowth nor preferred integrations in or next to genes previously implicated in HCC formation was observed.

PB 1095 | A Novel Strategy for Liver Cell-based Therapy in a Hemophilia B Model

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Background: Although hepatocyte transplantation has been demonstrated to be a promising approach for hemophilia B, the poor engrafted efficiency of donor cells in the recipient liver limits its clinical applications. In our previous study, we can control the diameter of the hepatic sinusoids by regulating hepsin levels without adverse effects. We hypothesized that this phenomenon can be applied in hepatocyte transplantation.

Aims: We tested whether the combination of temporally narrowing hepatic sinusoid with a hyperfunctional coagulation factor IX (FIX), FIX-Triple, could enhance the therapeutic efficiency of hepatocyte transplantation in hemophilia B.

Methods: The pretreatment of the anti-hepsin antibody or control IgG was administrated once daily for four days right before transplantation. Hepatocyte transplantation was implemented by the intrasplenic injection of the primary hepatocytes from FIX-WT or FIX-Triple donor mice into the recipient mice.

Results: The anti-hepsin pretreatment increased the transplanted cells in the liver of hemophilia B mice by 2 fold. Due to the enhanced engraftment rate, FIX clotting activity in the hemophilia B mice with the anti-hepsin pretreatment could increase from 0.84 to 1.99% by transplanting FIX-WT donor cells. When replacing FIX-WT with FIX-Triple donor cells, FIX clotting activity could further increase respectively to 4.40% with the control IgG pretreatment and 7.72% with the anti-hepsin pretreatment. Remarkably, the anti-hepsin pretreatment strategy coupled with FIX-Triple donor cells maintained the FIX clotting activity more than 5% after one month of transplantation. This strategy also produced a significant improvement of the bleeding phenotype by tail clipping assay.

Conclusions: Our data suggest that the strategy of controlling the diameter of hepatic sinusoids by the anti-hepsin pretreatment would be useful for improving the cell-based cure in hemophilia B.

PB 1096 | Immunity to AAV8 and Other AAV Serotypes in Healthy Individuals

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Background: A major challenge for Adeno-associated virus serotype 8 (AAV8) gene therapy is pre-existing anti-AAV8 immunity. Up to 40% patients with pre-existing neutralizing antibodies (Nabs) against AAV8 are currently excluded from clinical trials. The prevalence of anti-AAV8 Nabs and Nabs to other serotypes is not known due to the use of different and not standardized assay protocols and reagents. Development of strategies to circumvent pre-existing anti-AAV8 immunity requires careful assessment of the prevalence of pre-existing immunity to AAV8 and other serotypes with validated assays.

Aims: To determine and characterize the prevalence of anti-AAV8 immunity and co-prevalence of immunity to AAV1, AAV2, and AAV5 in healthy individuals using validated assays.

Methods: Three cohorts of healthy individuals from the US and Europe were selected to assess potential regional differences in pre-existing AAV immunity. The prevalence of anti-AAV8 Nabs was determined using a validated assay. Binding antibodies (Babs) and AAV8 T cell responses were assessed by ELISA and IFNg-ELISPOT, respectively. To assess co-prevalences, Nabs and Babs against AAV1, AAV2, and AAV5 were analyzed.

Results: In contrast to Boutin et al, who report an AAV8 Nab prevalence of about 20%, the prevalence of anti-AAV8 Nabs in this study was 30 - 45%. A high degree of co-prevalence with Nabs against AAV1, AAV2, and AAV5 was detected, which may be due to natural infections or development of cross-reactive antibodies upon infection with one serotype. Anti-AAV8 T cell responses appear to correlate only to some extent with anti-AAV8 Nab prevalence.

Conclusions: The high prevalence of anti-AAV8 Nabs and high co-prevalence of other serotypes independent of geographic region suggest that e.g. patients with pre-existing AAV8 immunity cannot be easily included in gene therapy trials using other AAV serotypes. Thus, strategies to circumvent pre-existing AAV8 immunity are required.

PB 1097 | Development and Application of Methods for the Selective Measurement of the Human Single Amino Exchange Variant Factor IX Padua

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Background: The BAX 335 hemophilia B gene therapy trial relies on the expression of FIX Padua, a naturally occurring single amino acid exchange (R338L) variant with a 10-fold higher activity than that of FIX wild type. Assessment of the gene therapy's success would benefit from methods that enable specific detection of the transgene product.

Aims: To develop FIX Padua-specific methods using a Fab2 mini antibody selectively binding this FIX variant and their application for testing of patient plasma samples of the BAX 335 study.

Methods: A FIX Padua-specific ELISA was established by using the Fab2 mini antibody to capture FIX Padua and a polyclonal anti-FIX antibody to detect bound protein. The performance of this ELISA was checked in terms of accuracy, precision and parallelism. A chromogenic FIX activity assay specific for FIX Padua was developed by similarly capturing FIX Padua with the Fab2 mini antibody.

Results: The ELISA which was designed to selectively capture FIX Padua covered a FIX Padua protein concentration range of 0.9 to 27.1 ng/mL. Spike-recovery carried out with representative patients' samples showed acceptable recoveries and there was no influence of the citrated plasma matrix on the assay performance. There was also a clear correlation between FIX Padua protein concentration and FIX activity, while the presence of functionally inactive FIX had no impact on the assay. The calibration curve of the FIX Padua-specific chromogenic activity assay, carried out after selective capture of FIX Padua with the Fab2 mini antibody, ranged from 0.1 to 3.3 mU FIX Padua/mL, while a normal reference plasma pool introduced in the assay at a FIX concentration of 0.1 U/mL showed no response.

Conclusions: The FIX Padua ELISA can be used in clinical settings to selectively quantify FIX Padua antigen levels. The Fab2 mini antibody offers also the possibility to set up a clinical FIX Padua-specific chromogenic activity assay.

PB 1098 | Biological Significance of Neutralizing Antibodies to AAV8

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Background: Pre-existing neutralizing antibodies (Nabs) against Adeno-associated virus 8 (AAV8) have been shown to efficiently inhibit AAV8 gene therapy, and thus to be a major hurdle in AAV8 gene therapy. Identifying a reliable and efficient anti-AAV8 Nab assay to screen patients who can be treated effectively is therefore crucial.

Aims: The present study was conducted to assess the biological relevance of *in vitro* anti-AAV8 Nab titers to determine those AAV8-Nab titers that can predict which patients are eligible for gene therapy.

Methods: Anti-AAV8 Nab titers in healthy donors were determined by a validated *in vitro* reporter assay using 2x serial dilutions starting at 1:5. Donors with a Nab titer < 1:5 were evaluated as negative. The biological relevance of *in vitro* Nab titers was assessed in comparative *in vivo* studies by injecting NOD/SCID mice with human plasma containing anti-AAV8 Nabs prior to administering AAV8 gene therapy.

Results: The *in vivo* mouse studies showed that an anti-AAV8 Nab titer of 1:5 in the circulation of mice almost completely blocked AAV8-mediated transgene expression in the liver, demonstrating that this titer is of high biological relevance. The 1:5 cut-off appears to be robust since the vast majority of Nab negative healthy donor samples were also negative at dilutions of 1:2.5 and 1:1.25.

Additionally, we could show that low antibody titers (1:5 to 1:40) can be overcome using significantly higher gene therapy doses.

Conclusions: An anti-AAV8 Nab titer of 1:5 is a valid exclusion criterion for clinical studies. Our data suggest that the *in vitro* assay is more

suitable for screening of patients, since the *in vivo* assay cannot detect lower Nab titers (< 1:160) due to dilution of human Nabs in the circulation of mice. Whether low titer anti-AAV8 Nabs can be overcome remains to be confirmed in an immune competent mouse strain.

PB 1099 | Nonclinical Safety Evaluation of a FVIII Gene Therapy Construct in Mice

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Background: Shire is developing a gene therapy to treat hemophilia A using an adeno-associated virus (AAV) vector and a codon optimized human BDD-FVIII transgene.

Aims: The present study was performed to determine the toxicity of our FVIII gene therapy candidate SHP654 (AAV8.BDD-FVIII) following a single intravenous administration at dose levels of $4x10^{12}$, $8x10^{12}$, or $2x10^{13}$ capsid particle/kg to C57BL/6J mice.

Methods: Acute and delayed onset of toxicity and/or reversibility of toxicity during an 18-week observation period, biodistribution to tissues, FVIII activity and development of binding and neutralizing antibodies were assessed. Animals were sacrificed on day 3, in week 3, or week 18 after injection of SHP654 to evaluate toxicity and biodistribution.

Results: Single intravenous administration of SHP654 to C57BL/6J mice was well-tolerated at all dose levels, with no clinical signs, toxicologically relevant effects, or changes in clinical or anatomical pathology observed. In all of the tissue samples harvested, the distribution of SHP654 DNA was dose-related and generally highest at the earliest time point, decreasing significantly by week 18. Consistent with the hepatic tropism of AAV8, SHP654 DNA was predominantly detected in the liver and higher levels were still maintained at this time. In contrast, SHP654 DNA levels in the brain and testes samples were evaluated as negative by week 18. Animals treated with SHP654 showed higher levels of FVIII activity and human FVIII antigen compared to baseline, thus confirming exposure to human FVIII.

Conclusions: On the basis of these toxicological results, the NOAEL in this study was considered to be $2x10^{13}$ cp/kg, which was the highest dose tested.

PB 1100 | Preliminary Results of SPK-9001 Gene Transfer Demonstrate Statistical Improvements on the Health-related Quality-of-Life in Adults with Haemophilia B

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Background: Consistent and sustained FIX activity levels >12% after gene transfer may eliminate spontaneous joint bleeding, reduce factor injections and risks of non-adherence. However, the effectiveness of gene transfer on such patients' health-related quality-of-life (HRQoL) after gene transfer is unknown.

Aims: To determine the impact of gene transfer on HRQoL in haemophilia B adults with ≤2% of FIX activity levels.

Methods: Nine haemophilia B (endogenous FIX level of ≤2 IU/dL) adults (mean age 33.6±13.6 years) received a single $5x10^{11}$ vg/kg infusion of *SPK-9001*, an investigational gene transfer product. HRQoL was assessed pre-vector (baseline) and post-vector (weeks 4, 12, 26 and 52) infusions using the generic EQ-5D and the haemophilia-specific Haem-A-QoL questionnaires. Differences were calculated from baseline (B) to post-vector (PV: recent follow-up assessment). As minimal clinically important differences (MCID) for evaluating subject-level HRQoL improvements responder definitions (RDs) of the Haem-A-QoL domains and EQ-VAS were estimated, based on the one-half standard deviation criterion at baseline.

Results: As of 1/25/2017 data cut-off, nine participants have been followed-up for 12 - 52 weeks after gene transfer. All participants reported in general good HRQoL in the EQ-5D (B: M=80.56±8.8 vs. PV: M=88.22±10.3; no statistical significant improvement). By contrast, significant mean improvements from B to PV were found for the Haem-A-QoL domains ‚Work' ($p< .030$), ‚Treatment' ($p< 0.011$), ‚Future' ($p< 0.004$) and ‚Total Score' ($p< 0.003$). At baseline, 50% thought that ‚never/seldom' "things will be better in the future" compared to 0% at PV. All patients (100%) were defined as HRQoL responders according to the RDs of the Haem-A-QoL ‚Total Score' (3.6-point decrease) and 7 out of 9 patients according to EQ-VAS (4.4-point increase).

Conclusions: Improvements in Haem-A-QoL domains ‚Treatment' and ‚Future' and ‚Total Score' suggest a single infusion of SPK-9001 resulted in meaningful HRQoL improvements.

PB 1101 | Dose Response and Long-term Expression of a FVIII Gene Therapy Construct in Hemophilia A Mice

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Background: Shire is developing a gene therapy to treat hemophilia A, using an adeno-associated virus (AAV) vector and a codon optimized B-domain deleted *F8* transgene.

Aims: The presented studies were performed to investigate the dose response in FVIII activity/efficacy and the long-term expression of FVIII gene therapy candidate AAV8.BDD-FVIII (SHP654) in hemophilic mice.

Methods: Twelve male FVIII knockout mice per group received a single intravenous dose of $1x10^{12}$, $4x10^{12}$, or $1x10^{13}$ vg/kg SHP654 or

10 mL/kg buffer. Human FVIII activity level was determined every 2 weeks for 8 weeks. At the end of the observation period, the animals' bleeding phenotype was assessed using a tail-tip bleeding assay. Long-term expression of SHP654 was assessed in human FVIII transgenic (huFVIIItg) mice. These mice carry a knockout of murine *F8* gene and transgenically express human FVIII mRNA transcripts in multiple tissues from human *F8* cDNA [van Helden et al., 2011], but lack detectable circulating FVIII protein. Thirty huFVIIItg mice received a single intravenous bolus injection of $4x10^{12}$ cp/kg SHP654, and blood samples, taken over 24 weeks, were analyzed for FVIII activity and anti-FVIII antibodies.

Results: Treatment of FVIII knockout mice with $4x10^{12}$ or $1x10^{13}$ vg/kg SHP654 yielded a mean FVIII activity of 0.6 and 2.1 IU/ml on day 56, and significantly reduced blood loss in the tail-tip bleeding assay. After treatment of huFVIIItg mice with $4x10^{12}$ cp/kg SHP654, a mean FVIII plasma activity of 2.9 - 3.8 IU/mL was determined, which persisted over 24 weeks.

Conclusions: SHP654 doses above $1x10^{12}$ vg/kg were shown to be efficacious in FVIII knockout mice, and resulted in persistent FVIII expression in huFVIIItg mice.

PB 1102 | Pre-existing Anti-AAV5 Neutralizing Antibodies Measured Using a Highly Sensitive Assay in Sera of Hemophilia B Patients in a Phase I/II Clinical Trial of AMT-060 Do Not Predict Efficacy of AAV5-mediated Liver-directed Gene Transfer

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Background: Gene transfer via adeno-associated viral (AAV) vectors is currently believed to be impaired by the presence of anti-AAV neutralizing antibodies (NAB). Patients (pts) are screened for NAB and excluded from treatment if determined as positive in the assay implemented; however, assays vary in sensitivity. We previously treated 10 pts with severe hemophilia B who tested negative for pre-existing anti-AAV5 NABs as measured by a green fluorescent protein-based assay with an AAV5-hFIX (AMT-060) in a Phase I/II study. Recently a novel, highly sensitive NAB assay based on a luciferase reporter vector became available.

Aims: To re-assess pre-existing anti-AAV5 NAB status in our Phase I/II pts using the luciferase-based assay and determine the relationship of any titers detected to therapeutic outcomes.

Methods: Pre-treatment screening samples from 10 pts with factor IX (FIX) activity < 2% of normal enrolled in a Phase I/II study of AMT-060 were analyzed for anti-AAV5 NAB with the luciferase-based NAB assay. Therapeutic outcomes included FIX activity, AAV5-specific T-cell mediated immunity assessed by human interferon gamma Elispot assay, and liver enzyme levels.

Results: By the luciferase NAB assay, 7/10 pts returned below the limit of detection and 3/10 had positive titers (all in low dose cohort $5x10^{12}$ gc/kg). The pt with the highest NAB titer (1:341) presented the highest FIX activity (6.8%) in the dose cohort. The other 2 pts presented titers of 1:211 and 1:22 and FIX activity of < 2% and 3.0%, respectively. FIX activity spanned the range seen in the entire cohort (< 2-6.8%; n=5). None of the 3 positive pts experienced elevations in liver enzymes post-treatment. No clinically relevant T-cell responses to the capsid were detected.

Conclusions: NAB titers detected by the higher-sensitivity luciferase-based assay in pts with hemophilia B previously screened with the GFP-based assay did not predict efficacy or safety outcomes of AAV5-based gene transfer.

PB 1103 | Manufacturing Scale AAV Vectors: Quantification Strategy for Clinical Trials

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Background: Classical potency assignment for adeno-associated virus subtype 8 (AAV) clinical trial material aims to quantify the AAV vector by measuring the vector genome DNA. This approach often involves high assay variability, i.e. a coefficient of variability (CV) of ≥ 60%, and thus renders it difficult to control dosing in dose-escalation clinical trials.

Aims: To develop a strategy for potency assignment based on surrogate measurement (AAV8 ELISA) supported by a complementary assay portfolio and consistent large scale manufacturing process.

Methods: The production process for gene therapy vector (AAV8) in a HEK293 cell line was internally developed and scaled up to manufacturing scale for clinical trials. The AAV vector lots produced in pilot (200L) and manufacturing (500L) scale were fully characterized by qPCR, AAV8 ELISA, cryoEM, native agarose gel electrophoresis, and in vivo potency testing of the FVIII gene therapy vector in FVIII ko mice.

Results: Methods qualification and/or validation showed a CV of ≤10% for AAV8 ELISA, ≤43% for qPCR, and ≤1% for cryoEM. The lots produced in pilot and manufacturing scale achieved comparable and constant amounts of full AAV8 vector (73 ±3%) as measured by cryoEM. Since the AAV8 ELISA showed lower variability than qPCR, and the fact that production achieved a constant amount of full AAV8 in all preparations, this assay was used for potency assignment for these lots. The correctness of AAV8 ELISA potency assignment was confirmed in the biological activity assay by dosing AAV8 vector based on the AAV8 ELISA.

Conclusions: AAV8 ELISA appears suitable for potency assignment of clinical trial material to allow greater precision and consistency in dosing during trials, if manufacturing can demonstrate lot-to-lot consistency.

PB 1104 | Gold Nanoparticles: A New Approach for Non-viral Gene Therapy of Hemophilia

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Background: Current genetic approaches for hemophilia focuses on viral vectors as transport system to the liver but might lead to malignant transformation. Gold nanoparticles (AuNP) as DNA transport system are completely inert and do not cause immune reactions. The pEPI vector for stable long-term expression contains a scaffold matrix attachment region (SMAR) that facilitates episomal replication without integration. The vector DNA is bound to gold using polyethylenimin (PEI) as linker. This ensures that after cellular uptake, the DNA escapes endosomal degradation.

Aims: To develop AuNPs as carrier system for a non-viral episomally replicating vector in liver cells.

Methods: AuNPs were complexed with PEI and DNA and incubated with different cell lines. Different PEI variants and different relative ratios of the three components were tested. pEPI contains a CMV promotor driving *GFP* as reporter and a G418 resistance gene. A shorter SMAR and the hEF1a promotor were also tested. The transfection efficiency and GFP expression were evaluated by FACS.

Results: 5nm AuNPs were superior to 50nm AuNPs to transport cargo into the cells. The optimal ratio of 5nm AuNP:PEI:DNA was 30µg:9µg:3µg. Coating 5nm AuNP with 25kD linear PEI achieved superior expression compared to branched PEI or JetPEI®. For long-term expression studies using pEPI with short, long and without SMAR, cultures were split 3d after transfection and cells continuously grown for 10 weeks without or with short- or long-term G418 selection. Expression analysis revealed that the short SMAR was always superior to the plasmid with long SMAR. Comparing the CMV with the EF1a promoter in pEPI revealed that EF1a was less subject to silencing. Finally, using AuNP to stably introduce pEPI with a human FVIII cDNA with the short SMAR showed FVIII activity of up to 8% in the cell culture supernatant.

Conclusions: These results show that gold nanoparticles as carriers for DNA are a promising approach for non-viral genetic therapy for hemophilia and other liver disorders.

PB 1105 | Health-related Quality of Life in Pediatric Hemophilia B Patients Treated with rIX-FP

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Background: Bleeds, frequent infusions and pain can impact the health-related quality of life (HRQoL) in children with hemophilia, limiting their ability to lead a normal life. In a multi-centre Phase III study (NCT01662531) pediatric patients received weekly prophylaxis with rIX-FP (IDELVION).

Aims: To determine the impact of rIX-FP prophylaxis on HRQoL in pediatric patients.

Methods: 27 children (mean age 5.9±2.93 years) were enrolled. HRQoL was assessed at baseline and at the end of study (EOS) (≥12 months later). Children completed the hemophilia-specific HRQoL questionnaire (HAEMO-QOL) and their caregivers the hemophilia-specific treatment satisfaction (TS) questionnaire (HEMO-SAT$_P$). Scores range from 0-100; decreasing scores indicate improvements in HRQoL and TS. Minimal important difference (MID) between baseline and EOS were calculated based on the Cohen's *d* effect size (ES). ES of *d=0.2* indicate small effects, *d=0.5* medium, and *d=0.8* large effect size.

Results: At baseline 19 children (age group I: 4-7 years (n=12), age group II: 8-12 years (n=7)) completed the respective age group version of the Haemo-QoL, 23 parents filled in the Hemo-Sat$_P$. Children reported good HRQoL (I: M=19.07±16.9; II: M=26.09±6.3); parents showed a high TS in the Hemo-Sat$_P$ (M=14.45±11.2). HRQoL differences between baseline and EOS were not statistically significant in age group I, but a medium ES was found for ‚physical health' (*d*=.601) and ‚treatment' (*d*=.491). Age group II showed a statistically significant improvement from baseline to EOS in the Haemo-QoL total score (p< .037), medium to big ES were found for most of the domains. Parents reported a statistically significant TS improvement in the dimension ‚burden' of the Hemo-Sat$_P$ (p< .034), small ES were found for most of the domains.

Conclusions: Children showed HRQoL improvement between baseline and EOS, this difference reached statistical significance in age group II. Parents reported a significant improvement in TS.

PB 1106 | Hyperfibrinolysis in Children. Prevalence of Hyperfibrinolysis in Mucocutaneous Bleeders of Unknown Cause in a Pediatric Cohort

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Background: The fibrinolytic system includes a broad spectrum of proteolytic enzymes with a critical role in hemostatic-thrombotic balance. It is also involved in pathophysiological conditions associated with acute bleeding. Mucocutaneous bleeding is a major cause of consultation for bleeding at the outpatient clinic. Von Willebrand (vW) disease and platelet function defects are the most frequent congenital coagulation disorders found in this setting. However, a definitive

cause is not found in 60% of bleeders patients, who therefore are categorized as bleeders of unknown cause (BUC).

Aims: Considering the clinical improvement of bleeding in BUC in response to tranexamic acid, an antifibrinolytic agent, and that standard diagnostic procedures do not routinely include evaluation of fibrinolysis, it appears relevant to determine the potential occurrence of hyperfibrinolysis in BUC.

Methods: Methods. Ninety-three BUC patients aged 2-18 years were recruited from January 2011 to December 2013 in an outpatient clinic. A bleeding assessment tool (ISTH-BAT) score and a second hemostatic workup were performed, including platelet count, platelet aggregation and secretion, PT and aPTT, vW disease diagnostic tests and clot lysis time in plasma rich in platelets activated with ristocetin (CLT-PRP-Ris).

Results: Hyperfibrinolysis was found in 36 patients (38%) and of these patients, 23 exhibited an isolated defect (24%). Forty patients were diagnosed as BUC (40%).

Conclusions: There is a high prevalence of hyperfibrinolysis in pediatric BUC patients after secondary hemostasis assessment including CLT-PRP-Ris. However, a cause of bleeding was not found in 40%. Further studies are warranted to confirm our findings.

PB 1107 | Pregnancy in Factor XI-deficient women

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Background: Factor XI (FXI) deficiency is a rare bleeding disorder, probably underdiagnosed because patients with severe or partial FXI deficiency do not suffer from spontaneous bleeding but may do so only after hemostatic challenge. In particular, women are exposed to a hemostatic challenge during their menstrual period. The other common challenge for women is childbirth.

Aims: The aims of our study was to evaluate retrospectively the presence of postpartum hemorrhage and possible treatment, in pregnant women with FXI deficiency, of varying severity.

Methods: 22 female patients were available for study. Between these, 12 patients had one or more pregnancies (on the whole 26 pregnancies).The average values were of FXI was 0.30 IU/ml (0.002-0.40 IU/ml). Three patients were homozygous resulting in a severe deficiency of FXI (0.002; 0.002; 0.005; respectively), 19 patients were heterozygous resulting in a mild deficiency of FXI (0.25-0.40 IU/ml).

Results: In our center, just three patients received replacement therapy with fresh-frozen plasma (FFP) at delivery time in six pregnancies (three pregnancies in a bleeder patient with FXI :C 0.005 IU/ml, two pregnancies in another bleeder patient with FXI :C 0.002 IU/ml, and just one pregnancy in another patient with FXI:C 0.002IU/ml) without bleeding symptoms at the delivery. Further pregnancies (20 in nine patients) were carried out without replacement therapy because the patients were no bleeders and with FXI :C levels between 0.25 and 0.40 IU/ml. None of the patients have had bleeding manifestations.

Conclusions: In our patients, no postpartum hemorrhages were depicted, but the three patients with severe deficiency were previously treated with FFP. We believe that pregnancy and labor should be managed in close collaboration with the local hemostasis center. Severely deficient individuals and heterozygotes with a clear history of abnormal bleeding and low FXI level usually require blood product support (FFP or FXI concentrate)for labor, especially if delivery is operative.

PB 1108 | Factor XIII Deficiency and Noonan Syndrome: A Tricky Association

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Background: Noonan syndrome (NS) is an autosomal dominant disorder associated with different coagulopathies including thrombocytopenia, platelet function disorders and coagulation factor (F) deficiencies. Haemostatic investigations are recommended in all patients newly diagnosed with NS. Only one case of mild FXIII deficiency in a girl with NS was reported so far.

Aims: -

Methods: -

Results: A 6-year-old girl presented with fever and acute abdominal pain. On admission, she underwent diagnostic laparoscopy with resection of a normal appendix and Meckel's diverticulum. Short after surgery, bleeding from the trocar wounds occurred. Her medical history so far was uneventful. There was no personal or family history of bleeding disorders. Laboratory tests revealed a FXIII of 4% with no other haemostatic abnormalities. Daily FXIII replacement of 30 IU/kg during 5 days was required to overcome rapid FXIII consumption with trough levels below 5%, and to resolve bleeding. Further investigations showed normalization of FXIII activity by mixed plasma test, no mutations on FXIII gene and normal FXIII activity in both parents. These results ruled out the presence of an inhibitor and congenital FXIII deficiency, respectively. A more accurate clinical examination of the girl revealed facial features suggestive for NS, which was confirmed by genetic testing. On follow-up, FXIII activity normalized within 2 months and no further bleeding occurred.

Conclusions: We report the first case of a severe FXIII deficiency in a patient with NS. In this patient, NS was diagnosed only after a first bleeding complication occurred. In addition, FXIII deficiency was transient and recovered spontaneously 2 months after the bleeding episode. Our case suggests that a negative bleeding history or normal haemostatic results do not exclude the occurrence of bleeding complications in risk situations in patients with NS. Thus, timely postoperative monitoring of haemostasis is required to prevent bleeding complications in these patients.

PB 1109 | Dysprothrombinemia Caused by the Homozygote Mutation Ala563Val in the *F2* Gene Was Associated with Reduced Thrombin Generation in a Patient with Bleeding Disorder

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Background: Prothrombin (FII) deficiency is a very rare bleeding disorder (1:2 mill), which may be inherited or acquired. Inherited dysprothrombinemia is an autosomal recessive disorder associated with a moderate to severe bleeding tendency.

Aims: We have characterized a patient with frequent bleeding and low FII activity levels, and his close family, for mutations in the *F2* gene and the effect on FII antigen level and thrombin generation (TG).

Methods: Coagulation assays, prothrombin antigen/activity and TG were performed to characterize the mutation. Mutation analysis of the *F2* gene was performed by Sanger sequencing of all the 14 exons and the exon/intron boundaries.

Results: The index patient was a three year old boy suffering from frequent nose bleeds. The boy had an evident prolongation of the prothrombin time and APTT, a strongly reduced FII activity of ~1%, but the FII antigen was normal, thus he had a Type II prothrombin deficiency (dysprothrombinemia). The patient was homozygous for a missense mutation in exon 13 at position c.1688 C>T (Ala563Val), a mutation only previously described in a compound heterozygous patient. The parents and one of the two sisters turned out to be carriers. The parents were first cousins. In the TG assay, lag time and peak were not detected during the first 90 min, suggesting that homozygosity of the mutation leads to an evidently reduced ability to generate thrombin compared to the heterozygous family members. The heterozygous family members had a FII activity of ~50% and the peaks were reduced by 50% in the TG assay. All heterozygotes were asymptomatic and the last sister was unaffected.

Conclusions: Treatment with Octaplex® was started every fortnight, and TG was monitored at through concentration (14 days), at peak concentration and after 1, 2, and 7 days. The TG was markedly reduced already after 7 days and treatment with Octaplex® was changed to once a week. No bleeds have been observed after initiation of prophylactic treatment with Octaplex®.

PB 1110 | Severe Acquired Hemophilia A, B and C Associated with Lupus Anticoagulant in a Child. A Case Report

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Background: Acquired haemophilia (AH) is a very rare disease in childhood, with an incidence of about 0.045/million/year. It is mainly caused by autoantibodies acting as neutralizing inhibitors anti-factor FVIII, very few cases being reported with inhibitors, concomitently targeting multiple other coagulation factors. The association of lupus anticoagulant is exceptional.

Aims: Our *objective* is to present an extremely rare case.

Methods: We performed coagulation tests associated with immunoserological tests for infections and autoimmunity.

Results: We report a 4 years old girl, without past hystory of bleeding disorder, autoimmunity, infection, malignancy or use of medications. She was admitted with a suggestive purpura for Schoenlein Henoch vasculitis, without clinical or exploratory findings for other diseases. The symptoms were mild (only cutaneous manifestations) and the evolution to resolution took about 7 days. But in contrast with the benign clinical expression, the biological exploration revealed major hemostatic alterations, registered in Table 1.

TABLE 1 Hemostatic alterations

	APTT(s)	PT(s)	TT(s)	FVIII(%)	FIX(%)	FXI(%)	FXII(%)
Day 1	113.2	15.9	18.1	0.1	0	0	70
Day 14	97	12.3	16.1	13.5	8.5	7.3	65

The test for inhibitors assessed the results mentioned in Table 2.

TABLE 2 Plasmatic level of inhibitor titre (Bethesda Units - BU)

	Inhibitors anti-FVIII (BU/ml)	Inhibitors anti-FIX (BU/ml)	Inhibitors anti-FXI (BU/ml)
Day 1	3	3.2	-
Day 14	28.85	36.90	5

The child was negative for antinuclear antibody, but she was positive for lupus anticoagulant: anti-cardiolipin and anti-beta 2 glicoprotein remained negative. The child remained clinically asymptomatic, justifying our „ watch and wait" attitude.

Conclusions: In *conclusion*, an AH with multiple coagulation factors inhibitors and biological image of associated hemophilia A, B and C, hypotheticaly connected to a vasculitis and lupus anticoagulant had a spontanous clinical recovery with maintance of low levels of factor VIII, IX and XI. Such favourable asymptomatic evolution could lead to the hypothesis that in children the prevalence of that potentially life-threatening disease could be subject of underdiagnosis.

PB 1111 | A Novel Thrombin-activable Factor X Corrects Acquired Hemophilia A in Rabbit

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Background: Rendering the factor X sensitive to thrombin was demonstrated *in vitro* to correct FVIII deficient plasma *(Louvain-Quintard et al. J Biol Chem, 2005)*. The advantages of this product are to be a zymogen molecule, possessing a longer half-life than factor VIII and factor IX.

Aims: To generate a novel factor X, more efficiently activated by thrombin and demonstrates its potential *in vivo*.

Methods: A modified factor X was generated by inserting a thrombin cleavage site between the activation peptide and the heavy chain. The molecule was expressed in HEK293-FS cells and purified using an anti-gamma carboxyglutamic domain aptamer.

Results: The molecule was analyzed in mass spectrometry and showed the expected post-translational modifications (11 gla domains, a beta hydroxylation and the activation peptide glycosylations). The presence of the peptide rendered the molecule sensitive to thrombin and allows to maintain the FVIIIa/FIXa complex and the fraction-X of the venom of the Russell's viper activations. In contrast, the activation by the FVIIa/tissue factor complex was diminished. The molecule (15 µg/ml) corrects *in vitro* factor VIII-, factor IX- and factor XI-deficient plasmas with Endogenous Thrombin Potential similar to that when plasmas were spiked with 1U/ml of missing factors. The lag times was increased, however. The pharmacokinetic of the molecule was evaluated in wild-type mice. The half-life was similar than for plasmatic factor X. It was assayed in hemophilia A mice providing a minor correction due to the poor potency of human FXa in mice plasma. A rabbit model of hemophilia A was set up by inhibiting factor VIII with a cocktail of two monoclonal antibodies. The presence of the modified FX *ex vivo* in plasma samples (20 µg/ml) or *in vivo* in treated rabbits (1.7 mg/kg) normalized the blood defect.

Conclusions: A factor X rendered susceptible to thrombin cleavage can correct *in vivo* hemophilia A and can be a novel hemophilia treatment alternative.

PB 1112 | Recombinant Factor VIIa-Albumin Fusion Protein Undergoes Endothelial Cell Protein C Receptor Mediated Internalization and Recycling

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Background: Recombinant activated coagulation factor VII (rFVIIa) is used to treat bleeding episodes in Hemophilia patients with inhibitory antibodies to FVIII or FIX. Multiple injections are normally required due to the short in vivo half-life of rFVIIa (2-3 hrs). A recombinant FVIIa-albumin fusion protein (rVIIa-FP) has been developed that is well tolerated in healthy volunteers and has a 3- to 4-fold half-life extension relative to rFVIIa. Recycling of rVIIa-FP via interaction of the albumin moiety with the neonatal Fc Receptor, FcRn, has been demonstrated [1] and is a likely mechanism for half-life extension in vivo. Interestingly, FVIIa interacts with endothelial cell protein C receptor (EPCR) and internalised EPCR/FVIIa complex is recycled via the recycling endosome [2]. EPCR is important for the protein C (PC)/activated protein C (APC) mediated anticoagulation pathway and it has been proposed that therapeutic concentrations of FVIIa may augment the coagulation cascade by competitively reducing EPCR binding sites for PC/APC.

Aims: To investigate the impact of albumin fusion on EPCR-mediated internalization and recycling of FVIIa.

Methods: Internalization, intracellular trafficking and recycling of rVIIa-FP was examined by confocal microscopy in 293 cells stably expressing human EPCR.

Results: We demonstrate that rVIIa-FP binds to EPCR in a calcium-dependent manner, is internalized into early endosomes and quickly moves into Rab11a^{+} recycling endosomes. With constant extracellular Calcium, rVIIa-FP remains bound to EPCR and undergoes continuous cycles of internalization and recycling, whereas removal of extracellular Calcium results in the dissociation and release of recycled rVIIa-FP from the cells.

Conclusions: The half-life extended protein rVIIa-FP interacts with EPCR and is recycled in complex with EPCR via the Rab11+ endosome.

[1] Chia et al, WFH 2016 (poster)

[2] Nayak et al, Blood 2009

PB 1113 | Treatment of Hemophilia A by Injection of FVIII-encoding mRNA

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Background: Treatment of patients with hemophilia A with exogenous therapeutic FVIII is complicated by important cost, the low half-life of FVIII (10 to 15 hours) and the need for life-long treatment. Injection of erythropoietin-encoding in vitro transcribed mRNA (IVT mRNA) (1) allows the endogenous production of transgenic erythropoietin for few days without innate immune activation.

(1) Kariko, Molecular Therapy, 2012

Aims: Investigate whether the injection of FVIII-encoding IVT mRNA allows the endogenous production of FVIII.

Methods: HEK293 cells were transfected with IVT mRNA encoding B-domain deleted FVIII (FVIII-HSQ), codon optimized FVIII (CoOpSQ) and CoOp FVIII-226/N6 (CoOpN6) formulated in TransIT. TransIT-formulated IVT mRNA was also injected intravenously to

FVIII-deficient mice. FVIII in cell culture supernatant or plasma was detected by ELISA (FVIII:Ag) and chromogenic assay (FVIII:C) over 72 hours.

Results: Cells transfected with CoOpSQ-encoding mRNA produced more FVIII than cells transfected with HSQ or CoOpN6 (0.30±0.02, 0.22±0.01 and 0.16±0.01 nM, respectively) after 48 hours. However, FVIII:C in supernatant was equivalent for the three constructs (0.07±0.01, 0.05±0.01 and 0.07±0.01 nM, respectively). Levels of circulating CoOp SQ in FVIII-deficient mice were 0.71±0.40 nM FVIII:Ag and 0.18±0.15 UI/ml FVIII:C, 48 hours after *in vivo* transfection.

Conclusions: Intravenous injection of FVIII-encoding mRNA permits an endogenous production of transgenic FVIII ≥10% of normal values for up to 72 hours. We will now confirm whether such FVIII levels protect mice in tail clipping assays, and whether the endogenous FVIII production induces a neutralizing immune response upon repeated treatments. At term, our works will allow to compare the efficiency and safety of IVT mRNA with that of alternative therapeutic approaches that are currently under development: gene therapy, bi-specific antibodies and long-lasting FVIII products.

PB 1114 | Clinically-relevant Bioavailability of rIX-FP after Subcutaneous Administration to Rodent and Non-rodent Species

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Background: rIX-FP, a recombinant fusion protein linking coagulation factor IX with albumin, has recently been approved for intravenous (i.v.) prophylaxis and on-demand treatment of hemophilia B patients. A subcutaneous (s.c.) administration of a factor IX (FIX) product that has a prolonged half-life may allow patients to maintain adequate trough levels without the need for frequent i.v. injections.

Aims: Aim of these studies was to investigate the bioavailability of rIX-FP following s.c. administration to hemophilia B mice, rabbits and pigs.

Methods: Hemophilia B mice received either a single rIX-FP dose of 125IU/kg (i.v. or s.c.) or 250IU/kg s.c. (rIX-FP and rFIX, a marketed recombinant FIX product as study comparator). Thereafter, blood samples were drawn up to four days. In the next study, rabbits obtained a single i.v. dose of 150IU/kg rIX-FP or a s.c. dose of 150 and 500IU/kg rIX-FP, respectively, followed by a 10 day follow up. In the third study, pigs received a single i.v. dose of 250IU/kg or a single s.c. dose of 50, 100 or 241.5IU/kg rIX-FP. Blood samples were drawn up to 17 days. For all studies, determination of human FIX antigen and activity (mice only) plasma concentrations was performed using an ELISA technique and an aPTT-based clotting assay, respectively.

Results: In all species, a dose-dependent increase of rIX-FP plasma levels was observed following s.c. dosing reaching peak plasma levels between 16 and 32 hours post dosing. The respective s.c. bioavailability of rIX-FP was in the range of 22% to 55%. Furthermore, maximum plasma levels reached following rIX-FP dosing were up to seven times higher and AUC (area under the curve) values up to 15 times higher in mice in comparison to rFIX treatment.

Conclusions: Taken together, s.c. administration of rIX-FP to hemophilia B mice, rabbits and pigs was well tolerated and resulted in a clinically-relevant s.c. bioavailability, whilst s.c. pharmacokinetics of rIX-FP were superior to a marketed rFIX.

PB 1115 | Rescue of Multiple Hemophilia B-causing Mutations in F9 Exon 3 by a Unique ExSpeU1

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Background: RNA splicing modulation is a promising approach to develop therapies for genetic diseases, with advantages (i.e maintenance of physiological gene regulation; small therapeutic transgene) over gene replacement therapy. Splicing mutations in severe coagulation factor disorders are relatively frequent and we have shown that can be rescued by exon-specific variants of the spliceosomal U1snRNA (ExSpeU1).

Aims: To select a unique ExSpeU1 able to rescue multiple mutations at the 5' splice site (5'ss) of *F9* exon 3, which cause hemophilia B (HB).

Methods: Expression of ExSpeU1s and *F9* minigenes, harboring the c.277G>A, c.277+1G>A/T, c.277+2T>C/G, c.277+4A>G and c.277+5G>A/C mutations, in HEK293 cells and evaluation of *F9* mRNA splicing (RT-PCR).

Results: Minigene expression studies demonstrated that all mutations induce exon 3 skipping, with the +4 and +5 variants also associated to trace level of correct transcripts (~10%). In co-transfection experiments, we screened the rescue activity of a panel of ExSpeU1s, targeting the poorly conserved IVS3 sequences downstream of the 5'ss, to minimize potential off-target effects. We identified an ExSpeU1 targeting the region at +6 intronic position (ExSpeU1sh6) able to remarkably recover correct splicing for the +4 (from ~10% to 19% of transcripts) and particularly the +5 variants (from ~8% to 90%). Intriguingly, this ExSpeU1sh6 appears to be active, albeit in preliminary experiments, also on the +1 and +2 variants (from 0% to ~10%), commonly considered not rescuable.

Conclusions: These data further expand the potential of RNA therapeutics based on ExSpeU1, particularly for disease such as coagulation factor disorders in which even a modest of protein levels can be beneficial to patients. If confirmed, they also provide the first insight into the ability of targeting and rescue the severest as well as most common splicing mutations occurring at the conserved +1 +2 GT dinucleotide.

PB 1116 | Dose-response and Duration of Effect of a Half-life Extended Recombinant Factor VIIa in the Tail Vein Transection Bleeding Model in Haemophilia A Rats

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Background: Recombinant factor VIIa (rFVIIa) is a safe and reliable option for treatment in haemophilia patients with inhibitors. A rFVIIa variant (NNC947) carrying two mutations (L288Y T239) and a 40-kDa heparosan polymer (HEPtune®, Caisson Biotech) has been shown to be resistant to inhibition by antithrombin, has a 15 fold prolonged half-life in rats and has an *in vitro* activity on par with wild-type rFVIIa.
Aims: Here we test the *in vivo* effect of a rFVIIa variant (NNC947) in a Tail Vein Transection model in haemophilia a (HA) rats.
Methods: Acute effect and dose-response relationship were determined in HA rats by administration of rFVIIa and NNC947 in a blinded fashion 5-min after transection of the left lateral tail vein. Haemostatic effect was assessed from the accumulated blood loss measured in 40 min with vehicle treated wild-type and HA rats as controls. The duration of effect of NNC947 was compared to rFVIIa by administration of 2.7 mg/kg at time points up to 96 hrs before injury.
Results: For both compounds clear dose-response relationships were observed with normalization of blood loss at 2.7 mg/kg. The estimated ED50-values (mean and 95%CI) for NNC947 and rFVIIa were 0.31 [0.11-0.80] and 0.47 [0.20-1.09] mg/kg, respectively, and found not to be significantly different. For both compounds, a time dependent effect on blood loss was observed. In the case of NNC947, normalization of the blood loss was maintained at 6 and 24-hrs time points, whereas animals receiving rFVIIa had already returned to the haemophilic baseline at 6 hrs.
Conclusions: NNC947 is efficacious in stopping an ongoing bleed in HA rats and exhibits a dose-response relationship not significantly different from rFVIIa using the Tail Vein Transection model. A significantly prolonged duration is observed compared to rFVIIa reflecting the prolonged half-life.

PB 1117 | Defining Extended Half-life rFVIII: A Critical Review of the Evidence

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Background: Key therapeutic advances in hemophilia include new modified recombinant FVIII products designed to extend half-lives (EHL). There is no uniform definition of what constitutes an EHL rFVIII. Such a definition would be useful to understand the properties of standard and EHL rFVIIIs and help enable physicians and payers make informed choices when selecting rFVIIIs.
Aims: To critically assess the published evidence on new and emerging rFVIII products in order to propose a definition to classify EHLs
Methods: We systematically searched PUBMED, EMBASE, and FDA/EMA/Health Canada clinical pharmacology reports for publications, conference presentations, and regulatory submissions on rFVIII products. We included all publications on prospective crossover pharmacokinetic (PK) studies evaluating rFVIIIs with modifications to increase half-lives in adults and adolescents with severe hemophilia A.
Results: Different criteria have been used to define EHL rFVIIIs, including absolute half-life and dosing regimen. In our critical analysis of the published data, a more holistic approach to classify rFVIIIs has evolved to a definition that requires the following:
1. EHL ratio close to the biological limit imposed by von Willebrand factor (1.4) and reduced clearance ratio measured in a PK comparator crossover study
2. Use of technology designed to extend circulating half-life
With additional evidence:
3. Lack of bioequivalence with a standard rFVIII comparator—above the FDA/EMA cutoff of 125% for the 90% confidence intervals for area under the curve ratio
4. Reduced dosing frequency with retention of hemostatic efficacy compared to standard rFVIII for the majority of patients
Conclusions: In this systematic review, a pragmatic definition of EHL rFVIII has emerged. This EHL definition should provide better clarity in clinical discussions surrounding the appropriate use of rFVIII products. At present, only products with fusion molecules or pegylation fulfill the proposed criteria for EHL.

PB 1118 | Nonclinical Immunogenicity and Safety of SHP656 (BAX826), a Next Generation Extended Half-life Recombinant Factor VIII Product

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Background: Hemophilia A is a genetic bleeding disorder caused by missing or defective factor VIII (FVIII). Shire is developing SHP656 (BAX826) as the first EHL rFVIII in which polysialic acid (PSA) is conjugated to Baxalta´s licensed rFVIII product (ADVATE) to extend the circulation FVIII half-life.

Aims: To assess the safety and immunogenicity of SHP656 in different animal species.

Methods: The toxicity and immunogenicity of SHP656 were evaluated in mice, rats, and cynomolgus monkeys. Acute toxicity was assessed in a dose-escalation study in monkeys IV administered 350 and 1800 IU SHP656/kg; repeat-dose toxicity in rats at 80-800 IU/kg, in monkeys at 800 IU/kg for 4 weeks, and in monkeys at 80-600 IU/kg for 31 days (incl. cardiovascular and respiratory safety). Thrombogenicity was evaluated after a single IV administration (900 IU/kg BW) to rabbits (Wessler test). The licensed rFVIII was used as comparator in most of these studies. Comparative immunogenicity was assessed in three mouse models and in two in vitro studies using human primary cells and plasma.

Results: SHP656 was well-tolerated in all species, with no clinical signs directly caused by the test item observed *in vivo* or histopathologically. The NOAEL in the RDT studies was the highest dose tested: 800 IU and 600 IU SHP656/kg BW in rats and monkeys. Toxicokinetics indicated dose-proportional pharmacokinetics. Exposure to FVIII decreased after repeated dosing due to anti-FVIII antibody formation in rats and monkeys. Development of anti-FVIII antibodies is an expected immune response after repeated application of heterologous proteins. In all three mouse models, at all doses, SHP656 showed a reassuring immunogenicity profile.

Conclusions: These results indicate sufficient safety margins to support the maximum clinical dose of SHP656, and a FVIII immunogenicity profile that supports further development.

PB 1119 | Pharmacokinetics and Pharmacodynamics of Subcutaneously Administered Marzeptacog Alfa (Activated) in Hemophilia B Mice

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Background: The rapid clearance of factor VIIa (FVIIa) requires daily intravenous (IV) administrations to attempt prophylaxis for patients with hemophilia A or B with inhibitors. Subcutaneous (SQ) dosing is a preferred route of administration for convenience and less pain, but has been limited by low bioavailability and potency of the currently marketed FVIIa products. Marzeptacog alfa (activated) (MarzAA), a rFVIIa with enhanced biological properties was developed using a rational protein design approach. Compared to recombinant wild-type FVIIa, MarzAA has 7-fold increased catalytic activity, measured by the rate of Factor Xa generation *in vitro*, in the presence and absence of tissue factor and prolonged duration of effect *in vivo*. A human IV single-dose escalation study up to 30mg/kg showed terminal half-life of 3.5 hrs and dose-dependent pharmacodynamic effects on prothrombin time, aPTT and thrombin generation, along with a good safety profile.

Aims: Determine pharmacokinetics and pharmacodynamics of single and daily dose SQ MarzAA.

Methods: MarzAA was injected into hemophilia B mice IV at 0.5 mg/kg or SQ at 0.5, 0.75 or 1 mg/kg and pharmacokinetics (PK) and aPTT were measured at 1, 2, 4 and 6 hrs. MarzAA was injected daily SQ for 4 days at 0.5 or 1 mg/kg and was sampled at 24, 48, 72 and 74 hrs. aPTT was measured using an assay kit from Sekisui Medical Co and MarzAA antigen using Abcam Factor VII ELISA Kit.

Results: Daily SQ dosing 0.5 mg/kg had trough levels of MarzAA 29.9-76.9 (mean 43.4) ng/mL and increased 2 hours after administration to 267.4-362 (mean 323.9) ng/mL. Daily SQ dosing 1 mg/kg achieved trough levels of MarzAA 50-80.9 (mean 63.7) ng/mL and increased 2 hours after administration to 230.8-729.5 (mean 471.9) ng/mL.

TABLE 1 Antigen levels with Daily Subcutaneous Dosing

Daily dose (mg/kg)	Time (hours)	Mean Antigen level (ng/mL)
0.5	24	38.6
0.5	48	48.8
0.5	72	43.0
0.5	74	323.9
1	24	73.7
1	48	59.4
1	72	58.0
1	74	471.9

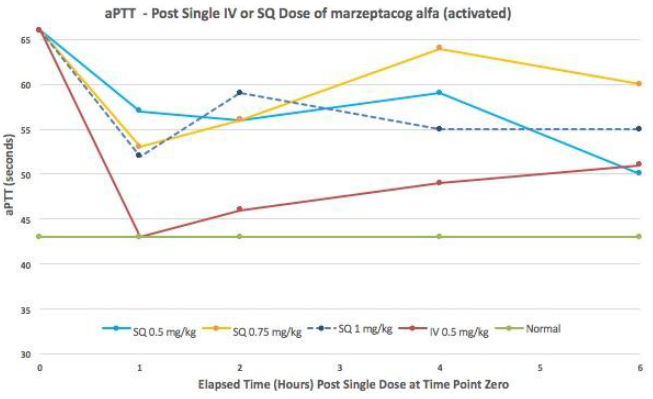

FIGURE 1 aPTT reduction by SQ route compared with IV

Conclusions: The blood levels achieved with daily dosing SQ and increased potency of MarzAA supports the initiation of the Phase 2/3 SQ dosing study in individuals with hemophilia B with inhibitors with a target of achieving normal coagulation pharmacodynamics.

PB 1120 | Underestimation of N-glycoPEGylated Factor IX (N9-GP) Activity in One-stage Clotting Assay Owing to Contact Activator Impairment of N9-GP Activation

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Background: Factor IX (FIX) activity is routinely quantified by measuring the activated partial thromboplastin time (APTT) in a one-stage (OS) FIX clotting assay. Available APTT reagents provide many combinations of contact activator and phospholipid surface. The recovery of N9-GP, also named nonacog beta pegol, activity against a FIX standard depends on the reagent used. Diverse reagents, containing silica, ellagic acid, or kaolin as the contact activator, may underestimate N9-GP activity.

Aims: To identify the mechanism responsible for the N9-GP activity underestimation obtained with a heterogeneous group of APTT reagents.

Methods: Activated FXI (FXIa) amidolytic activity was measured to assess the influence of APTT reagents/contact activators on the enzymatic activity. The clotting phase of the OS assay was mimicked (contact activation phase omitted, FXIa added to the APTT reagent) by mixing equal volumes of FIX-deficient plasma, N9-GP/FIX sample, APTT reagent/reagent filtrate/contact activator/buffer, and $CaCl_2$ and quantifying the amount of activated FIX (FIXa) formed. Cleavage of N9-GP by FXIa was also monitored by SDS-PAGE.

Results: In the presence of underestimating APTT reagent or isolated contact activator, but not APTT reagent filtrate, clotting phase activation rates of N9-GP and FIX were decreased, N9-GP activation more than FIX activation. Reagent and activator impaired FXIa enzymatic activity. The negative effects are likely owing to FXIa adsorption to the contact activator surface, and activation of FIX then apparently poses a greater steric problem after polyethylene glycol (PEG) conjugation.

Conclusions: Contact activators impair FXIa activity and, of relevance for the OS clotting assay, reduce FXIa-mediated activation of N9-GP to a larger degree than that of FIX. This is likely due to aggravated steric challenges, potentially pertinent to other bulky, long-acting FIX molecules, and leads to underestimation of N9-GP activity.

PB 1121 | Analysis of the Novel Recombinant Factor VIII-SingleChain Protein Predicts A Lower Immunogenic Potential as Compared to Full-length Recombinant FVIII

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Background: Hemophilia A (HA) is a hereditary bleeding disorder. Replacement therapy with plasma-derived (pd) or recombinant (r) FVIII reduces bleeding episodes. However, HA patients require repeated injections resulting in inhibitor development in ~30% of severe HA patients. The rVIII-SingleChain is a novel B-domain-truncated (BDT) molecule purposely designed to express covalently linked FVIII heavy & light chains resulting in increased dosing intervals, stability, homogeneity & clinical efficacy.

Aims: To investigate the immunogenic profile of rVIII-SingleChain compared to other FVIII products.

Methods: rVIII-SingleChain biochemical & immunogenic characteristics were compared with other pd & rFVIII products using biochemical & human-based assays.

Results: rVIII-SingleChain is highly homogeneous & efficiently binds vWF. It is less efficiently internalized by human dendritic cells compared to other rFVIIIs. In contrast, full-length (FL) rFVIII products are more heterogeneous with dissociated FVIII chains & fragments that poorly bind vWF & are potentially available for immune recognition. In the current study, rVIII-SingleChain triggered responses in fewer linker region-specific T cells compared to other BDT or FLrFVIIIs (15% vs 25-35%). The entire rVIII-SingleChain sequence generated fewer HLA-DR/DP/DQ-restricted epitopes compared to pd & FLrFVIII. Adding pdVWF greatly reduced rVIII-SingleChain-derived epitopes (by 67%, 71%, 90% for DR, DP, DQ) but was less effective for FLrFVIII (23%, 22%, 0% for DR, DP, DQ). rVIII-SingleChain had a lower immunogenic profile once bound to pdVWF than either pd or rFVIIIs. These findings are in accordance with clinical data in previously treated patients (PTP) where no rVIII-SingleChain inhibitors developed. An ongoing extension study in previously untreated patients (PUP) is investigating clinical benefit in this population.

Conclusions: These preclinical data warrant testing of rVIII-SingleChain in PUPs to determine clinical benefit.

PB 1122 | Identification of Amino Acid Substitutions in the D´D3 Region of von Willebrand Factor that Increase the Binding Affinity for FVIII

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Background: Current therapies for severe haemophilia A require intravenous administration of coagulation factor VIII (FVIII) 2-3 times a week for effective prophylactic treatment. FVIII circulates in the bloodstream as a complex with von Willebrand factor (VWF) such that the clearance of FVIII is essentially determined by VWF clearance mechanisms. This association has limited strategies that aim to extend the half-life of the FVIII molecule itself. Alternate strategies to address the VWF component are in turn constrained by FVIII exchange to endogenous VWF generally present in haemophilia A patients. A shift in

this exchange equilibrium through the use of a VWF molecule with enhanced FVIII binding properties may lead to further improvements in the half-life of co-administered FVIII.

Aims: To use both directed and non-directed mutagenesis approaches to identify residues in the D'D3 region of VWF that increase the affinity of VWF for FVIII.

Methods: The N terminal region of mature VWF has been previously identified as a key region for FVIII binding. More than 200 individual and combination mutations at residues S764, L765, S766, P769 and S806 were generated as VWF D'D3 region fragments and expressed in either HEK293 or CHOK1 cells. The resulting proteins were screened by Surface Plasmon Resonance to identify those with improved FVIII binding kinetics. In addition, a VWF D'D3 mammalian display library subjected to random mutagenesis was screened for enhanced FVIII binding variants.

Results: Substitutions at residues S764 and S766 within the VWF D' region and at one position within the D3 region were found to increase FVIII binding affinities, primarily through significant decreases in off-rates.

Conclusions: We have identified amino acid substitutions at a number of VWF residues that increase the affinity for FVIII. These VWF variants may be of value in novel therapeutic strategies to further increase the half-life of FVIII for treatment of haemophilia A.

PB 1123 | Administration of rFVIIa to Concizumab-dosed Monkeys Is Safe and Concizumab Does Not Affect the Potency of rFVIIa in Haemophilic Rabbits

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Background: Concizumab is a high affinity monoclonal antibody directed against the Kunitz2-domain of TFPI. It is currently in clinical development intended for subcutaneous prophylaxis in haemophilia A/B patients with and without inhibitors. A dose-dependent effect of concizumab has previously been demonstrated in haemophilic rabbits (Hilden, Blood 2012 119:5871-8). In inhibitor patients, potential break-through bleedings may be treated with rFVIIa. Both concizumab and rFVIIa are procoagulants; therefore it is considered important to investigate any potential interaction.

Aims: To examine 1) the safety of rFVIIa in concizumab-dosed monkeys and 2) whether concizumab modifies the effect of rFVIIa *in vitro* and *in vivo* in haemophilic rabbits.

Methods: In a safety study, 3 doses of 0.25, 0.5 or 1.0 mg/kg rFVIIa were dosed (iv) to cynomolgus monkeys with 2 hrs intervals in the presence of steady state concentrations of concizumab, obtained by daily sc dosing for 28 days, followed by macro- and microscopic pathological examination.

Concizumab, rFVIIa and their combination was tested in thrombin generation test (TGT) and thrombelastograhy (TEG) using human plasma or whole blood.

Cuticle bleeding was induced in haemophilic rabbits and after 30 min rFVIIa or concizumab+rFVIIa was dosed iv.

Results: No treatment related adverse findings were found in the safety study, i.e. no thrombi or signs of excessive coagulation. Both concizumab and rFVIIa caused increased concentrations of thrombin-anti-thrombin (TAT) and D-dimer, indicating a pharmacological activity of the compounds.

In TGT and TEG, the combined effect of rFVIIa and concizumab was increased as compared to rFVIIa and concizumab alone.

In haemophilic rabbits, rFVIIa dose-dependently reduced blood loss, with no effect on the dose-response curve of rFVIIa by dosing of concizumab immediately before rFVIIa.

Conclusions: Administration of rFVIIa to concizumab-dosed monkeys was safe and the presence of concizumab did not affect the dose-response of rFVIIa in haemophilic rabbits.

PB 1124 | A Monoclonal Antibody with TFPI Neutralizing Activity, Improves the Coagulation Parameters of Hemostatic Assays Performed with Hemophilic Whole Blood

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Background: TFPI is an endogenous inhibitor of Factor Xa (FXa) and Factor VIIa (FVIIa), both critical to thrombin generation during coagulation. Restoring FXa and FVIIa activities by inhibiting TFPI may enhance coagulation in hemophilia, where deficiencies in Factor VIII (FVIII) or Factor IX (FIX) impair thrombin generation. Physiologically relevant pools of TFPI that affect coagulation exist free in plasma and released from platelets upon activation.

Aims: We investigated the effect of PF-06741086, a TFPI neutralizing human monoclonal antibody, in comparison to recombinant FVIII or FIX in hemostatic assays run in whole blood from hemophilic patients with and without inhibitors. We also investigated the baseline variability and reproducibility of PF-06741086 in restoring hemostasis by comparing its effect in whole blood collected from the same patients on differing days.

Methods: PF-06741086, recombinant FVIII or FIX, vehicle or isotype control was dosed into whole blood collected from hemophilia A, hemophilia A inhibitor or hemophilia B patients. Whole blood was analyzed in rotational thromboelastography (ROTEM) and thromboelastography (TEG) using tissue factor to activate coagulation.

Results: PF-06741086 induced dose-dependent pro-coagulant responses in ROTEM and TEG assays when added to whole blood from hemophilia patients with and without inhibitors including decreased clotting times and increases in clot firmness parameters. We observed the pro-coagulant effects of PF-06741086 assessed on different days

from the same patient to be consistent and reproducible while noting baseline variability in day to day responses within individual patients.

Conclusions: These studies demonstrate both the in vitro potency and reproducibility of PF-067410867 in improving coagulation parameters in whole blood which contains the two major sources of TFPI affecting coagulation. PF-06741086 is currently under evaluation as a treatment for hemophilia.

PB 1125 | Protein Engineering of Factor X to Restore Coagulation: Building an Alternate Amplification Loop

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Background: Activated Factor VIII (FVIIIa) and Factor IX (FIXa) form the main complex responsible for generation of sufficient amounts of activated Factor X (FXa) that support normal clot formation. In haemophilia this is impaired by a deficiency of either factor. Current treatments rely on replacement of the missing clotting factors; however, development of neutralizing antibodies has created a need for bypassing therapies. Thrombin-Activatable Factor X (TA-FX) variants are one strategy to re-establish a mechanism for producing adequate FXa and circumvent the lack of a functional FVIIIa/FIXa complex.

Aims: To use rational design and screening of substrate libraries containing thrombin cleavage sites to design novel TA-FX variants that restore coagulation.

Methods: The challenge is to identify a specific and potent thrombin cleavage site. We measured cleavage rates of peptide substrates based on natural thrombin sites as well as a library of potential substrate sequences. Optimal cleavage sequences were introduced into the activation peptide of Factor X in combination with protein engineering strategies expected to enhance cleavage rates as well as minimize the insertion size and number of substitutions. The TA-FX variants were evaluated with respect to in vitro thrombin activation rates, thrombin generation and thromboelastography. In vivo efficacy was evaluated in a tail vein transection model in FVIII-KO mice.

Results: A strong correlation between thrombin activation rates and effect in plasma and whole blood assays was observed. Several variants demonstrated normalized thrombin generation parameters and full restoration of clotting in haemophilic whole blood at concentrations of 50-100 nM. The most potent were further shown to normalize bleeding in a tail vein transection model in haemophilic mice.

Conclusions: These results demonstrate that design of Factor X variants, which can be effectively activated by thrombin, is a feasible concept to restore coagulation.

PB 1126 | Pharmacodynamic Efficacy of a Recombinant Fusion Protein Linking Activated Factor VIIa to Human Albumin (rVIIa-FP) in FVIII and FIX Depleted Plasma with or without Inhibitors

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Background: Recombinant fusion protein linking coagulation factor VIIa with albumin (rVIIa-FP) is currently in clinical development for treatment of patients with congenital hemophilia A or B with inhibitors. Due to its prolonged plasma and tissue half-life compared to rFVIIa, rVIIa-FP is expected to improve on current available treatment for these patients.

Aims: The aim of this study was to evaluate and compare the efficacy of rVIIa-FP and rFVIIa in factor VIII (FVIII) and factor IX (FIX) depleted plasma with or without inhibitors in vitro.

Methods: rVIIa-FP or rFVIIa were added at concentrations of 0.1 to 500 IU/mL FVIIa activity to human FVIII and FIX depleted plasma with or without neutralizing antibodies. Pharmacodynamic activity in spiked plasma was determined by measuring the activated partial thromboplastin time (aPTT, Pathromtin® SL) on a BCS XP analyzer. Thrombin generation (calibrated automated thrombogram, Thrombinoscope BV, The Netherlands) was quantified after intrinsic activation using a mixture of Phospholipids and Pathromtin® SL.

Results: Data confirmed a clear hemostatic impairment of FVIII and FIX depleted plasma with and without inhibitors as indicated by a prolonged aPTT, delayed onset of thrombin generation (lagtime) and lower peak thrombin levels. Addition of 0.1 to 500 IU/mL rVIIa-FP or rFVIIa in vitro resulted in a concentration dependent correction of coagulation parameters in both, the absence or presence of inhibitory antibodies.

Conclusions: The study demonstrates in vitro comparable, concentration dependent pharmacodynamic efficacy of rVIIa-FP and rFVIIa based on key parameters of hemostatic function, i.e. correction of aPTT and thrombin generation in FVIII and FIX depleted plasma with inhibitors. The potential of rVIIa-FP to improve on the available treatment options of patients with FVIII or FIX inhibitors is currently being evaluated in clinical studies.

PB 1127 | Effect of Phospholipid Vesicle Composition on Activity of FVIIIa-mimetic Bispecific Antibodies

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Background: As part of the tenase complex, activated factor VIII (FVIIIa) binds to exposed phosphatidylserine (PS) on cell membranes and assembles with activated factor IX and factor X. It has been shown

that FVIIIa binds preferentially to phosphatidylcholine (PC) phospholipid vesicles (PVs) containing both phosphatidylserine (PS) and phosphatidylethanolamine (PE). Recently, a FVIIIa-mimetic bispecific antibody (emicizumab) was developed as a potential treatment for hemophilia A patients with and without inhibitors. We have generated a novel FVIIIa-mimetic bispecific antibody, BS-027125, with improved target specificity over emicizumab. Given that, unlike FVIIIa, FVIIIa-mimetic antibodies do not directly bind phospholipids, it is unclear whether these antibodies will show similar phospholipid preferences.

Aims: To compare the effect of varying PV compositions and concentrations on the activity of an emicizumab biosimilar (Emi-bsim), BS-027125, and recombinant FVIII (rFVIII).

Methods: The procoagulant activity of Emi-bsim, BS-027125 and rFVIII were assessed by a thrombin generation assay triggered with factor XIa. Synthetic PVs tested were composed of either PC/PE/PS (40/40/20%) or PC/PS (80/20%).

Results: As expected, rFVIII activity was ~2.5-fold higher on PE-containing phospholipid vesicles and decreased when either PV was limiting or in excess. Notably, whereas Emi-bsim activity was similar on both PVs, BS-027125 was ~3 fold more active on PE-containing PVs. The PV concentration that supported peak activity was higher for Emi-bsim and BS-027125 than for rFVIII.

Conclusions: The different relative activities of rFVIII and bispecific antibodies on different phospholipid surfaces complicate direct comparisons between these molecules. These results highlight the influence of assay design when benchmarking the activity of bispecific antibody mimetics of FVIIIa. Furthermore, these results suggest Emi-bsim and BS-027125 may function via different mechanisms.

PB 1128 | A New Long-acting Pegylated Recombinant Factor VIII Derived from Single-Chain Factor VIII Engineered to Be Highly Expressed in CHO Cell Line

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Background: Prophylactic treatment with coagulation factors requires frequent administration leading to the high cost of therapeutic drugs and patient inconveniences.

Aims: This study aims to develop the affordable long-acting recombinant factor VIII.

Methods: The recombinant single-chain FVIII (scFVIII) constructed by joining the heavy chain to light chain via varying lengths of N-terminus of B-domain were transiently expressed in HEK293 cells to screen the scFVIII with a high expression level and appropriate specific activity. The scFVIII (G4) selected from them was modified with cysteine at the B-domain for pegylation and transferred into CHO-S cells. The Cys-introduced single-chain FVIII (scFVIII (G4_cys)) was then pegylated with maleimide-PEG (40kDa), producing a pegylated scFVIII.

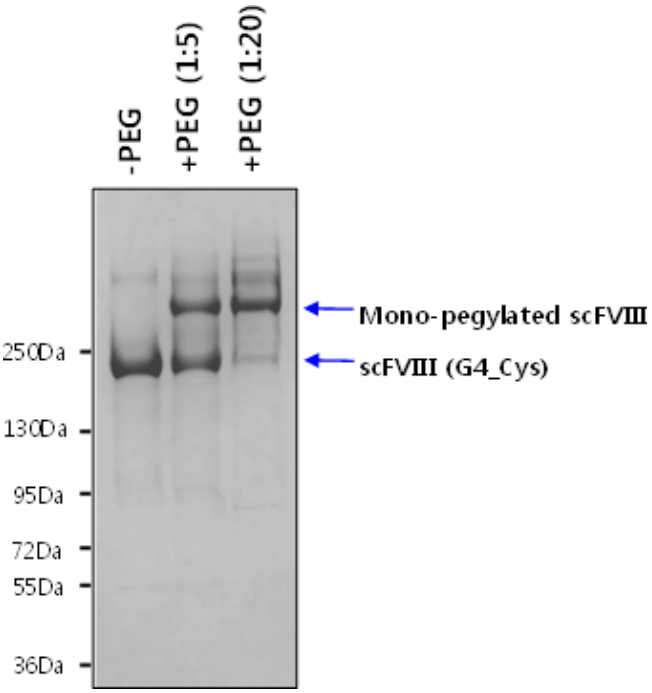

FIGURE 1 Site-specific pegylation of scFVIII (G4_cys) with maleimide-40kDaPEG The molar ratio of scFVIII to PEG at pegylation is 1:5 and 1:20

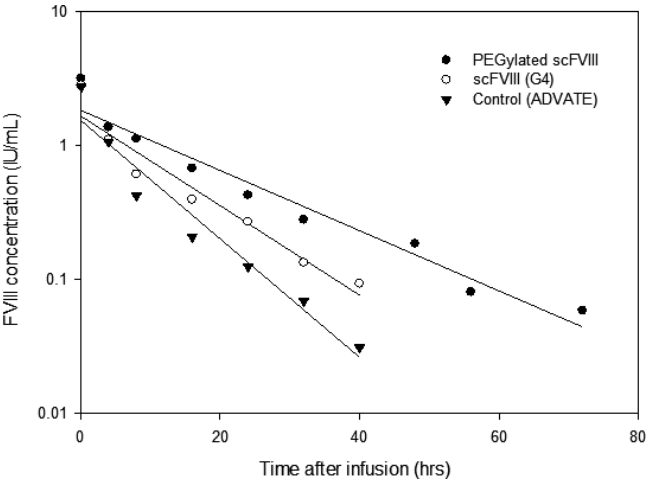

FIGURE 2 Pharmacokinetic study in hemophilia A mice

Pharmacokinetic study (n=3/group, 125 IU/kg) and blood coagulation tests using tail clipping (n=4~13/group, 50, 100, 200 IU/kg) were conducted for the pegylated scFVIII in hemophilia A mice.

Results: The expression level and specific activity of scFVIIIs produced from HEK293 cells varied according to the length of B-domain. The selected clone, scFVIII (G4) has an aPTT/CS ratio close to 1. scFVIII (G4_Cys) was produced in CHO-S cell line at the level of ~90 IU/mL/day in flask culture exchanging whole media daily (pseudo perfusion culture) and site-specifically pegylated to produce the mono-pegylated form.

The pegylated scFVIII has the specific activity (CS, aPTT) comparable to full length recombinant factor VIII and its aPTT/CS ratio was

approximately 1. The half-lives of scFVIII (G4) and its pegylated form were 1.2 and 1.9 fold longer, respectively, than the full-length recombinant FVIII (control).
The acute hemostatic efficacy of the pegylated scFVIII was equivalent to that of control.
Conclusions: The scFVIII (G4_cys) was highly produced from CHO cell line and the site-specifically pegylated scFVIII derived from it reserved the hemostatic efficacy and circulated longer than the control recombinant FVIII in hemophilia A mice.

PB 1129 | Pharmacodynamic Efficacy of a Recombinant Fusion Protein Linking Activated Factor VIIa to Human Albumin (rVIIa-FP) in FVII Depleted Plasma

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Background: Congenital factor VII deficiency is a rare autosomal recessively inherited bleeding disorder. Replacement with FVII concentrates or plasma products is the mainstay of treatment for these patients. The novel recombinant fusion protein comprising activated human coagulation factor VIIa linked to human albumin (rVIIa-FP) is currently in clinical development. Due to its prolonged plasma and tissue half-life versus rFVIIa, rVIIa-FP is expected to improve on current available treatments.
Aims: The aim of this study was to evaluate the pharmacodynamic efficacy of rVIIa-FP and rFVIIa in factor VII depleted plasma mimicking severe FVII deficiency in vitro.
Methods: rVIIa-FP or rFVIIa were added to human FVII depleted plasma at concentrations of 0.1 to 500 IU/mL based on FVIIa activity. Primary parameters for the evaluation of pharmacodynamic activity in vitro were prothrombin time (PT) and thrombin generation. PT was measured using two different reagents (Innovin® and Thromborel® S) on a BCS XP analyzer. Thrombin generation was quantified by calibrated automated thrombogram triggered by the extrinsic pathway using tissue factor (5 pM) and phospholipid containing PPP-Reagent (Thrombinoscope BV, The Netherlands).
Results: Hemostasis of FVII depleted plasmas was impaired as indicated by prolonged PT, delayed onset of thrombin generation (lag-time) and decreased peak thrombin levels. Addition of 0.1 to 500 IU/mL rVIIa-FP or rFVIIa resulted in dose dependent correction of coagulation parameters including PT and correction of thrombin generation.
Conclusions: The study demonstrates comparable correction of key coagulation parameters by rVIIa-FP and rFVIIa in a FVII deficient in vitro system. Current clinical trials are evaluating rVIIa-FP's potential for improving treatment of FVII deficient patients.

PB 1130 | Extended Hemostatic Efficacy of rIX-FP is Confirmed in a Hemophilia B Mouse Model of Arterial Injury

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Background: rIX-FP, a recombinant fusion protein linking coagulation factor IX with albumin, has recently been approved for intravenous (i.v.) prophylaxis and on-demand treatment of hemophilia B patients.
Aims: This study was conducted to compare the pharmacokinetic (PK) and pharmacodynamic profile of rIX-FP and a marketed recombinant factor IX (rFIX) product in an arterial injury model in hemophilia B mice.
Methods: A clinically-relevant i.v. dose of 50 IU/kg of rIX-FP or rFIX was given to hemophilia B mice. A vehicle-treated group served as negative control. Hemostatic efficacy was assessed in vivo at 3, 24, 48 and 72 hours (h) post administration (p.a.). Arterial injury was induced by placing a ferric chloride-saturated patch of filter paper (10%) on the carotid artery for three minutes. Thereafter, blood flow was monitored over 40 minutes or until thrombotic occlusion using by Doppler sonography. Afterwards, blood samples were drawn and analysed for factor IX (FIX) activity using a one-stage clotting assay.
Results: Absence of occlusive thrombosis was confirmed in all vehicle-treated animals. In FIX-treated groups, hemostatic efficacy was comparable at 3 h p.a. and declined over time. However, treatment with rIX-FP resulted in sustained hemostatic efficacy until 72 h p.a. while rFIX-treated animals had already returned to control levels at this time point ($p = 0.014$). The results were in line with the plasma activity levels of FIX with peak levels determined at 3 h for both treatment groups followed by a time-dependent decrease ($p = 0.0013$). Thereby, FIX plasma activity was in the range of vehicle-treated animals at 24 h after rFIX application. However, an improved PK profile was observed for rIX-FP indicated by a significantly higher FIX exposure over the time ($p = 0.0013$) and activity values above the vehicle control until 72 h p.a..
Conclusions: Prolonged hemostatic efficacy of rIX-FP was demonstrated in hemophilia B mice in comparison to rFIX.

PB 1131 | A Model of the Minimum Half-life Extension Ratio Needed to Reduce the Dosing Frequency of Extended Half-life Recombinant FVIII Products

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Background: With new recombinant FVIII (rFVIII) products available, it is crucial to differentiate which should be considered extended half-life (EHL) versus standard products. This supports assessment by physicians, payers, and consequently, appropriate and adequate access for patients. A key defining criterion of an EHL product is having an

EHL ratio. However, a minimal clinically-meaningful half-life (HL) extension ratio has not been defined until now.

Aims: To identify the HL extension ratio required to make a clinically meaningful (one day) reduction in dosing interval while maintaining the same percentage of patients always above a target rFVIII concentration of 1 IU/dL

Methods: The population PK model for standard rFVIII by Björkman *et al*. (2012) was used to estimate the percentage of patients always > 1 IU/dL using a benchmark regimen for rFVIII dosed 3 times weekly (the most commonly prescribed prophylactic dosing frequency in most countries). Then, dosing frequency was reduced to twice weekly and rFVIII HL extended until the percentage of patients always > 1 IU/dL was equal to the benchmark regimen. The result is an estimate of the minimal clinically-important HL extension ratio required to meet the definition of an EHL rFVIII product.

Results: Benchmark doses for rFVIII of 100 IU/kg/week were tested to reflect common rFVIII utilization. This benchmark regimen resulted in 56.6% of patients always > 1 IU/dL. Comparing the benchmark to doses of 80 and 90 IU/kg/week, the fold HL extension required to achieve 56.6% of patients > 1 IU/dL was 1.30 and 1.26, respectively (Table 1).

TABLE 1 The minimum EHL ratio required for a 2x/wk EHL rFVIII to achieve the same proportion of patients always above 1 IU/dL as 3x/wk standard rFVIII

Dose regimen	Proportion of patients always above 1 IU/dL	Half-life extension ratio
Three times per week (benchmark); 30 IU/kg on Day 1 and 3, and 40 IU/kg on Day 5	56.6%	Not applicable
Twice weekly; 40 IU/kg on Days 1 and 3	56.6%	1.3
Twice weekly; 45 IU/kg on Days 1 and 3	56.6%	1.26

Conclusions: Per this model, rFVIII products should show a minimum HL extension ratio of 1.3 for a meaningful reduction in dosing while maintaining the same percentage of patients always above 1 IU/dL FVIII concentration. Future research could investigate the extent to which EHLs that exceed the minimum HL extension ratio criteria may improve rFVIII coverage versus current prophylactic regimens.

PB 1132 | The Safety and Efficacy of IB1001 in Previously Treated Children 12 Years of Age or Younger with Hemophilia

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Background: IB1001 is a third generation recombinant factor IX approved in the US for the control and prevention of bleeding episodes and for perioperative management of patients ≥12 years of age with hemophilia B. This study evaluated the pharmacokinetics, safety, and efficacy of IB1001 when used in previously treated patients ≤12 years old.

Aims: To evaluate the pharmacokinetics, safety and efficacy of IB1001 in previously treated patients ≤12 years of age with hemophilia B.

Methods: This was a multi-center, non-randomized, open-label study. Patients were assigned either a prophylactic or on demand regimen based on investigator discretion. Bleed control efficacy was evaluated using annualized bleeding rate, subject's rating of efficacy for the degree of bleed control, and the number of infusions required to treat a bleed. Subjects were monitored for adverse events and regularly assessed for the development of inhibitors. The study was approved by an independent ethics committee at each study site. Written informed consent for all patients was provided prior to study entry.

Results: The study included nine patients, all male, with a median age of 9 (range 2-11). All patients were assigned a prophylactic regimen. One patient erroneously received on demand treatment.

The median exposure days for patients on prophylactic treatment was 221 (range 111-404) and the median time between first and last treatment was 46 months. The median total number of bleeds per patient was 1 (range 0-6); two patients experienced no bleeds. The median annualized bleed rate was 0.3 (range 0-1.6). The patient who received treatment on demand experienced 23 bleeds with an annualized bleed rate of 11.

No adverse events related to IB1001 were reported. None of the patients developed factor IX inhibitors during the study.

Conclusions: In this small study, IB1001 appeared safe and effective in preventing and controlling bleeding episodes in previously treated patients ≤12 years of age with hemophilia B.

PB 1771 | Construct and Known Group Validity of Patient-reported Outcome (PRO) Instruments in US Adults and Caregivers of Children with Hemophilia B: Results from the Bridging Hemophilia B Experiences, Results and Opportunities into Solutions (B-HERO-S) Study

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Background: B-HERO-S was a pilot study evaluating the impact of hemophilia on people (men/women) with mild-severe hemophilia B (PWH) and caregivers (CG) of boys/girls with hemophilia B, via standardized and disease-specific PROs.

Aims: To assess construct and known group validity of PROs.

Methods: US PWH completed a 1-hour Web-based survey including EQ-5D-5L (mobility [MO], self-care [SC], usual activities [UA], pain/discomfort [PD], anxiety/depression [AD], calculated Index, VAS), Brief Pain Inventory (BPI; pain interference [PI], pain severity [PS]), Hemophilia Activities List (HAL), and PHQ-9 (depression). CG completed PHQ-9 and GAD-7 (anxiety). Construct validity was assessed using Pearson product-moment correlation (threshold, >0.37). Known group validity was assessed by comparisons to self-reported characteristics based on Kruskal Wallis test (*P* values).

Results: B-HERO-S enrolled 299 PWH and 150 CG of children; most had mild-moderate hemophilia B. In PWH, EQ-5D-5L items/VAS/Index correlated with all BPI items (**Table 1**) and PS/PI domain scores. EQ-5D-5L items (except AD) and VAS correlated with HAL domain scores. EQ-5D UA/PD/AD/Index correlated more highly with PHQ-9 than EQ-5D MO/SC/VAS. BPI PS correlated with most HAL domains and overall scores; BPI worst/least/average/current pain correlated with all HAL domains, composite and overall scores. BPI PI correlated with all HAL scores. PHQ-9 scores correlated with HAL transportation and household tasks. In CG, PHQ-9 and GAD-7 scores were correlated. All known groups had significant differences in EQ-5D items/VAS/Index and BPI PS/PI (**Table 2**). In CG, PHQ-9 had significant differences across all known groups; GAD-7 was significant for all except for age.

Conclusions: The PROs administered in B-HERO-S showed high construct validity with expected directionality. Nearly all PRO domains/scores discriminated between known groups, with greatest significance between items/domains closely matching self-reported characteristics.

TABLE 1 Construct Validity in PWH (Pearson Correlation Coefficient); all values P<0.001

	EQ-5D-5L							BPI	
	Mobility	Self-care	Usual Activities	Pain/ Discomfort	Anxiety/ Depression	Health Utility Index	Overall Health VAS	Pain Interference	Pain Severity
BPI Pain Interference	0.595	0.561	0.686	0.629	0.729	-0.753	-0.668	-	-
BPI Pain Severity	0.517	0.560	0.669	0.638	0.715	-0.741	-0.690	-	-
PHQ-9	0.330	0.292	0.549	0.611	0.664	-0.468	-0.271	0.655	0.596
HAL Overall Score	-0.703	-0.595	-0.569	-0.512	-0.497	0.695	0.635	-0.602	-0.531
HAL Upper Extremity	-0.619	-0.574	-0.535	-0.427	-0.460	0.643	0.651	-0.562	-0.520
HAL Basic Lower Extremity	-0.717	-0.532	-0.498	-0.482	-0.447	0.637	0.549	-0.518	-0.442
HAL Complex Lower Extremity	-0.599	-0.435	-0.428	-0.481	-0.362	0.544	0.415	-0.420	-0.303

BPI, Brief Pain Inventory; HAL, Hemophilia Activities List; PHQ-9, Patient Health Questionnaire; PWH, people (men/women) with mild-severe hemophilia B; VAS, visual analog scale.

TABLE 2 Known Group Validity in PWH (P-values)

	EQ-5D Index	EQ-5D VAS	BPI Pain Interference	BPI Pain Severity	PHQ-9	HAL Upper Extremity	HAL Basic Lower Extremity	HAL Complex Lower Extremity
Self-reported anxiety (yes/no)	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	0.048	0.192
Self-reported depression (yes/no)	<0.001	<0.001	<0.001	<0.001	<0.001	0.008	0.002	0.008
Self-reported arthritis (yes/no)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Self-reported pain (yes/no)	<0.001	0.002	<0.001	<0.001	<0.001	0.874	0.093	0.009
Age, years (<30, 30-45, >45)	0.062	0.014	0.035	0.001	<0.001	0.370	0.300	0.058
Hemophilia severity (mild/moderate/severe/inhibitor)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Functional status (unrestricted/full, limited/no)	<0.001	<0.001	<0.001	<0.001	0.024	<0.001	<0.001	<0.001
Hemophilia treatment (on-demand, prophylaxis, on-demand plus)	<0.001	<0.001	<0.001	<0.001	0.805	<0.001	<0.001	<0.001
Percentage of life on prophylaxis (0%, 1%-49%, 50%-99%, 100%)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	0.616

PB 1772 | High and Sustained Observed trough FIX Activity Levels with Prophylactic Dosing of IDELVION (rFIX-FP) in Patients with Hemophilia B

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Background: rIX-FP, a long-acting fusion protein genetically linking recombinant human coagulation Factor IX with recombinant human albumin, is used in the treatment of hemophilia B patients. Maintaining higher FIX activity trough levels enables patients to transition from severe to moderate or mild disease states, thereby reducing bleed frequency. Sustained FIX activity levels are critical to achieving persistent pharmacological effects from FIX replacement therapies.

Aims: To evaluate mean observed trough FIX activity levels in pediatric and adult patients with hemophilia B during prophylaxis with rIX-FP in two clinical trials.

Methods: Adult/adolescent patients (≥12 years) with hemophilia B were assigned to prophylaxis with rIX-FP once every 7- (35-50 IU/kg) or 14-days (75 IU/kg). Pediatric patients (< 12 years) received prophylaxis once every 7 days (35-50 IU/kg). Adult trough FIX activity levels were measured every four weeks before each infusion over a maximum period of ~70 weeks. Pediatric trough FIX activity levels were measured at 4, 12, 24 and 36 weeks. Patients with ≥1 measurement obtained at observed trough were included in the analysis.

Results: A total of 45 and 18 adult patients were included in the analysis of observed trough levels with rIX-FP prophylaxis once every 7 and 14 days (mean doses 47 and 74 IU/kg), respectively. Pediatric patients (N=24) received prophylaxis once every 7 days (mean dose 47 IU/kg). In adult/adolescent patients, once every 7- and 14-day prophylaxis dosing regimens provided mean observed trough FIX levels of ~23% and ~13%, respectively, where >96% of samples were above 5%. Pediatric patients had mean FIX activity levels of ~14%.

Conclusions: This analysis demonstrates that rIX-FP provides consistently high observed trough FIX activity levels with both 7- and 14-day prophylaxis dosing regimens. These findings are consistent with low median annualized bleeding rates observed in both adult and pediatric patient populations.

PB 1773 | Pharmacokinetics of Recombinant Fusion Protein Linking Activated Factor VIIa to Human Albumin (rVIIa-FP) and Eptacog Alfa in Hemophilia Patients with Inhibitors

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Background: Recombinant fusion protein linking activated factor VIIa to human albumin (rVIIa-FP) is a therapeutic option designed to prevent and treat bleeding events in hemophilia patients with inhibitors. It aims to offer reduced infusion frequency and thus address the limitations associated with the short half-life of current rVIIa treatments.

Aims: To compare single-dose pharmacokinetics (PK) of rVIIa-FP to eptacog alfa (rFVIIa) and assess safety of rVIIa-FP in on-demand treatment in hemophilia patients with inhibitors.

Methods: In a Phase II/III multicenter, open-label, dose escalation study of rVIIa-FP for on-demand treatment in hemophilia patients with inhibitors, the first 12 patients underwent single-dose PK evaluation. Patients received a single injection of 90 (n=6) or 270 µg/kg (n=6) rFVIIa (day 1) followed by a single injection of 1500 µg/kg rVIIa-FP (day 3 to 15). FVIIa activity was assessed using StaClot assay. PK parameters evaluated included plasma half-life, area under plasma concentration time curve (AUC) and maximum plasma concentration (C_{max}). The safety of rVIIa-FP during on-demand treatment was also assessed.

Results: Mean baseline-corrected FVIIa activity at 24 h was higher for rVIIa-FP (11.0 IU/ml) compared to rFVIIa (0.06 IU/ml [90 µg/kg] and 0.11 IU/ml [270 µg/kg]). rVIIa-FP had an extended half-life of 8.5 h, more than two-fold higher than rFVIIa (3.0 [90 µg/kg] and 2.6 h [270 µg/kg]). rVIIa-FP produced a larger AUC compared to rFVIIa (863 vs. 112 [90 µg/kg] and 325 [270 µg/kg] IU·h/mL). C_{max} for rVIIa-FP was 76 IU/mL, in between that of the 90 (44.1 IU/mL) and 270 µg/kg (131 IU/mL) rFVIIa groups. No patients developed inhibitors to native FVII and there were no serious adverse events.

Conclusions: rVIIa-FP has an improved PK profile with a longer half-life and prolonged FVIIa activity compared to rFVIIa. In addition, rVIIa-FP showed good tolerability.

PB 1774 | Efficacy and Safety of rFVIIIFc Prophylaxis in Pediatric, Adolescent, and Adult Subjects with Severe Hemophilia A over 3-4 Years: The ASPIRE Study

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Background: The ASPIRE extension study evaluates long-term safety and efficacy of rFVIIIFc in previously treated subjects with severe hemophilia A.

Aims: To present safety and efficacy data from the third interim data cut (Y3) of ASPIRE (11 Jan 2016).

Methods: Eligible subjects completing A-LONG (aged ≥12 y) received 1 of 4 treatments in ASPIRE: individualized prophylaxis (IP), weekly prophylaxis (WP), modified prophylaxis (MP), or episodic treatment (ET); or for those < 12 y who completed Kids A-LONG, IP or MP. Subjects could switch treatment groups at any time. The primary endpoint was occurrence of inhibitor development.

Results: Of 153 adults/adolescents who completed A-LONG, 150 enrolled in ASPIRE, and 78 remained on-study at Y3. Of 67 children who completed Kids A-LONG, 61 enrolled in ASPIRE and 45 remained on-study at Y3. No inhibitors were observed across age groups in ASPIRE. The safety profile of rFVIIIFc was consistent with the parent studies and prior interim analyses. Median (IQR) duration of treatment was 4.1 (3.0-4.2) y (A-LONG subjects) and 2.9 (2.3-3.1) y (Kids A-LONG subjects). Among A-LONG subjects, 19 (12.7%) changed treatment groups at least once. From the end of A-LONG (or Kids A-LONG), 70.3% (90.2%) had no change in dosing interval, while 23.4% (6.6%) extended and 6.3% (3.3%) shortened. Median (IQR) change in weekly consumption at ASPIRE Y3 was 0 (0-0) from end of A-LONG and 0 (0-3.2) from end of Kids A-LONG. Dosing characteristics (Table 1) and ABRs (Table 2) are summarized. Among adults/adolescents, 93.6% (IP), 97.8% (WP), 94.5% (MP), and 99.2% (ET) of bleeding episodes were controlled with 1-2 rFVIIIFc injections. Per subject, median doses to resolve bleeding episodes were 45.6 (IP), 34.1 (WP), 37.1 (MP), and 27.3 (ET) IU/kg. Results were generally similar for pediatric subjects.

Conclusions: The safety and efficacy of rFVIIIFc prophylaxis was confirmed over ~4y in adults/adolescents and ~3y in children. ABRs remained consistently low while maintaining extended prophylactic dosing intervals.

TABLE 1 Summary of dosing characteristics for subjects with an efficacy period treated with rFVIIIFc prophylaxis in ASPIRE (Y3)

	Adults/adolescents		Pediatric subjects (<6 years)[a]	Pediatric subjects (6 to <12 years)[b]	
Median (IQR)	IP (n = 107)	WP (n = 27)	MP (n = 21)	IP (n = 29)	IP (n = 30)
Average weekly dose (IU/kg)	79.2 (73.8, 99.9)	66.0 (62.5, 66.8)	70.8 (62.8, 90.4)	101.1 (88.8, 118.2)	95.3 (82.0, 110.2)
Annualized consumption (IU/kg)[c]	4200.4 (3913.7, 5264.1)	3507.3 (3377.9, 3644.9)	3750.4 (3300.0, 4717.8)	5336.5 (4677.3, 6196.8)	5016.8 (4306.7, 5844.9)
Dosing interval (days)	3.5 (3.5, 5.0)	7.0 (7.0, 7.0)	5.0 (4.0, 6.9)	3.5 (3.5, 3.5)	3.5 (3.5, 3.5)

IP, individualized prophylaxis; IQR, interquartile range; MP, modified prophylaxis; WP, weekly prophylaxis.

[a]Two subjects <6 years of age received MP with the following dosing characteristics: average weekly dose, 82.0 IU/kg and 118.7 IU/kg; annualized consumption, 4,640.6 IU/kg and 6,479.1 IU/kg; dosing interval, 2.3 days and 5.7 days.

[b]One subject 6 to <12 years of age received MP with the following dosing characteristics: average weekly dose, 84.5 IU/kg; annualized consumption, 4,572.3 IU/kg; dosing interval, 3.5 days.

[c]Annualized consumption is the total rFVIIIFc (IU/kg) received during the efficacy period extrapolated to a 1-year interval of time.

TABLE 2 Summary of overall ABR among subjects with an efficacy period in ASPIRE (Y3)

	Adults/adolescents				Pediatric subjects (<6 years)[a]	Pediatric subjects (6 to <12 years)[b]
Median (IQR)	IP (n = 107)	WP (n = 27)	MP (n = 21)	ET (n=13)	IP (n = 29)	IP (n = 30)
Overall on-study ABR[c]	0.8 (0, 2.7)	2.2 (0.4, 5.1)	4.1 (1.2, 10.4)	19.1 (13.9, 30.5)	1.5 (0.5, 2.4)	1.6 (0.8, 3.9)

ET, episodic treatment; IP, individualized prophylaxis; IQR, interquartile range; MP, modified prophylaxis; WP, weekly prophylaxis.

[a]Two subjects <6 years of age received MP and had overall on-study ABRs of 4.1 and 10.7.

[b]One subject 6 to <12 years of age received MP and had an overall on-study ABR of 1.0.

[c]The annualized bleeding rate (ABR) is the total number of bleeding episodes during the efficacy period extrapolated to a 1-year interval of time.

PB 1775 | Comparing the One-stage and Chromogenic Assay: Factor VIII Activity Assay Discrepancy at Baseline Does Not Reflect Assay Discrepancy after DDAVP in Non-severe Hemophilia A

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Background: Measurement of factor VIII activity (FVIII) is used to monitor treatment in hemophilia A (HA) patients. Two assays are generally used, i.e. the one-stage (OSA) and chromogenic assay (CSA). Assay discrepancies were shown in baseline FVIII in non-severe HA and during treatment with FVIII concentrates, but has not been studied in patients receiving DDAVP.

Aims: To investigate discrepancies between the OSA and CSA after DDAVP administration in non-severe HA patients.

Methods: Non-severe HA patients who received a DDAVP test dose (0.3 µg/kg) between 2011 and 2015 were included after informed consent. FVIII was measured with the OSA and CSA before (T0) and after DDAVP administration at 1 (T1) and 4 (T4) hours. Assay discrepancy at T0 was defined as a two-fold difference. Response to DDAVP was defined as complete response (CR) (FVIII ≥0.50 IU/mL), partial response (PR) (FVIII 0.30-0.50 IU/mL) or no response (NR) (FVIII < 0.30 IU/mL). Discrepancy in response at T1 and T4 was defined as a different response category after DDAVP.

Results: Twenty nine non-severe HA patients were included with a median age of 41 years [range 6-67]. At T0, median FVIII with the OSA was 0.20 IU/mL [IQR 0.09-0.27], median FVIII with the CSA was 0.19 IU/mL [IQR 0.09-0.28]. Four patients showed assay discrepancy at T0 (in 3 patients the OSA was higher, in 1 patient the CSA). At T1 17/29 patients (58%) had CR, 9 (31%) PR and 3 (10%) NR based on the OSA. Based on the CSA 22/29 (76%) patients had CR, 4 (14%) PR and 3 (10%) NR. Discrepancy in response was seen in 24% at T1 and 29% at T4. At T1 discrepancy was seen in 2 of the 4 discrepant patients from T0 and in 5 of the 25 non-discrepant patients. The assay resulting in the lowest FVIII:C at T0 also gave the lowest outcome after DDAVP in all these 7 patients.

Conclusions: Assay discrepancy before treatment does not reflect FVIII assay discrepancy after a DDAVP test dose. By using the assay with the lowest FVIII:C before treatment, overestimation of response to DDAVP may be prevented.

PB 1776 | Risk Differential in Inhibitor Development in the First Days of Treatment by Product Class: A SIPPET Analysis

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Background: Recently a randomized clinical trial (SIPPET) showed that the risk of inhibitor development is nearly doubled in previously

untreated and minimally treated patients with severe hemophilia A treated with recombinant products (rFVIII) compared with plasma-derived (pdFVIII) products containing von Willebrand factor (VWF).

Aims: The aim of this post-hoc analysis of the SIPPET study was to assess the early risk of inhibitor development every 5 exposure days (EDs) and to see whether or not there was a difference between the two groups over the course of time of FVIII exposure.

Methods: 251 children were enrolled in the SIPPET study and randomized to receive a single pd or rFVIII. The outcomes were any FVIII inhibitor levels ≥ 0.4 BU and high-titer inhibitors (peak levels > 5 BU). Survival analysis by Kaplan-Meier and Cox regression were repeated every 5 EDs to assess the association of source of FVIII with inhibitor development over time.

Results: 6 out of 251 patients developed an inhibitor and of those 50 were high-titer; all occurred before 40 EDs. Over the complete observation period, users of rFVIII products had a 87% higher rate of inhibitor development than users of pdFVIII (hazard ratio (HR) 1.87; 95% confidence interval (CI95) 1.17-2.96). However, this risk was particularly high during the first 5 EDs, for both all (HR 3.14, CI95% 1.01-9.74) and high-titer inhibitor development (HR 4.19, CI95% 1.18-14.8). After the first 5 EDs, the difference between two arms was less pronounced, i.e., around two times higher for rFVIII compared to pdFVIII. Table 1 shows the cumulative incidence per time interval for each product class.

TABLE 1 Cumulative incidence per time interval for each product class

	ALL INHIBITOR Cumulative incidence (%)		HIGH-TITER INHIBITOR Cumulative incidence (%)	
Exposure days (EDs)	pdFVII	rFVIII	pdFVII	rFVIII
0-5	3.3	10.3	2.5	10.3
6-10	12.2	10.0	11.7	10.0
11-15	2.7	12.9	0.9	4.4
16-20	3.9	5.1	2.0	2.2
21-25	0	2.3	0	1.5
26-30	2.4	0	0	0
31-35	1.3	2.5	1.5	0
36-40	0	1.4	0	0

Conclusions: The risk of inhibitor development during replacement therapy occurs earlier (0-5 EDs) in patients treated with rFVIII than in those treated with pdFVIII, and remains high until 15 EDs. For pdFVIII, the highest risk came later (6-10 EDs) and this peak lasted shorter. The results of this analysis show the relevance of the early exposure days for inhibitor development.

PB 1777 | Pocket Handheld Ultrasound Has High Accuracy in Identification of Effusions and Major Landmarks in Normal and Hemophilic Joints: Expanding Point of Care for Patients with Hemophilia with Joint Disease

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Background: Point-of-care musculoskeletal ultrasound (MSKUS) has proven to be valuable for evaluation of hemarthroses. We previously introduced the concept of handheld devices as a cost-effective alternative to stationary MSKUS, finding high similarity between the two modalities on standard views.

Aims: To determine recognition accuracy and qualitative assessments of landmarks and effusions by different providers with handheld compared to stationary MSKUS devices.

Methods: Ten providers (6 physicians, 3 physical therapists and 1 nurse) trained in MSKUS were provided blinded readings of 144 scrambled joint MSKUS images (72 knee, 40 elbow and 32 ankle views). Parallel images were acquired from healthy volunteers and hemophilia patients at the same session with the handheld GE V2 scan and stationary Logiq S8. Providers were asked to identify landmarks and effusions and qualitatively describe osteochondral integrity, fat pad and other soft tissue characteristics. Adjudicators were a radiologist and a MSKUS experienced hematologist. Study procedures complied with the UCSD Human Research Protection Program.

Results: From 1440 responses analyzed, accurate recognition of major anatomic landmarks was on average 97.6% on handheld compared to 98.6% on stationary images. Correct recognition of effusions was 96.7% on handheld images compared to 99.1% on stationary images (Table 1). Discrepancy between various providers was minimal. There was great variability when interpreting more subtle soft tissue and osteochondral characteristics in the 820 qualitative assessments, most likely due to poorer image quality of the handheld device for these more detailed evaluations.

Conclusions: Notwithstanding limited depiction of soft tissue and osteochondral characteristics, handheld ultrasound devices have high accuracy for recognition of major anatomic landmarks and effusions, highlighting its potential clinical applicability for rapid diagnosis of hemarthroses, especially in resource-restricted settings and patient homes.

TABLE 1 Recognition of various structures on the handheld and stationary ultrasound images

	Bony (% correct)	Cartilage (% correct)	Meniscus (% correct)	Fat pad (% correct)	Effusion (% correct)	Other (soft tissue, bursa, artery) (% correct)
Handheld ultrasound images	97.3	98.2	98.8	97.0	96.7	98.6
Stationary MSKUS images	98.4	100	100	97.0	99.1	98.7

PB 1778 | Influence of Family History on Mode of Delivery in Severe Haemophilia in the UK

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Background: The risk of intracranial haemorrhage (ICH) is significantly increased in neonates with severe haemophilia and is likely related to trauma at delivery. Whilst instrumental delivery is a well recognised risk factor and should be avoided, debate continues as to whether elective caesarean (CS) delivery is safer than spontaneous vaginal delivery (SVD). Where there is a family history of haemophilia this uncertainty may result in variation in practice.

Aims: To examine the influence of family history (FH) on MOD in severe haemophilia in children born in the UK from 2003-2015 and to assess whether this has changed over time.

Methods: Delivery data from 254 cases of severe haemophilia attending 6 comprehensive care centres was analysed to assess the influence of family history on MOD. Data from deliveries with a negative family history was also compared with data from NHS Maternity Statistics.

Results:

TABLE 1 Mode of Delivery

Mode of Delivery	NHS Maternity Statistics	FHx Negative (N=127)	FHx Positive (N=127)
SVD	343,979 (61%)	83 (65%)	59 (46%)
Instrumental	81,808 (13%)	15 (12%)	1 (1%)
CS - Elective	73,486 (12%)	15 (12%)	49 (39%)
CS - Emergency	92,595 (14%)	11 (9%)	16 (13%)
Unknown	0	3 (2%)	2 (1%)

In those with a negative family history of haemophilia, MOD was in keeping with national NHS Maternity Statistics. In severe haemophilia there was a significant difference in MOD between those with and without a known family history ($p < 0.0005$). In the presence of a positive family history there was a higher proportion of deliveries by elective caesarean section compared to vaginal deliveries. As expected in the positive family history group there were very few instrumental deliveries. Data on deliveries before and after 2010 demonstrate an increase in the proportion of elective caesarean deliveries from 25% prior to 2010, to 58% after 2010.

Conclusions: In the UK a positive family history of severe haemophilia is associated with a higher proportion of elective caesarean deliveries as compared to vaginal deliveries. This has been more marked in deliveries from 2010 onwards and has occurred despite continuing uncertainty on the safest mode of delivery.

PB 1779 | Inhibitor Development in Previously Untreated Patients Treated with Octocog Alfa (ADVATE)

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Background: Contradicting evidence is available on inhibitor rates in previously untreated patients (PUPs) treated with recombinant or plasma-derived products.

Aims: The aim of this analysis is to review the inhibitor frequency reported in PUPs treated with octocog alfa (ADVATE).

Methods: The relevant clinical studies for ADVATE in PUPs were identified. These included clinical data contained in the marketing authorization file, as well as an internal meta-analysis and relevant investigator-driven studies from published literature. The identified studies were assessed individually for information regarding the frequency of inhibitor development considering the length of follow-up in exposure days (EDs), frequency of inhibitor testing, and known environmental and genetic risk factors for inhibitor development. Also the published data from the SIPPET study were included in this review.

Results: Inhibitor rates in clinical studies are reported in the table.

Conclusions: Inhibitor PUP studies of this FVIII product, even with very thorough monitoring and central lab testing, have never shown an incidence rate greater than 34%, with a pooled estimate incidence in PUPs with severe hemophilia A (FVIII < 1%) of 0.26 (95%CI 0.21-0.30).

TABLE 1

Study	Inhibitor rate in PUPs	Follow-up	Frequency of inhibitor testing	Risk factors
ADVATE PUP study (060103)	16/55 (29.1%)	Median EDs: 76 (min-max 1-414).	Every 5 EDs until the 20th ED and then every 10 EDs or every 3 months	53/55 with FVIII<1%. 17/55 w/ family history of inhibitor. 29/55 with intron 22 inversion. 47/55 were on on-demand.
EPIC study (061002)	6/19 (31.6%)	Early terminated	On EDs (±1 ED) 3, 6, 10, 15, 20, 30, 40, 50	19/19 with FVIII<1%. 12/19 with high risk gene mutation.
ADVATE PASS studies - meta-analysis	5/91 (5.5%) [a]	Median: 384 days	Variable, according to local clinical practice	Not available.
RODIN study	41/157 (28.2%)	75 EDs	In 92% of centers, after every 1 to 5 EDs during the first 20 EDs and every 3 months thereafter	157/157 with FVIII<1%. 22/157 w/ family history of inhibitors. 95/157 w/ high risk gene mutations. 40/157 with peak treatment. 46/157 were on on-demand tx.
FranceCoag study	33/97 (34.0%)	75 EDs	On average, every 6.3 EDs during the first 25 EDs and every 9.9 EDs during the overall follow-up period.	97/97 with FVIII<1%. 9/97 w/ family history of inhibitors. 31/97 had a peak treatment. 41/97 were on on-demand tx.
UKHCDO study	42/172 (24.4%)	75 EDs	Not available (guidelines: every fifth ED until 20 EDs and then less frequently)	172/172 with FVIII<1%. 103/172 with high risk mutations. 26/172 had a peak treatment.
EUHASS registry	13/59 (22.0%)	Not available	Not available	Not available
SIPPET study	13 on Advate. No. of inhibitors not available	Not available	Not available	13/13 with FVIII<1%
PUP inhibitor meta-analysis [b]	104/401 (26%)	Variable	Variable	401/401 with FVIII<1%

[a] Patients with less than 50EDs;
[b] Mantovani et al. Blood 2015 126:289.

PB 1780 | Predictors of Patients with 0 Bleeds during Every-7-days Prophylaxis with BAY 94-9027 in PROTECT VIII

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Background: PROTECT VIII was a partially randomized, open-label, phase 2/3 trial that investigated the use of BAY 94-9027 for routine prophylaxis and treatment of bleeds in adolescents and adults with severe hemophilia A. Previously treated males aged 12–65 years with severe hemophilia A received BAY 94-9027 for 36 weeks on demand or as prophylaxis at intervals determined after a 10-week run-in period of 25 IU/kg 2x/wk BAY 94-9027 prophylaxis. Patients with ≤1 spontaneous joint or muscle bleed during the run-in were eligible for randomization to 45–60 IU/kg prophylaxis every 5th day or 60 IU/kg every 7th day for 26 weeks (weeks 11-36). As shown in PROTECT VIII, 60 IU/kg BAY 94-9027 every 7th day is feasible and generally well tolerated in select patients (*J Thromb Haemost* 2016:Epub).

Aims: To identify clinical predictors for an annualized bleeding rate (ABR) of 0 during every-7-days prophylaxis with BAY 94-9027 in PROTECT VIII.

Methods: In this post hoc analysis, best responders were defined as patients with an ABR of 0 who did not discontinue or change dosing frequency during weeks 11–36.

Results: In PROTECT VIII, 43 patients were randomized to every-7-days prophylaxis. Of these 43 patients, 11 switched to more frequent dosing after a mean ± SD of 84±38 days (range, 22-131). Sixteen of 32 patients (50%) who remained on every-7-days prophylaxis had 0 bleeds; 15 patients were responders with an ABR of 0. Responders receiving every-7-days prophylaxis who had an ABR of 0 during weeks 11-36 had fewer total and joint bleeds in the 12-month period before study entry and fewer target joints at baseline compared with patients who switched from every-7-days prophylaxis to more frequent dosing **(Table)**. Most or all patients were previously treated with prophylaxis in both groups.

TABLE Baseline Characteristics of Patients Who Received BAY 94-9027 Prophylaxis Every 7 Days

	Responders With ABR of 0 During Every-7-Days Prophylaxis (n=15)*	Patients Who Switched From Every-7-Days to More Frequent Prophylaxis (n=11)	All Patients Receiving Every-7-Days Prophylaxis (n=43)
Age, y, median (Q1; Q3)	31.0 (26.0; 52.0)	38.0 (36.0; 47.0)	37.0 (26.0; 50.0)
Number of bleeds in the last 12 months, median (Q1; Q3)	2.0 (0; 11.0)	3.5 (2.0; 6.0)†	3.0 (1.0; 9.0)
Number of joint bleeds in the last 12 months, median (Q1; Q3)	1.0 (0; 11.0)	2.0 (1.0; 5.0)†	2.0 (0; 8.0)
Presence of target joint, n (%)	10 (66.7)	9 (81.8)	31 (72.1)
Number of target joints per patient, median (Q1; Q3)	1.0 (0.0; 3.0)	2.0 (1.0; 4.0)	2.0 (0.0; 3.0)
Previous prophylaxis treatment, n (%)	13 (86.7)	11 (100)	38 (88.4)

*16 patients who remained on every-7-days prophylaxis had 0 bleeds; 1 patient treated every 7 days discontinued from the study early with 0 bleeds recorded and was not included in the responder group. †Calculated from 10 patients with available data. ABR=annualized bleeding rate.

Conclusions: Number of previous bleeds in the immediate prior 12-month period and number of target joints may be used to identify patients who may benefit and could expect low spontaneous bleeding rates from once-weekly prophylaxis with BAY 94-9027.

PB 1781 | Individualizing Hemophilia Prophylaxis using Thromboelastography (TEG)

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Background: Prophylaxis is the standard of care approach to prevent bleeding/joint disease in severe hemophilia. Individualized dosing, when attempted, is based on factor levels and ignores other components of the coagulation system that vary between individuals. Global hemostasis assays have been studied in hemophilia patients, but these tests have not been used to guide prophylactic dosing regimens.
Aims: To utilize TEG to individualize prophylaxis regimens in patients with severe hemophilia A.
Methods: Subjects on prophylaxis who signed informed consent to participate in an IRB-approved institutionally-funded study were enrolled. All subjects were on standard half-life rFVIII concentrates. After appropriate washout, each subject had kaolin-activated TEG and FVIII PK sampling (pre-dose, peak, and q24 hours) until they reached a predetermined TEG endpoint as follows: when a subject's R time was within 20% of their abnormally prolonged baseline (signifying insufficient clotting activity), they were required to redose. Patients whose prophylaxis regimen was extended beyond their previous regimen were followed for 6 months to monitor for bleeding events.
Results: 18 subjects enrolled (5 withdrew prior to PK testing due to the time commitment), 1 withdrew after declining to extend his dosing interval per his PK results leaving 12. Of these, 10 had their dosing regimen extended (7 to every 72 hours and 3 to every 96 hours) and 7 have completed the study and remained on their extended regimen. Two are still in the follow up period and 1 had a second spontaneous bleed within 28 days before reaching 6 months and was thus considered a failure and resumed his pre-study dosing regimen. See table for data including bleeding in the 9 subjects that did not reach this endpoint.

TABLE 1 Study summary for the subjects who had their dosing frequency extended

Subject ID	Age	Factor	Dose (IU/kg)	Previous prophylaxis regimen	Study dosing regimen	Spontaneous bleeds during the follow up period (6 months)	Completed 6 month follow up	Continued study extended dosing regimen
HTI 3	12	Helixate	35	3 x per week	every 72 hours	2	Yes	Yes
HTI 4	14	Helixate	40	3 x per week	every 96 hours	0	Yes	Yes
HTI 7	17	Advate	35	3 x per week	every 72 hours	0	Yes	Yes
HTI10	5	Advate	50	2 x week	every 72 hours	0	Yes	Yes
HTI 13	12	Advate	40	every other day	every 96 hours	1	Yes	Yes
HTI 14	8	Helixate	45	every other day	every 72 hours	0	Yes	Yes
HTI 15	5	Advate	50	3 x per week	every 72 hours	0	Yes	Yes
HTI 17	9	Advate	30	3 x per week	every 72 hours	0	Ongoing	N/A
HTI 18	23	Advate	35	3 x per week	every 72 hours	1	Ongoing	N/A

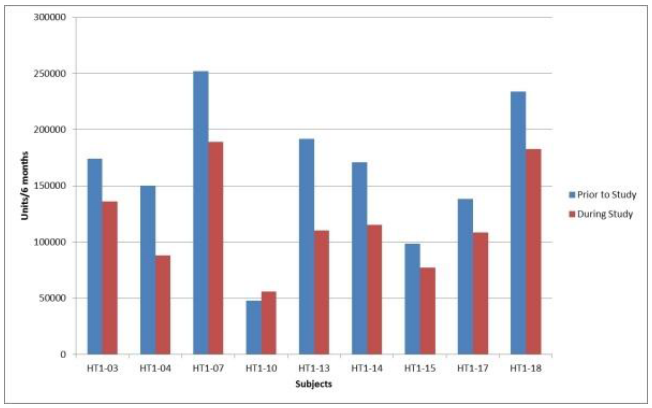

FIGURE 1 Factor consumption pre- and post-regimen adjustment

As a result of the less frequent dosing regimen, overall factor consumption decreased.

Conclusions: These results suggest that TEG is a promising tool which can be used to bring a personalized approached to prophylactic factor therapy in hemophilia.

PB 1782 | Impact of a Product-Specific Reference Standard for the Measurement of a PEGylated rFVIII Activity: The Swiss Multicentre Field Study

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Background: Measuring factor VIII (FVIII) activity can be challenging when it has been modified, such as when FVIII is pegylated to increase its circulating half-life. Use of a product-specific reference standard may help avoid this issue.

Aims: Evaluate the impact of using a product-specific reference standard for measuring the FVIII activity of BAX 855 — a pegylated FVIII — in eight of Switzerland's main laboratories.

Methods: FVIII-deficient plasma, spiked with 5 different concentrations of BAX 855, plus a control FVIII sample, were sent to the participating laboratories. They measured FVIII activity by using either with a one-stage (OSA) or two-stage assay (TSA) against their local or a product-specific reference standard.

Results: When using a local reference standard, there was an overestimation of BAX 855 activity compared to the target concentrations, both with the OSA and TSA. The use of a product-specific reference standard reduced this effect: mean recovery ranged from 127.7% to 213.5% using the OSA with local reference standards, compared to 110% to 183.8% with a product-specific reference standard; and from 146.3% to 182.4% using the TSA with local reference standards compared to 72.7% to 103.7% with a product-specific reference standard.

Conclusions: In this *in vitro* study, the type of reference standard had a major impact on the measurement of BAX 855 activity. Evaluation was more accurate and precise when using a product-specific reference standard.

PB 1783 | Long-term Quality-of-Life Outcomes with rFVIIIFc Prophylaxis in Adult Subjects with Severe Hemophilia A

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Background: Long-term quality-of-life (QOL) effects of rFVIIIFc are being evaluated in the A-LONG clinical trial and ongoing ASPIRE extension study of subjects with severe hemophilia A.

Aims: To present longitudinal QOL data from Hemophilia A subjects at A-LONG study entry through the third ASPIRE interim data cut.

Methods: Subjects were evaluated using the Haem-A-QOL patient-reported outcome. QOL outcomes were analyzed over the duration of the A-LONG study and the third ASPIRE interim data cut (11 Jan 2016) for subjects who had Haem-A-QOL total score change from baseline through ASPIRE month 24. Last observation carried forward imputed missing data. Within-group t-tests compared mean change over time on Haem-A-QOL total score and sub-domain scores.

Results: Out of 105 A-LONG prophylaxis subjects (age≥17) with baseline Haem-A-QOL score, 80 subjects had total change scores from baseline to ASPIRE month 24 (mean age 35, SD 11.4). The sample included 52 patients on prophylaxis prior to trial enrollment and 28 on on-demand regimen. Patients were from North America (34%), Europe (25%), and other continents (41%), and included 69% White, 24% Asian, 4% African-American, and 4% other. Most (66%) had target joint bleeding at baseline and the median pre-study annualized bleeding rate was 13.5 (IQR 4, 30). The sample exhibited improved QOL from A-LONG baseline to ASPIRE baseline (median follow-up 7.2 months, IQR 6.5-8.3), with a mean change of 4.6 ($p< 0.01$). These overall QOL improvements were maintained over 24 months of follow-up ($p< 0.01$). An examination of the sub-domain scores over time suggested that the most pronounced improvements were in Physical Health, Sports and Leisure, and Feeling.

Conclusions: Subjects treated with rFVIIIFc reported significant QOL improvements that were maintained for 24 months of follow-up. The biggest QOL gains reflected Physical Health, Sports and Leisure, and Feeling sub-domains assessed by the Haem-A-QOL.

PB 1784 | Bleeding Events and Safety Outcomes in Persons with Hemophilia A (PwHA) with Inhibitors: The First Large, Prospective, Multicenter, Non-interventional Study (NIS) from a Real World Setting

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Background: Management of PwHA and anti-FVIII neutralizing antibodies (inhibitors) (PwHAwI) remains an unmet need, with only two bypassing agents (BPAs; aPCC and rFVIIa) available for bleed control/prevention. Emicizumab, a bispecific humanized monoclonal antibody, bridges FIXa and FX to promote coagulation by replacing the function of missing FVIII, and is being developed to treat/prevent bleeds in PwHA with/without inhibitors. This global NIS was designed to assess real-world data (RWD) on bleed rates and treatment (tx) in PwHA, understand disease behavior, and enhance clinical development of emicizumab. The NIS previously showed high annualized bleed rates (ABRs) with episodic and prophylactic BPAs, and a high proportion of bleeds in PwHAwI were not treated (42.7%).

Aims: Present additional prospective RWD on bleed and safety outcomes in PwHAwI, including BPA tx exposure and purpose, from a multicenter NIS prior to rollover to a Phase 3 emicizumab study (NCT02622321). Results by country will be presented for all/treated bleeds and prophylaxis compliance.

Methods: Inclusion criteria: ≥12 y.o. with congenital HA and high-titer FVIII inhibitor history (≥5 Bethesda units/mL); signed informed consent/assent; coagulation product use ≥6 mo.; ≥6 or ≥2 bleeds on episodic or prophylactic tx in last 6 mo., respectively.

Results: 103 PwHAwI on episodic (n=75) or prophylactic (n=28) regimens enrolled from 12 countries; median (range) age, 31 (12-75) years. Coagulation product (episodic and prophylaxis, respectively) was used in 93.3 and 100% of pts; most common, aPCC in 75.7% of all pts-70.7 and 82.1% of pts for bleeds, and 6.7 and 82.1% of pts for usual prophylaxis (**Tables 1-2**).

TABLE 1 Exposure to hemophilia treatments

Regimen	Episodic (n=75)	Prophylaxis (n=28)	All patients (N=103)
aPCC exposed patients (%)	70.7	89.3	75.7
aPCC median number of doses/ median cumulative dose, unit	11 / 34,000	70 / 332,278	20 / 94,029
rFVIIa exposed patients (%)	45.3	39.3	43.7
rFVIIa median number of doses/ median cumulative dose, ug	15 / 112,515	14 / 84,000	14 / 110,030
FVIII exposed patients (%)	4.0	17.9	7.8
FVIII median number of doses/ median cumulative dose, unit	46 / 64,000	67 /137,000	52 / 110,125
DDAVP (intravenous) / Cryoprecipitate/Fresh frozen plasma or whole blood, exposed patients (%)	6.7	0	6.7

Median cumulative dose reported is based on exposure during the NIS

TABLE 2 Purpose of hemophilia treatment

	Episodic (n=75)		Prophylaxis (n=28)		All patients (N=103)	
Hemophilia Medications	% patients who reported use as treatment for bleed*	% patients who reported use as usual prophylaxis*	% patients who reported use as treatment for bleed*	% patients who reported use as usual prophylaxis*	% patients who reported use as treatment for bleed*	% patients who reported use as usual prophylaxis*
aPCC	70.7	6.7	82.1	82.1	73.8	27.2
rFVIIa	45.3	4.0	28.6	25.0	40.8	9.7
FVIII[†]	4.0	0	10.7	17.9	5.8	4.9
DDAVP (intravenous) [‡]	1.3	0[§]	0	0	1.0	0
Cryoprecipitate	1.3	0[§]	0	0	1.0	0
Fresh frozen plasma or whole blood	1.3	0	0	0	1.0	0

*Collected through bleed/medication questionnaire completed by patient/legal guardian via an electronic handheld device. [†] No patients received FVIII long acting treatment. [‡]No patients received DDAVP intranasal treatment. [§]1 patient in the episodic arm received one-time prophylaxis.

Conclusions: Global RWD on coagulation product use show remaining high ABR, proportion of untreated bleeds, factor consumption, and median number of infusions, and therefore a high unmet need for more efficient and easy to use HA tx. Upcoming results will offer new insight on the management of PwHAwI by country.

PB 1785 | Disparities in Age at Diagnosis and Initial Treatment between People with Moderate and Severe Haemophilia A: Evidence from the KAPPA Register

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Background: Early diagnosis and treatment for haemophilia are critical for maintaining healthy joints and optimal health related quality of life (HRQL). Recent studies have indicated that people with moderate haemophilia also suffer from arthropathy and reduced HRQL. Thus, delay in diagnosis and treatment may alter outcome in moderate haemophilia as well.

Aims: To compare the initial event/cause that led to the diagnosis and ages at first diagnosis and treatment between people with moderate and severe haemophilia A.

Methods: Eligible people with moderate (factor VIII [FVIII]: 1-5 kIU/L) to severe (FVIII: < 1 kIU/L) haemophilia A were included in this study from the KAPPA international register. Participants signed an informed consent prior to enrollment between 2013 and 2016. Chi-square tests, linear regression and Kaplan-Meier estimates were used for data analysis. The Regional Ethical Review Board of Lund University approved the study protocol.

Results: Overall, 94 (17.3%) participants with moderate and 449 (82.7%) with severe haemophilia A were included (Table 1). Median (inter-quartile range: IQR) age was 28 (IQR: 16-43) and 24 (IQR: 11-36) years for people with moderate and severe haemophilia, respectively. Participants with moderate haemophilia got diagnosed and received treatment 2.5 (95% CI: 1.1 - 3.9) and 2.7 (95% CI: 1.1 - 4.3) years later compared to those with severe haemophilia, respectively (Figure 1). The reason for evaluation for haemophilia was more often bleeding events (67.1 vs. 55.0%) and medial/surgical procedures (11.7 vs. 6.2%) in moderate versus severe haemophilia A.

TABLE 1 Characteristics of participants haemophilia severity

	Moderate, n(%)	Severe, n(%)	P
Country of residence			0.053
Nordic*	16 (17.0)	119 (26.5)	
Non-nordic**	78 (83.0)	330 (73.5)	
Reason for diagnosis			0.008
Family investigation	10 (10.6)	91 (20.3)	
Bleeding events	63 (67.1)	247 (55.0)	
Medical/surgical procedures	11 (11.7)	28 (6.2)	
Unknown	10 (10.6)	83 (18.5)	

* Denmark, Norway, Sweden

** Lithuania, Oman, Tunisia, Turkey and United Arab Emirates

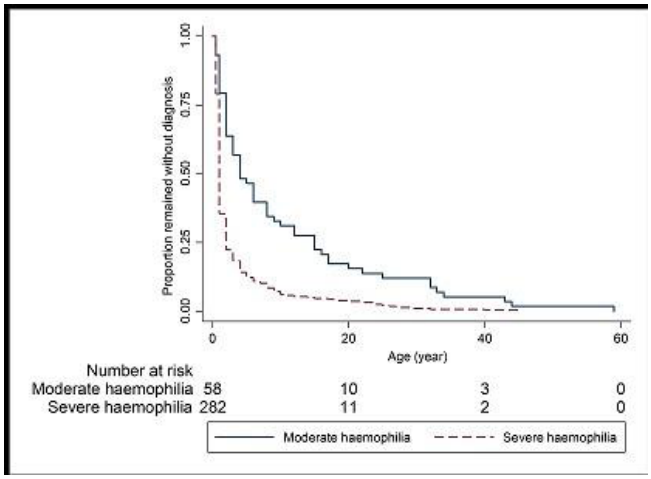

FIGURE 1 Kaplan-Meier estimates of the age at first treatment among people with moderate and those with severe haemophilia A

Conclusions: The majority of participants with moderate haemophilia A received diagnosis and treatment prior to age 5, however later than those with severe haemophilia. Information on early bleeding symptoms likely reflects severity of the bleeding phenotype and can help in planning an optimized long-term treatment strategy for people with moderate haemophilia A to improve their long-term outcomes.

PB 1786 | Low Dose Prophylaxis for Children With Haemophilia - One Year Experience from a Haemophilia Comprehensive Treatment Centre, Kerala, India

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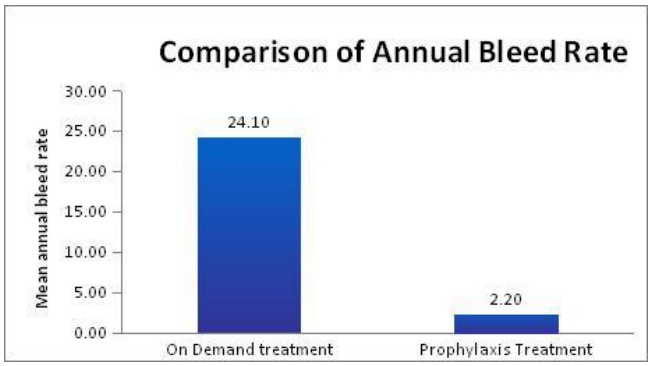

FIGURE 1 Comparison of annual bleed rate

Background: Haemophilia is currently the most effectively and safely treated monogenic inherited disorder in developed nations. Prophylaxis for Haemophilia is a challenge in low and middle income countries due to resource limitations and cost barriers.

Aims: To compare the clinical outcomes of On demand therapy (ODT) and Prophylactic therapy (PT) in children with Haemophilia.

Methods: Ten children with severe Haemophilia A (n=7) and severe Haemophilia B(n=3) under the age group of 5-15years with clotting factor concentrate(CFC) level < 1% were started with low dose plasma derived CFC prophylaxis . All were inhibitor negative with mean exposure of 36.45 days. The dosage was 10-20 IU/kg twice weekly for Haemophilia A and 25-40 IU/Kg once weekly for Haemophilia B. During ODT children received CFC in standard recommended doses. The data collection period was 18 months (6 months retrospectively for ODT and 12 months prospectively for PT).

Results: The mean consumption of CFCs for ODT & PT were 608.49 and 1661.34 IU/kg/year .The cost of CFCs during ODT and PT were USD 1841.16 & 5436.36/person/year respectively. A significant reduction in annual bleed rate was seen from the transition of ODT to PT (24.10 vs 2.20, p < 0.001)(Fig 1) . Similar reductions were observed for number of days of hospital visits/stays (27.40 vs 2.60, p 0.005) (Fig 2) and school absenteeism (52.40 vs 1.40, p 0.008) respectively. The changes in Haemophilia Joint Health Score (HJHS, 4.60 vs 1.45, p 0.08) and Functional Independent score in Haemophilia (FISH, 28.50 vs 31.70, p 0.10) were not significant. Inhibitors were absent for all children throughout the period.

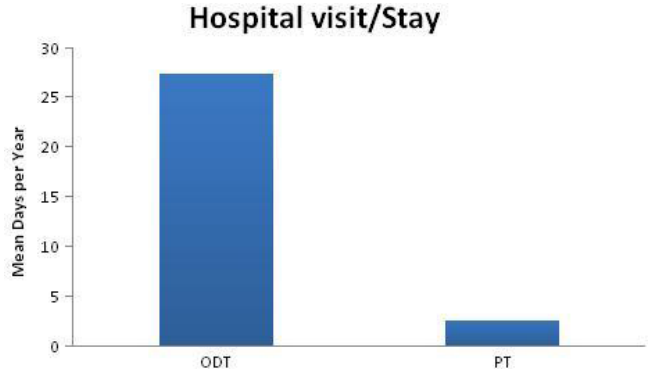

FIGURE 2 Comparison of days of hospital stay/visit

Conclusions: Low dose prophylaxis for severe Haemophilia has clinical benefits and is feasible in resource poor countries. This should be considered as a treatment strategy for severe haemophilia in the developing world.

PB 1787 | Final Results from the PUP-GCP Clinical Trial: A Low Inhibitor Rate in Previously Untreated Patients with Severe Haemophilia A Treated with Octanate

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Background: Octanate is a human plasma-derived, von Willebrand factor (VWF) -stabilised coagulation factor VIII (FVIII) concentrate which demonstrated haemostatic efficacy in previously treated patients (PTPs) with haemophilia A.

Aims: To assess immunogenicity in previously untreated patients (PUPs), 50 PUPs with severe HA in an open, prospective, multi-national GCP study for an observation time of 100 exposure days, or for maximum 5 years.

Methods: Patients with severe HA without previous exposure to any FVIII or FVIII-containing products were enrolled. Efficacy and tolerability were assessed. Inhibitor assay, according to modified Bethesda method, was frequently tested: at screening, prior to first exposure to Octanate, every 3-4 exposure days (ED 1-20), and afterwards every 10 EDs (ED 21-100), at least every 3 months.

Results: Patients were followed for a mean of 2.8 years (136-2387 days). More than 7,000 EDs were documented. Five (9.8%) of the 51 patients developed inhibitors during the study, 4 of which (7.8%) were high-titre. Of these, 3 (5.9%) were considered to be clinically relevant (those affecting FVIII therapy); other 2 were transient inhibitors that disappeared during regular unchanged Octanate treatment. All clinically relevant inhibitors developed under on-demand treatment and before ED 20. Patients who developed inhibitors had either intron 22 inversions or large deletions. Octanate was well-tolerated and the adverse event profile was consistent with the population studied. The hemostatic efficacy in prophylaxis and treatment of bleeding episodes was generally rated as "excellent".

Conclusions: The data indicate a low overall inhibitor rate for Octanate in PUPs. Five out of 51 patients (9.8%) developed inhibitors, of which 3 (5.9%) were clinically relevant. The data are furthermore consistent with findings in PTPs demonstrating that Octanate is an efficacious and well-tolerated human FVIII for patients with HA.

PB 1788 | Intracranial Hemorrhage in Hemophilia Patients: The Status of Ongoing Retrospective-Prospective Italian Registry (2009-2016)

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Background: .Intracranial hemorrhage (ICH) is the most serious event in patients with hemophilia (PWHs) which leads to disability and in some cases to death. ICH is more frequent in children < 2 years and in adults >50 years. Severe hemophilia and inhibitors proved to be the major risk factor for ICH in PWHs.

Aims: To evaluate incidence, mortality, management and risk factors for ICH.

Methods: From Jan 2009 to Nov 2016 we retrospectively and prospectively (from Nov 2012) collected all ICHs occurred in the PWHs treated in Italian Hemophilia Centers. Clinical features of PWHs, data of ICH management and outcomes were evaluated ctor for ICH in PWHs.

Results: 29 ICH events were found in 4630 PWHs. ABR was 0.89 per 1000 patients. 6 ICHs occurred in children ≤2 yrs (none of the children was on prophylaxis), one in a 4-year-old child during immune-tolerance induction and the remaining 22 in PWHs ≥40 yrs, 50% of whom affected by mild hemophilia. Mean age of adults was 52.7 yrs (± 17.4), 81.8% were treated only on-demand, 42.8% of severe patients were on prophylaxis. 2/22 had inhibitors, 13/22 adults suffered from hypertension, 2 were obese, one had diabetes, 2 hyperlipidemia and one was affected by chronic renal failure. In 51.7% of PWHs, ICH was spontaneous. Surgery was required in 10/29 patients for cerebral hematoma evacuation. 62,1% of patients were treated with FVIII/FIX concentrates or rFVIIa for a mean of 31.7 days (± 5.5), while 37.9% subjects died before starting or during treatment. In all survivors prophylaxis was then continued either lifelong (72.7%) or for 4-6 months (27.3%). 44.4% of survivors became permanently disable.

Conclusions: Up to today the results obtained confirm high incidence of ICH in children ≤2 yrs and in adults ≥40 yrs with hypertension. High incidence was also found in mild hemophilia patients usually considered at very low risk for spontaneous bleeding. 81% of patients were treated on-demand, which indicates the important role of prophylaxis in preventing ICH.

PB 1789 | Evaluation of Outcomes from Long-term Prophylaxis or Episodic Treatment of Patients with Haemophilia A: A Systematic Review

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Background: While short-term efficacy of episodic and prophylactic treatments for haemophilia A (HA) is well documented, there is a need to better understand long-term outcomes of existing treatment strategies in real-world clinical practice.

Aims: To evaluate clinical and humanistic outcomes from long-term prophylaxis or episodic treatment of HA.

Methods: A systematic literature review was conducted using EMBASE, MEDLINE and conference abstracts. Eligible studies were published in English between January 1, 2006 and December 15, 2016. Studies reporting at least 5 years of follow-up for patients with HA receiving prophylaxis and/or episodic treatment were included.

Results: Seventeen studies from 18 publications met the inclusion criteria with 15/17 (88%) including children. Of these, we qualitatively assessed characteristics and outcomes from 9 studies reporting all bleeding events (n=5 studies), joint bleeds (n=6), joint health (n=7), health-related quality of life (HRQoL, n=6) or burden (n=3). Seven studies were limited to severe HA (FVIII < 1%) and 5 to patients without inhibitors. Median follow-up ranged from 5.3-20.3 years. Mean annual bleeding rates ranged from 2.6-11.7 for prophylaxis and from 12.0-33.4 for episodic treatment. Mean annual hemarthrosis episodes ranged from 0.5-3.4 and 13.7-16.6, respectively. Prophylaxis patients had higher HRQoL overall and for several subdomains across studies, missed fewer days from school/work and had greater functional independence. All results were consistent across studies examining subgroups based on prior treatment exposure, age (adults and children), and the effects of switching between prophylaxis and episodic regimens.

Conclusions: Prophylaxis, whose short-term benefits on bleed control are well-documented, conferred better long-term outcomes including fewer bleeding episodes, better joint health and better quality of life. Results from real-world studies suggest higher bleeding rates than clinical studies and remaining unmet needs for long-term management of HA.

PB 1790 | Comparison of the Relationship between Factor IX Activity and Bleeding Risk during Prophylaxis with Nonacog Beta Pegol (N9-GP)

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Background: N9-GP (recombinant glycoPEGylated FIX with an extended half-life) has been developed to prevent and treat bleeds in haemophilia B with less frequent dosing and achieve higher FIX activity levels compared to non-modified FIX products. Safety and efficacy of N9-GP for prophylaxis (PPX) and treatment of bleeds were investigated in paradigm™2, the pivotal, multicentre, single-blind, non-controlled, randomised, phase 3 trial.

Aims: To evaluate the impact of FIX activity profile on bleeding risk in patients receiving once-weekly N9-GP PPX, taking N9-GP dose level and time since last dose into consideration.

Methods: Male patients aged 13-70 years with haemophilia B (FIX activity ≤2%) were randomised 1:1 to receive 10 or 40 IU/kg once-weekly N9-GP PPX for 52 weeks. *Post-hoc* analysis compared annualised bleeding rates (ABR) for each arm. FIX activity was assessed at steady-state in a subset of patients (n=59). Bleeding risk by time since last dose was analysed using Cox regression, including regimen and shared frailty to account for within patient correlation.

Results: Estimated ABR for patients in the 40 IU/kg arm was 49% lower versus the 10 IU/kg arm (p=0.033, Table 1). For all bleeds, the instantaneous bleeding risk by time since last dose was 1.93 times higher in the 10 IU/kg arm versus the 40 IU/kg arm (p=0.0123), and 2.72 times higher for spontaneous bleeds (p=0.0143, Table 2). The peak FIX activity at steady-state ranges were 0.72-1.44 IU/ml and 0.23-0.55 IU/ml for 40 and 10 IU/kg, respectively; while the steady state trough ranges were 0.25-0.43 IU/ml and 0.06-0.20 IU/ml. Lower bleeding risk appears to be associated with higher FIX activity, in favour of 40 IU/kg.

Conclusions: Patients achieved greater prophylactic protection with 40 IU/kg N9-GP once-weekly dosing than 10 IU/kg. Achieving close to physiological FIX activity levels for extended time periods could be important in delaying the progression of underlying joint disease for patients with haemophilia B.

Acknowledgements: The authors acknowledge the data analysis contributions of Judi Møss and Silke Ehrenforth (Novo Nordisk employees).

TABLE 1

Table 1. ABR by prophylaxis dose

	Prophylaxis		
	10 IU/kg	40 IU/kg	Both
Number of patients	30	29	59
Number of patients with bleeds, N (%)	25 (83.3)	16 (55.2)	41 (69.5)
Number of bleeds	132	70	202
Bleeds per patient (min; max)	0.0; 17.0	0.0; 17.0	0.0; 17.0
Mean treatment period (years)	0.97	0.96	0.96
Individual ABRs			
N	30	29	59
Median	2.93	1.04	2.04
Interquartile range	0.99; 6.02	0.00; 4.00	0.00; 5.00
Poisson estimate of ABR	4.56	2.51	3.55
95% CI	3.01; 6.90	1.42; 4.43	2.53; 4.98
p-value*	0.402	0.013	0.040
Estimated ABR reduction (adjusted)**			
40 IU/kg versus 10 IU/kg	–	–	0.49
95% CI	–	–	0.05; 0.73
p-value***	–	–	0.033

ABR, annualised bleeding rate; CI, confidence intervals.
ABR estimates based on a Poisson regression model with dose as a factor allowing for over-dispersion and using treatment duration as an offset.
*p-values are from the one-sided test of the null hypothesis that the ABR is at least 4.8 evaluated at the 2.5% level.
**Reduction: 1–ABR relative risk. Adjusted estimates are based on a model with covariate adjustment for prior treatment and historical ABR. The adjusted estimates exclude two patients on 10 IU/kg who are missing their historical ABR. Positive values indicate a decrease in bleeding rate and negative values indicate an increase.
***A two-sided test of the null hypothesis that there is no difference between the two doses evaluated at the 5% level.

TABLE 2

Table 2. Hazard ratio of bleeds by time since last N9-GP dose – for all dosing intervals

	Prophylaxis 10 IU/kg versus 40 IU/kg
All bleeds	
Hazard ratio	1.93
p-value	0.0123
Spontaneous bleeds	
Hazard ratio	2.72
p-value	0.0143
Traumatic bleeds	
Hazard ratio	1.15
p-value	0.4205

The cumulative hazard of bleed versus time since last administered dose was estimated in a Cox-proportional hazard model with treatment included as a fixed effect and patient modelled as a shared frailty. In this shared frailty model, the within-patient correlation is taken into account by assuming patient frailty follows a log-normal distribution.

PB 1791 | Efficacy of rVIII-SingleChain in the Treatment of Adult and Adolescent Patients with Severe Hemophilia A in Europe

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Background: rVIII-SingleChain is a novel B-domain truncated recombinant Factor VIII comprising covalently bonded FVIII heavy and light chains, and has high binding affinity for von Willebrand factor. The safety, efficacy and pharmacokinetics of rVIII-SingleChain were evaluated in the AFFINITY program involving patients from around the world.

Aims: To evaluate the efficacy of rVIII-SingleChain in the treatment of hemophilia A patients in Europe.

Methods: Participants ≥12 years with severe hemophilia A (FVIII < 1%) were enrolled in a pivotal trial and received either on-demand or prophylactic infusion of rVIII-SingleChain. Dose and regimen were at the investigator's discretion based on clinical bleeding phenotype and previous FVIII treatment and could be adjusted any time during the study.

Results: Of the 173 patients included in the efficacy population, 85 (49.1%) from 11 European countries were included. 69 patients received prophylaxis and 16 on-demand treatment with

rVIII-SingleChain. Median annualized total and spontaneous bleeding rates (ABR/AsBR) were significantly lower with prophylaxis than with on demand treatment (ABR: 0.00 vs 28.08; p< 0.0001; AsBR: 0.00 vs 22.71; p< 0.0001). These low bleeding rates with prophylaxis in this subset of patients were comparable with those for the overall study population (median ABR: 1.14 and median AsBR: 0.00). Hemostatic efficacy of rVIII-SingleChain was excellent/good in 92.7% of the 563 bleeds treated in Europe compared with 93.8% of 835 bleeds treated in the overall study population.

Conclusions: These data show the efficacy of rVIII-SingleChain in hemophilia A patients in Europe, both as prophylactic therapy and for treatment of bleeding events. Results are consistent with findings from the overall study population and show very low annualized bleeding rates during rVIII-SingleChain prophylaxis.

PB 1792 | Chronic Liver Disease in Elderly People with Hemophilia

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Background: Chronic liver disease (CLD) is frequently seen in the hemophilia population.

The ADVANCE Working Group conducted a cross-sectional, epidemiological study of Hematuria and Hypertension in Hemophilia A and B (the H3 Study) in which patients with hemophilia (PWH) aged

≥ 40 years were included.

Aims: Using data from the H3 Study, the main objective has been to assess statistical associations between CLD and its risk factors, and to suggest implications for treatments.

Methods: Data from 13 European countries were collected at a single time-point (2011-13).

Univariate (UVA) and multivariate (MVA) logistic regression analysis were performed to test for associations with CLD.

Results: 532 PWH were included with either Hemophilia A (n=467) or Hemophilia B (n=65).

127 (24%) were identified with CLD. In the MVA, we have included HCV (OR=21.1, p< 0.001), diabetes (OR=3.0, p=0.02), HAART (OR=1.9, p=0.04) and total cholesterol (OR=0.6, p=0.002) as explanatory variables.

HCV, HAART, total cholesterol, and severe hemophilia were highly statistically significant in UVA.

Age was not significant in either UVA or MVA.

HCV Ab+ was significantly positively associated with CLD. Diabetes was not significant in UVA, but significantly positively associated with CLD after controlling for HCV. HAART was significantly associated with an increased likelihood of liver disease in the MVA. Total cholesterol is strongly negatively associated with CLD.

We found no evidence of interaction effects among the explanatory variables.

No significant associations with age, type of or severity of hemophilia were observed in MVA.

Conclusions: In our data HCV was the most significant factor leading to CLD, consistent with previous research.

We found diabetes significantly associated with liver disease after controlling for HCV.

To prevent CLD, intensified eradication therapy for HCV seems advocated. Furthermore, intensified monitoring and treatment of diabetes could be warranted and represents an interesting candidate for further research.

PB 1793 | Once Weekly Rfix Prophylaxis for Severe-Phenotype Haemophilia B in Normal Clinical Practice: Data from UKHCDO and Finland

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Background: New understanding of the pharmacokinetics (PK) of factor IX (FIX) in haemophilia B (HB), trials of weekly rFIX prophylaxis (WP) and the advent of enhanced half-life rFIX (EHL-IX) have focussed interest on less frequent prophylaxis with rFIX.

Aims: To collate data from a UK and Finnish cohort with severe HB using WP between 2014/ 2016.

Methods: UK data was derived from Haemtrack diary system. Finnish patients were identified by the Helsinki Haemophilia Centre and data taken from treatment diaries/ medical records. Pearson's coefficient was used to assess relationships between dose, genotype and ABR.

Results: 19 patients aged med. 37 years were selected empirically for WP. Where known, indications were patient choice (50%); poor venous access (15%); inability to self-inject (10%); poor compliance (10%). 11 patients did not comply with prophylaxis or used on-demand treatment prior to the study period (Med. ABR 12.5, Mean 13.0, IQR 5.5 - 20.0). Compliant WP was effective, with a med. ABR of 0.5 (Mean 1.7, IQR 0 - 2.3) using a med. dose of 67 IU/kg/wk; (IQR 40 -74). ABR did not correlate with dose (R= 0.32, p >0.1) or genotype. 8 patients had a trough FIX of < 0.01 - 0.12 IU/ml at 5 to 7-days; 4 trough levels of 0.06 - 0.11 IU/ml at 2 - 4 days. 1 patient had a t1/2 of 12 hrs.

Conclusions: WP with rFIX provided efficacy similar to that reported in clinical trials of WP and EHL-IX., although our cohort used lower doses. It was effective even in patients with a previous high ABR.

Most would have had FIX < 0.01 IU/ml at 7 days, yet still had a low ABR. Bleed protection appeared to extend beyond the point at which circulating F IX could no longer be measured. This supports suggestions that plasma rFIX level and t1/2 are poor surrogates for clinical efficacy. Although not suitable for all, WP may be used effectively in a subgroup of HB patients and could be used more widely. Further comparisons of rFIX WP and EHL-IX are justified.

PB 1794 | Pharmacokinetic Guided Personalized Prophylaxis with Human-cl rhFVIII in Adult Patients with Severe Hemophilia A

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Background: GENA-21 studied pharmacokinetic (PK)-guided personalized prophylaxis with a human cell-line derived recombinant FVIII concentrate (Human-cl rhFVIII) in 66 previously treated adults (PTPs) with severe hemophilia A predominantly treated on-demand before study entry. With Human-cl rhFVIII, the median dosing interval was 3.5 days, 58% of patients received ≤2 infusions per week and 73% of patients did not bleed during the 6 month prophylactic period.

Aims: GENA-21b is assessing the benefit of PK-guided prophylaxis in patients on routine prophylactic treatment prior to study start.

Methods: The ongoing study will enroll 55 adult PTPs with severe hemophilia A from USA, Canada, Europe, and Japan. The study will be approved by local ethics committees and all patients will provide written informed consent. Each patient will receive Human-cl rhFVIII for PK evaluation followed by 1-3 months of routine prophylaxis (30-40 IU/kg every other day or 3 times per week, Phase I). Individual PK data are analyzed from FVIII:C plasma levels to determine the dose and injection interval which should result in a trough FVIII level of ≥1%. Thereafter, prophylaxis will continue for 6 months based on the individually recommended treatment schedule (Phase II).

Results: Snap shot data on approximately 40 patients will be presented at the ISTH meeting. So far, 29 patients have undergone PK with a median half-life of 15.2 hours. The median treatment interval in Phase I regular prophylaxis is 3 times per week, with a median weekly dose of 105.2 IU/kg. For the individualized prophylaxis, 58.6% of patients were recommended to use Human-cl rhFVIII 2 times per week or less with a median weekly dose of 86.6 IU/kg, an 18% dose reduction.

Conclusions: Both in patients treated previously on-demand and in those prophylactically, PK-guided prophylaxis resulted in an extension of the treatment intervals in more than 50% of the patients while reducing the weekly dose as compared to routine prophylaxis.

PB 1795 | Application of Pharmacokinetic-tailored Approach to Prophylaxis in Routine Hemophilia Care

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Background: Best practices for switching from conventional to extended half-life (EHL) factor products have not been defined for hemophilia. Data are lacking to guide individualized approaches for transition to EHL products, incorporating personal half-life assessment, physical activity level, and bleed frequency.

Aims: To examine "real-world" experience transitioning patients to EHL FVIII prophylaxis regimens informed by sparse-sampling estimation of terminal half-life.

Methods: We performed a retrospective chart review of patients considering transition from conventional to EHL products collecting data on prescribed prophylaxis regimen, bleed rate, and activity level. Half-life (t1/2) was estimated by first order decay based on at least 2 post-infusion samples in the terminal portion of the PK curve for both conventional and EHL products.

Results: 22 patients had t1/2 estimation on a conventional FVIII product. Of these, 7 underwent t1/2 estimation following first dose of an EHL product. Post-infusion samples were drawn at mean times of 0.5, 25, and 45 hours. Mean half-life extension was 1.4x baseline (0.99-1.79). Five elected to switch products. Median conventional product dosing was 91.5 IU/kg/week (range: 88-476) which decreased to 62 IU/kg/week (62-179) on EHL product. Median annualized bleed rate (ABR) was 0 prior to product switch and 0 afterward. No patients returned to conventional product. 2 patients did not switch to an EHL product due to minimal change in t1/2; remaining patients expressed satisfaction with conventional product prophylaxis regimen.

Conclusions: Estimation of an individual's factor product half-life supports a tailored prophylaxis regimen. Prescribed prophylaxis regimens based on three relevant parameters (t1/2, bleed history, and activity) rarely match the label recommended regimen. Switching is not for everyone. Comparative effectiveness studies examining health outcomes including quality of life, and economic impact of PK-tailored versus empiric dosing approaches are needed.

PB 1796 | Prevalence of Cerebral Microbleeds and Macrobleeds in Neurologically Asymptomatic Patients of Congenital Hemophilia

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Background: Intracranial hemorrhage (ICH) is one of the most serious complication of hemophilia.

Aims: To study whether presence of cerebral microbleeds (CMB) predict future risk of ICH in patients with hemophilia.

Methods: We prospectively studied 53 patients of congenital hemophilia between age group of 12-45 years who had no past history of ICH and who were neurologically asymptomatic. All patients underwent GE-MRI Brain(1.5 Tesla MR unit) to look for the presence of CMB. The brain imaging was done using a T1 spin echo, T2 Fast spin echo & T2 weighted gradient echo sequences in axial plane. In addition, sagittal T1 spin echo & coronal sections were also used. The gradient echo images were analyzed for presence of microbleeds which was defined as small areas (size < 1cm) of rounded area of marked and homogeneous signal loss. Information on severity of hemophilia, presence of inhibitors, sites of bleed and type of therapy (prophylaxis or on demand) was collected from all patients. Informed consent was taken from all patients and the study was approved by institute ethics committee.

Results: There were 47 patients of hemophilia A & 6 were of hemophilia B. Seventeen 17 patients (32%) were having severe hemophilia, 29 (54%) moderate & 7 (14%) had mild hemophilia. Three patients had evidence of ICH (1 CMB + 2 macrobleed) on MRI with a prevalence of 5.7%. Mean factor level among ICH group was lower (0.33%) as compared to non ICH group (3.37%) (p value non significant). Prevalence of inhibitors among study group was 16% and none of the patients with ICH had presence of inhibitors. There was no occurrence of ICH in any patient during 6 months of follow up.

Conclusions: The prevalence of CMB and microbleed in neurologically asymptomatic patients with congenital haemophilia was 5.7%. Their presence did not predict the future risk of ICH in these patients. However, more patients with longer follow up is required to clearly delineate the association between CMB and ICH in patients with congenital haemophilia.

PB 1797 | Long-term Safety and Efficacy of Recombinant Factor VIII Fc (rFVIIIFc) for the Treatment of Severe Haemophilia A: European Subgroup Interim Analysis of the ASPIRE Study

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Background: The ongoing rFVIIIFc extension study, ASPIRE (#NCT01454739), evaluates the long-term safety and efficacy of rFVIIIFc in adults, adolescents, and children with severe haemophilia A.

Aims: To report interim outcomes for European subjects in ASPIRE.

Methods: Subjects who completed A-LONG or Kids A-LONG could enrol in 1 of 4 treatment groups in ASPIRE: individualised prophylaxis (IP; 25-65 IU/kg every 3-5d, or 20-65 IU/kg on D1 and 40-65 IU/kg on D4 if twice weekly); weekly prophylaxis (WP; 65 IU/kg every 7d); modified prophylaxis (MP; subjects not achieving optimal prophylactic dosing with IP or WP); or episodic treatment (ET). Subjects could change groups at any time. Subjects < 12y could enrol only in IP and MP groups. Primary endpoint: development of inhibitors. Secondary endpoints included annualised bleeding rate (ABR) and rFVIIIFc exposure days (EDs). Data from the 3rd interim data cut (11 January 2016) are reported here.

Results: Of 67 subjects (36 from A-LONG; 31 from Kids A-LONG) enrolled from 12 European countries,

60 remained on ASPIRE as of the 3rd interim data cut. From start of parent study to the interim data cut, subjects had a median of 217 (A-LONG) and 152 (Kids A-LONG) wks of treatment with rFVIIIFc, and a median of 341 (A-LONG) and 312 (Kids A -LONG) cumulative rFVIIIFc EDs. No inhibitors were observed; adverse events were typical of a general haemophilia A population. Median (IQR) ABRs were low with rFVIIIFc prophylaxis (Table 1). Median (IQR) change in weekly prophylactic consumption from end of A-LONG was 0.00 (-7.00, 0.00) and Kids A-LONG 0.00 (0.00, 16.67). In subjects from A-LONG and Kids A-LONG, 92.2% and 92.6% of bleeding episodes, respectively, were controlled with 1-2 injections.

Conclusions: Interim data from European ASPIRE subjects are consistent with the phase 3 parent studies and overall ASPIRE interim analysis. The results confirm the long-term safety of rFVIIIFc and maintenance of low ABR with extended prophylactic dosing intervals in individuals with severe haemophilia A.

TABLE 1 ABRs in European ASPIRE subjects as of the 3rd interim data cut

	A-LONG subjects (N=36)*				Kids A-LONG subjects (n=31)
ABR, Median (IQR)	Individualised prophylaxis, n= 29	Weekly prophylaxis, n =8	Modified prophylaxis, n=2	Episodic treatment, n=2	Individualised prophylaxis n=31
Overall ABR	0.85 (0.00, 2.25)	2.91 (1.35, 5.40)	3.41 (0.00, 6.82)	22.16 (13.86, 30.46)	1.90 (0.50, 3.90)
Spontaneous ABR	0.27 (0.00, 1.87)	2.05 (0.43, 2.52)	3.13 (0.00, 6.25)	11.83 (10.88, 12.77)	0.40 (0.00, 0.90)
Joint ABR	0.57 (0.00, 1.95)	1.65 (0.58, 2.58)	3.13 (0.00, 6.25)	9.65 (6.25, 13.05)	0.76 (0.00, 1.87)

ABR, annualised bleeding rate; IQR, interquartile range. *Subjects may be included in >1 treatment group

PB 1798 | Evaluation of the Impact of Polymorphism/Mutations in Genes Associated to Hemostasis in Clinical Diversity in Patients with Severe Hemophilia

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Background: Patients with severe hemophilia A and B (< 1% FVIII or FIX) present important clinical variability, mainly in relation to the frequency of bleeding events. The cause of this is still unclear.

Aims: To investigate the possible influence of polymorphisms/mutations in genes of proteins related to blood coagulation on clinical diversity of hemophilia A and B.

Methods: Polymorphisms/mutations on the genes of Factor VII (A353G), promoter region of Protein C (A/G -1641 e A/T -1476), Protein S (A2148G), Factor V Leiden (R506Q) and Prothrombin mutant (G20210A) were investigated in blood samples from 50 patients with severe hemophilia A (HA, n=44) or hemophilia B (HB, n=6) from Fundação HEMOMINAS, Minas Gerais, Brazil. Genomic DNA was extracted and amplified for analysis of polymorphisms and mutations by PCR-RFLP. Annual number of bleeding events was obtained from medical records and compared with the polymorphisms and mutations results by using T test. Brazilian ethics committee approved this study and informed consent was obtained from all patients.

Results: The frequency of polymorphisms/mutations in genes associated with hemostasis is presented in Table 1.

The annual bleedings events mean for 50 patients investigated retrospectively at the period between 2006 and 2016 was 11,5 (SD ± 10,35; CI = 2,86). The number of bleeding events was stratified in values below and above 11,5 and we identified 21 cases above and 29 below this mean value. When both groups were compared as to presence of polymorphisms/mutations, significantly difference was not observed.

Conclusions: The variability in the number of bleeding events in patients with hemophilia A or B, if above or below the mean, could not be related to the genetic profile based on polymorphisms/mutations in proteins related to coagulation process, therefore, other factors must be investigated. Financial support: CAPES, CNPq and FAPEMIG

PB 1799 | The Influence of F8 Mutation and Thrombophilic Genetic Markers on Bleeding Phenotype of Patients Affected with Severe Hemophilia A in the SIPPET Cohort

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TABLE 1 Frequency of homozygous, heterozygous and wild type (*) forms of polymorphisms/mutations studied

Factor VII A353G	N (%)	Protein S A2148G	N (%)	Factor V Leiden R506Q	N (%)
AA*	3 (6%)	AA*	30 (60%)	AA*	50 (100%)
AG	9 (18%)	AG	19 (38%)	AG	0 (0%)
GG	38 (76%)	GG	1 (2%)	GG	0 (0%)
Protein C A/G -1641	N (%)	Protein C A/T -1476	N (%)	Prothrombin G20210A	N (%)
AA*	8 (16%)	AA*	10 (20%)	GG*	50 (100%)
AG	17 (34%)	AT	23 (46%)	GA	0 (0%)
GG	25 (50%)	TT	17 (34%)	AA	0 (0%)

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Background: Severe hemophilia A (HA) presents a wide spectrum of bleeding tendency and previous studies suggested that bleeding may be influenced by factor VIII gene (*F8*) mutations and genetic thrombophilic risk factors.

Aims: To evaluate the role of *F8* mutations, prothrombin G20210 mutation (PT) and factor V Leiden (FLV) as determinants of bleeding tendency.

Methods: Bleeding tendency was evaluated considering the occurrence of the first bleeding and the first joint bleeding from birth to the start of prophylaxis or inhibitor development in a cohort of 251 patients with severe HA enrolled in the SIPPET study. Survival analyses were performed to calculate the risk of bleeding according to *F8* mutations and the presence of a mutated prothrombotic allele.

Results: 242/251 (97%) children with severe HA experienced their first bleeding, within the age of 56 months at a median of 7 months (IQR, 2-16); hematoma was the most common type occurring in 80/242 (33%). 115/251 (46%) experienced their first joint bleeding, within 84 months at a median of 21 months (IQR, 10-33). Out of 234 patients with a known *F8* mutation, 197 (84,1%) carried a null-mutation. Table reports the age at bleeding by type of *F8* mutation.

TABLE 1 Age in months at bleeding occurrence, median (interquartile range)

Bleeding event (number)	All patients	Patients with null mutations	Patients with non-null mutations	p-value
First bleeding (242/251)	7 (2-16)	8 (2-16)	6 (2-16)	0.869
First joint bleeding (115/251)	21 (10-33)	21 (10-35)	25 (10-33)	0.667

Patients with a null-mutation did not differ to patients carrying a non-null mutation with a HR of 0.94 (CI95% 0.66-1.33) for the first bleeding and a HR of 0.67 (CI95% 0.41-1.11) for the first joint bleeding. Prevalences of PT and FLV were 2.2% (5/231) and 6.9% (16/231), respectively and no difference has been observed in carriers and non-carriers (for the first bleeding HR 1.35, 0.84-2.16; for the first joint bleeding HR 1.02, 0.47-2.20).

Conclusions: The results of this cohort showed that previously untreated patients bled for the first time mainly within 2 years, and hematoma was the most common site. The age of the first bleeding and of the first joint bleeding did not differ between patients carrying a null or a non-null mutation. *F8* mutations and prothrombotic variants did not explain bleeding severity.

PB 1800 | Extensions of F9 Mutation Spectrum and Genotype-phenotype Heterogeneity in Haemophilia B

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Background: Hemophilia B (HB), a coagulopathy, is among the first to have been studied genetically. The World Federation of Hemophilia (WFH) Annual Global Survey 2014 reports a total 80 patients with HB. Figures for prevalence of HB in Pakistan reported annually by WFH since 1998 are very inconsistent the reason being lack of a centralized national registry. To date, only one study has been performed on genetic analysis of HB patients from Pakistan, identifying 11 different mutations.

Aims: This study was aimed to detect *F9* genetic alterations in haemophilia B (HB) patients of Pakistani origin. We also aimed at determining the genotype-phenotype relationships in these patients.

Methods: A total of 34 known HB patients from 22 unrelated families were enrolled into the study after obtaining informed written consent. There diagnoses were reconfirmed by determining the F.IX coagulation activity (F.IX:C). Genomic DNA was extracted and PCR amplification of all exons, flanking intronic regions, the promoter region and polyadenylation site was performed. All the mutations were analysed for functional consequences employing in-silico analysis tools. Genotype-phenotype correlation was determined.

Results: A total of 17 different mutations were found in 33 patients. Missense mutations were the most frequent, followed by nonsense mutations. Other mutations included a deletion, a splice site variation and a branch point change. A missense, a short insertion and nonsense novel mutations in exon 2, 6 and 7, respectively, were also identified. F.IX:C phenotypic heterogeneity was found in case of two mutations.

Conclusions: We concluded that the registered F.IX deficient population of Pakistan mainly comprises moderate HB. *F9* mutational spectrum in Pakistani HB patients is heterogeneous. Genotype-phenotypic correlation varies in a significant number of HB patients.

Keywords: Factor IX, Haemophilia B, F9 mutations, Genotype-phenotype heterogeneity

PB 1801 | Evaluate the Situation Diagnosis and Treatment of Hemophilia in Vietnam in 2015

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Background: In Vietnam, estimated, there are 6,000 patients with hemophilia, 30,000 carriers, in which only 40% is diagnosed and managed.

Aims: Evaluate the situation of diagnosis and treatment for hemophilia patients in Vietnam.

Methods: Cross - sectional survey, analysis without involvement, using questionnaires which was prepared.

Results: Following 506 hospitals received the questionnaires, there're only 149 hospitals responded to the survey (response rate is 39.3%). Survey response rate in central hospitals is 57.1%, in provincial hospitals is 64% and in district hospitals was 33%.

There are 54/ 149 hospitals that have treated and managed hemophilia patients (36.24%); of which there are 8 central hospitals and 32 provincial hospitals.

Most of surveyed hospital have implemented basic coagulation tests (PT, APTT, SLTC, Fib/TT) but in which there is only 62.5% of central hospitals and 18% of provincial hospital have implemented FVIII and FIX assay test. There's only 6 hospitals have implemented inhibitor assay test and there're only 8 hospital did the von Willerbrand diagnosis test.

There is a ratio of 100% of central hospitals surveyed have hematologists. 84% of provincial hospital surveyed have from 1 to 3 hematologists. There is a ratio of 10% of provincial hospitals surveyed have not hematologists and 82.4% district hospitals have not hematologists.

There are 6 hospitals in the survey (the ratio is 4%) had adequate of blood products including fresh frozen plasma, cryoprecipitate, factor concentrates: VIII, IX and VIIa to treat for hemophilia patients.

Conclusions: Most of patients with hemophilia in Vietnam were managed in National Institute of Hematology and Blood Transfusion (NIHBT) and Blood Transfusion of Hematology in Ho Chi Minh city (BTH). The ability to deploy the diagnostic tests of hemophilia in the majority of hospitals is still limited. Blood products (especially factor concentrates) for treatment are only available in some hospitals.

PB 1802 | Low Dose (20 iu/kg/week) Single Infusion Prophylaxis per Week Using Long Acting FVIIIc (ELOCTATE, Biogen) in Severe Hemophilia A: A Cost Effective & Feasible Protocol for Resource Constraint Situation

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Background: Various Intermediate to High dose (50 - 90 iu/kg/week) prophylactic protocols are well established.These are twice or thrice a week infusions. These protocols are out of reach in resource constraint countries.Episodic therapy is NO therapy for PWH.It may be better to use LOW dose prophylaxis than episodic therapy. Multiple infusions been the major issue of compliance.No data is available on once a week infusion of Long acting Eloctate with lower dose prophylaxis regimen.

Aims:

1) Assess efficacy of IV Eloctate 20 iu/kg/week single infusion prophylaxis regimen for Severe Hemophilia A

Outcome assessment at one year:

1) Reduction in Annualised Bleed Rate(ABR)

2) Reduction in days missed

Methods:

Study period: February16 -January17

Data at enrolment:

Number of PWH: 34

Age: 8 (5-11years)

Weight: 24 (19-32kg)

ABR in previous year: 19 (15-32)

Number of target joints: 1 (1-4)

HJHS score: 10/116 (0-22)

FISH score: 30/32 (26-32)

Days Missed: 26 (20-61)

All the patients had more than 50ED on episodic regimen and were Inhibitor Negative at enrolment.All patients were on regular physiotherapy schedule which was continued during study period.IV Eloctate 20iu/kg was infused once a week.Once in three months inhibitor tests were performed.All breakthrough bleeds were documented and treated with extra infusions of Eloctate. Days missed at were documented.Physical assessment was done once in 6months.Eloctate was received through Humanitarian aid Program of WFH.

Results: All patients completed the study showing 100% adherence to infusion schedule.

Outcome assessment for study period:

1) ABR: 3 (3-9)

2) Days missed: 9 (6-20)

3) Total bleeds: 102

4) Extra doses used: 127

5) Average Eloctate used: 1100 iu/kg/year

6) Inhibitor: Nil

There was marked reduction in ABR though no ZERO ABR achieved for any patient.There was no major change in HJHS or FISH score.

Conclusions: Once a week low dose IV Eloctate is feasible & effective prophylaxis regimen for resource constraint countries.

The reduction in ABR may lead to far better HJHS & QOL in long term for PWH.

PB 1803 | Development of the Education Interventions to Enhance Adherence to Prophylactic Treatment in Korean Hemophilia Patients

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Background: Prophylactic treatment is now considered as a standard management for severe hemophilia patients. Regarding good quality of life and the cost-effectiveness, a patient´s adherence to prophylactic treatment is one of the most important factors to desired outcomes.

Aims: The aims of this study are to know the present adherence of the patients in Korea and to develop the educational interventions to enhance adherence on the basis of the results.

Methods: We used the VERITAS-Pro as a measuring method for adherence to prophylactic treatment with permission to use from the authors of the VERITAS-Pro. We calculated individual scores of adherence and the average scores of six subscales of the patients who participated in this study.

Results: Ninety five eligible patients were recruited for participation from five hemophilia treatment centers. Of the ninety five patients with prophylactic treatment, eighty two patients were hemophilia A and thirteen patients were hemophilia B. The mean age of the were 20.9. (range: 1-63). The mean total score was 40.4 (range: 24-75). Subscale mean scores were 5.99 (Remember), 6.12 (Dosing), 6.22 (Skipping), 7.23 (Communicating), and 8.43 (Time) (Table 1).

TABLE 1 The mean scores of 6 subscales in VERITAS-Pro

Subscales	Scores (mean±standard deviation)
Timing	8.43±3.40
Dosing	6.12±2.33
Planning	6.43±2.48
Remembering	5.99±2.32
Skipping	6.22±2.76
Communicating	7.23±2.92

Conclusions: In this study we evaluated the present level of adherence to the prophylactic treatment in Korean hemophilia patients. We also recognized the need for education programmes focused on the proper time of the regular administration of clotting factor concentrates to the patients with prophylactic treatment. From the base of this result, we made the education card for presentation of importance of morning administration of clotting factor concentrates and guidance of education program of skill for self-infusion (Figure 1). We convinced this whole process can enhance adherence in patients with hemophilia in Korea.

FIGURE 1 Education card

PB 1804 | Immune Tolerance Induction in Patients with Haemophilia using Fc Fusion Recombinant Factor VIII

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Background: It has been reported that shorter duration immune tolerance induction (ITI) was achieved in three patients who were treated with rFVIIIFc, a long-acting recombinant factor VIII Fc fusion protein. ITI using rFVIIIFc is therefore expected to be effective for patients receiving haemophilia inhibitor treatment.

Aims: In this study, we describe the time course of the inhibitor titres of two Japanese patients who received initial and rescue ITI with rFVIIIFc.

Methods: This study was conducted at Ogikubo Hospital, Tokyo, Japan. Written informed consent was obtained from the two patient's parents. The two patients were diagnosed with severe haemophilia A (FVIII < 1%) and had anti-FVIII inhibitors.

Results: The clinical characteristics of these patients and the time course of their inhibitor titres are shown in the table and figure. [Case1] A 2-year-old male patient in whom ITI was initiated with rFVIIIFc. Inhibitors were detected with standard half-life rFVIII. After one month, ITI treatment was initiated with rFVIIIFc (110IU/kg, twice a week). This patient underwent an initial course of ITI. [Case 2] A 3-year-old male patient received rescue ITI. Before switching to rFVIIIFc,

the patient had previously received rFVIII for 12 months as ITI treatment; however, the treatment was unsuccessful.

The previous ITI dose was increased to four-fold that of the first regimen; however, the treatment failed. The patient received rescue ITI with rFVIIIFc (180 IU/kg, three times per week).

Conclusions: We reported the cases of two patients who received initial ITI and rescue ITI with rFVIIIFc, in which partially successful outcomes were achieved. rFVIIIFc has been reported to promote Tregs in animal models; accordingly, Tregs may be one of the factors associated with successful treatment. These results suggest that rFVIIIFc may be an effective choice for both initial and rescue ITI.

TABLE 1 The clinical characteristics of the patients undergoing ITI with rFVIIIFc

Case		1	2 ※
Age at inhibitor detection		2 years and 7 months	1 year and 2 months
Exposure days at inhibitor detection		15 days	5 days
Before ITI	First titre level (= peak titre)	6 BU	12 BU
During ITI	Prior ITI	No	Yes
	Age at start of ITI	2 years and 8 months	2 years and 1 month
	Inhibitor titre at initiation	6 BU	5BU
	Peak titre	21 BU	131 BU
	Last known titre	5 BU	32 BU

※The data include prior ITI and rescue ITI with rFVIIIFc

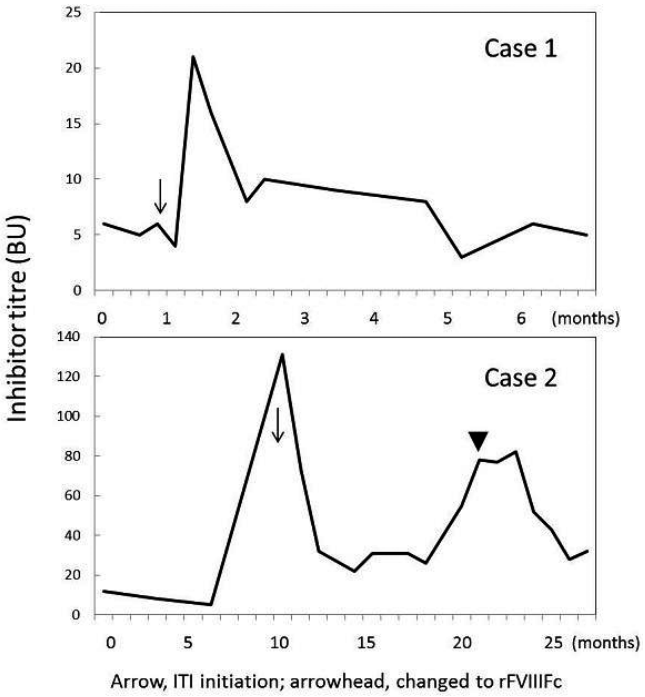

FIGURE 1 The time course of the inhibitor titres

PB 1805 | Spectrum and Origin of Mutations in Sporadic Cases of Haemophilia A in China

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Background: Many newly diagnosed Chinese haemophilia A patients are sporadic cases. In these sporadic HA families, it is important to confirm the origin of the mutation and carrier status.

Aims: In this report, we will introduce the spectrum and origin of mutations in 365 sporadic HA families in China and the methods of carrier and prenatal diagnosis in our center.

Methods: LD-PCR and PCR were adopted for the screening of the intron 22 and 1 inversion respectively. FⅧ sequences were analyzed by direct DNA sequencing. 7 STR sites related to F8 gene were combined together to do linkage analysis to define the origin of mutation in sporadic families. AccuCopy method was used to detect the copy number variations of the F8 gene to confirm deletions or duplications.

Results: In 365 sporadic families, 141 were caused by inversions, 129 were INV22 and 12 were INV1. For the inversions negative families, 127 were due to the point mutations including the splicing mutations, 65 were found small deletions/insertions, 32 were caused by large deletions/duplications. According to the linkage analysis, we confirm the mutation origin of these sporadic families and found that the most of cases got the mutation from the probands' maternal grandfathers (58.59%). For the mutations derived from the maternal grandfathers, the most common was INV22 which presented for 47%. While, for the de novo mutations, the INV22 was only found in 11% families but the point mutation was 54%. Combined with the linkage analysis, we did carrier diagnosis in these families and detected 327 carriers. For prenatal diagnosis, 68 fetuses were normal, 16 were female carriers and 33 were male patients.

Conclusions: According to the results, the mutation types of the sporadic families were characteristic in groups with different origins of the mutations. We can reasonably suggest that intron 22 and 1 inversions screening, F8 gene sequencing and CNVs detection combined with the linkage analysis are available for carrier and prenatal diagnosis in Chinese HA families.

PB 1806 | The Utility of Activated Partial Thromboplastin Time Clot Wave Form Analysis in the Investigation of Hemophilia A Patients Using Destiny Max TM Analyzer

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Background: It is difficult to diagnose the true severity of Hemophilia A (HA) by conventional one-stage aPTT based clotting assay when

FVIII:C level is 1.0 IU/dl or less. The clot formation waveform on a photo-optical detection system, provides information not only on clotting time but also coagulation velocity and acceleration denoted as Min1 and Min2 respectively.

Aims:

- To determine the best possible combination for aPTT clot waveform analysis (CWA) on Destiny Max^{TM} analyzer (Tcoag) with variation in reagents (aPTT S or aPTT HS), calcium chloride concentration (0.020 M or 0.025M), wavelengths (405 or 635 nm) and reagent dilutions by using STA-deficient FVIII plasma spiked with Kogenate at increasing concentrations of FVIII (0.3, 0.6, 1.0 and 2.5 U/dl).
- To assess if CWA could predict the bleeding phenotype independently from FVIII:C assay.

Methods: aPTT assay was performed using TriniClot aPTT S and HS (Tcoag). 29 patients with severe HA, 13 with moderate HA and 103 controls from blood bank donors were included. Severe clinical phenotype was defined as the presence of spontaneous bleeding episodes < 1-year old or joint or muscular bleeding < 3-years of age, ≥2 joints involved or ≥5 factor replacements in a year or the presence of intracranial bleeding.

Results: For the four combinations (S/405, HS/405, S/635, HS/635), the correlation coefficient between FVIII:C and Min1 were respectively 98.6 %, 97.1%, 95.8% and 98.4% (Figure 1). There was a better correlation between Min1 and FVIII:C while using 405 than 635 nm (p< 0.001). There was a significant (p=0.001) correlation of Min1 and Min 2 with the type of hemophilia and bleeding phenotype. Min1 showed a better discrimination of HA type and bleeding phenotype than other CWA parameters (Figure 2).

Conclusions: We conclude that aPTT waveform analysis will be able to define qualitative and quantitative differences in HA patients at FVIII levels < 1% and is useful for the investigation of the clinical phenotype and response to therapy.

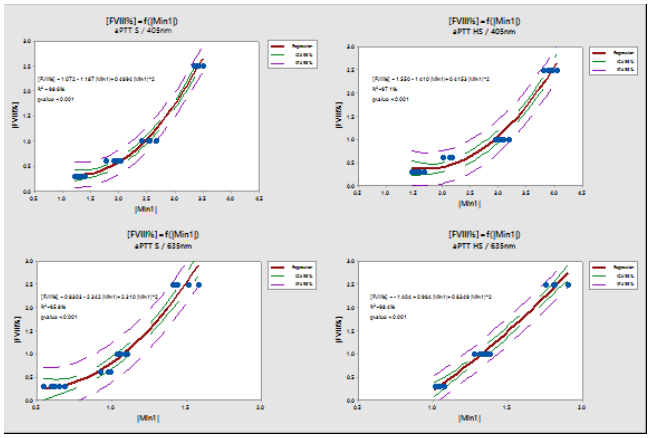

FIGURE 1 Correlation coefficient between FVIII:C and Min 1 based on two reagents (HS and S) and two wavelengths (405 and 635 nm)

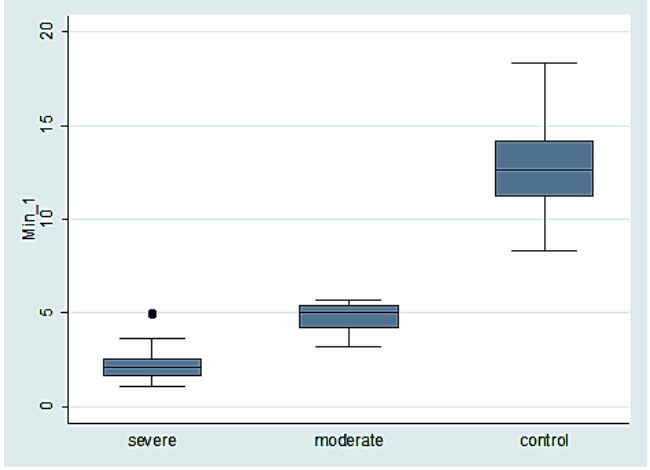

FIGURE 2 Box plot for Min 1 according to HA type (Interquartile range in brackets)

PB 1807 | Pharmacokinetics of Octocog α in Severe Hemophilia A Boys Aged Less than Six Years on Routine Prophylaxis

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Background: It is believed that personalized prophylaxis guided by: period of life, physical activity, bleeding phenotype and pharmacokinetic parameters (PK) may reduce the number of bleeds in severe hemophilia A (sHA) patients. Young children are physically active in uncontrollable manner and primary prophylaxis aims to prevent bleeds thus PK remain the only tool to „tailor" management. Based on bayesian probability myPKFiT has been developed to assess PK parameters in HA patients treated with octocog α (o-α).

Aims: To assess selected PK parameters (t1/2, time to < 1%) as well o-α activity at different time points in boys aged < 6 yrs. with sHA on prophylaxis with the use of o-α given at the dose 20-40 U/kg three times weekly.

Methods: The study comprised 39 Caucasian boys aged 33-71 (median 59.5) mo. enrolled in Polish nationwide prophylaxis. All patients were given o-α at the dose of 20.9-39.1 (median 31.7) IU/kg 3 x

weekly (usually Mon-Wed-Fri). Blood was sampled twice: 3 and 24 h. from a single dose infusion. o-α activity was assessed in local labs with the use of one-step coagulation assay and analyzed centrally with the use of myPKFiT application.

Results: Results are presented in Tab. 1 and 2.

PK parameters in the group studied were consistent with those presented by manufacturer. It has been shown that 31/39 (79.5%) pts had an o-α threshold >1% 48 h from drug infusion however after the following 12 h it remained >1% in 6/39 (15.4%) only. Moreover o-α thresholds >3% at 24h, 36h and 48 h from infusion were observed in 39/39 (100.0%), 39/39 (100.0%) and 8/39 (20.5%) pts, and >5% in 39/39, 6/39 (15.4%) and 0/39 (0.0%) pts respectively.

TABLE 1 PK parameters of octocog α in the group studied as measured by myPKFiT

	clearance (dL/h/kg)	volume in steady state (dL/kg)	FVIII half-life (h)	time to 1% above baseline (h)
mean	0,044	0,576	9,582	53,45
standard dev.	0,006	0,043	0,722	5,94
min.	0,030	0,500	8,500	43,00
max.	0,055	0,600	11,30	67,00
median	0,037	0,538	9,041	48,22

TABLE 2 Octocog α activity at different time points in the group studied

	0 h (%)	12 h (%)	24 h (%)	36 h. (%)	48 h. (%)
mean	79,55	20,93	8,80	3,79	1,73
standard dev.	16,56	4,76	2,46	1,26	0,61
min.	49,50	12,30	4,80	1,90	1,00
max.	116,10	31,00	14,50	6,80	3,30
median	64,53	16,61	6,80	2,48	1,73

Conclusions: For bleeding prophylaxis in boys younger than 6 years with sHA oc-α shoud be administered at intervals not longer than 48 h. These children would probably benefit if treated with extended half-life products in order to achieve higher FVIII trough level or prolonged intervals between infusion.

PB 1808 | The World Bleeding Disorders Registry: The Pilot Study

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Background: Significant gaps in evidence supporting optimal care of bleeding disorders still exist, which are difficult to address with conventional clinical study designs, such as RCTs. The World Bleeding Disorders Registry (WBDR) (ClinicalTrials.gov NCT02776826) is intended to fill these gaps by generating an unprecedented amount of real world data, which will be used for generating evidence to improve the quality of care worldwide.

Aims: The feasibility of conducting a global patient registry was assessed in a pilot study.

Methods: The pilot was an observational, global registry of patients diagnosed with hemophilia A or B, replicating the methodology of the planned WBDR, including the use of a web-based data entry system (McMaster University, Canada). Ethics and patient consents were obtained.

The feasibility of implementing the WBDR was assessed on HTC and patient interest, ability to obtain ethics and successful entry of quality data. Invited HTCs represented the 4 levels of economies based on World Bank´s classification by gross national income (GNI).

Results: Thirty-one of the 40 invited HTCs accepted to participate in the pilot study (78% participation rate). Two HTCs withdrew before applying for ethics, 2 HTCs were declined ethics approval, and 1 was still pending at study closure (Table 1). Twenty-six (90%) HTCs obtained ethics approval. As of database lock on 31 December 2016, 356 patients were enrolled and only 7 patients declined participation (98%). Data quality is currently being assessed. Acceptance of HTCs to participate in the WBDR pilot decreased with increasing GNI (Table 1).

TABLE 1 HTC Disposition: World Bank's Economic Classification by Gross National Income

	Disposition of HTCs by GNI n/N (%)				
HTC Disposition	Total	High-income	Upper middle-income	Lower middle-income	Low Income
Invited	40	14	8	13	5
Accepted	31/40 (78%)	8/14 (57%)	6/8 (75%)	12/13 (92%)	5/5 (100%)
Applied to ethics	29/31 (94%)	6/8 (94%)	6/6 (100%)	12/12 (100%)	5/5 (100%)
Obtained ethics approval	26/29 (90%)	5/6 (94%)	6/6 (100%)	11/12 (92%)	4/5 (80%)

Conclusions: This pilot confirms the interest of HTCs and patients in participating in a global registry, as well as the ethical acceptability and technological feasibility of a worldwide web-based patient registry. The learning from the pilot, including the relationship between GNI and retainment in the pilot, will inform the WBDR, expected to launch mid-2017.

PB 1809 | Data from the Austrian Haemophilia Registry: An Update

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Background: The Austrian Haemophilia Registry is a web-based patient registry initiated in 2007.

Aims: The primary aim of the Registry is to assess the demographic characteristics of patients with haemophilia (PWH). Secondary aims are to evaluate treatment modalities and potential side effects.

Methods: Data on the various characteristics and treatment modalities of patients from each of the eight Austrian Haemophilia Centers are recorded in a special Registry database.

Results: In total, 796 PWH were included in the Registry at the end of January 2017: 670 patients (84%) with haemophilia A (HA) and 126 (16%) with haemophilia B (HB).

The median age was 35 years (range: 1-94 years). Children (< 18 years of age) represent 19.5% and adults represent 80.5 % of the study population. Of all patients recorded, 39% have severe (factor VIII or IX levels < 1%), 10.5% have moderate (FVIII or FIX 1-5%) and 50.5% have mild (FVIII or FIX 5-50%) forms of haemophilia.

Data is available on the treatment modalities of 97% of patients with severe haemophilia: prophylaxis is applied in 70.5%, while 26.5% receive on demand treatment. The number of severe haemophiliacs on prophylaxis was higher among children/adolescents (91%) than among adults (63%).

Of all patients with severe haemophilia, 37.2% have shown an HCV infection and 12.3% are HIV positive. A co-infection with both, HIV and HCV, has been confirmed in 10.1%.

At present, 3.3% of all HA patients and 6.7% of severe HA patients show an inhibitor - a high-titer inhibitor (> 5.0 Bethesda Units (BU)/ml) was found in 50% of all HA patients with inhibitor and in 47% of severe HA patients with inhibitor. Currently 0.8 % of all our HB patients and 4.2% of severe HB patients have a low-titer inhibitor (< 5.0 BU/ml).

Conclusions: The Austrian Haemophilia Registry covers more than 80% of the assumed number of PWH in Austria. Most patients with severe haemophilia receive prophylaxis. HCV and HIV infection are still a major issue in the Austrian haemophilia population.

PB 1810 | Application of the Pattern and Parameters of the aPTT Clot Reaction Curve in Patients with Hemophilia A and Acquired HA

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Background: The diagnosis of Hemophilia A (HA) and Acquired Hemophilia A (HAadq) is usually performed by measuring the activity of FVIII. ACL TOP coagulometers show the aPTT coagulation reaction curve and calculate the parameters as Max1: maximum value of the first derivative of the curve (velocity), Max2: maximum value of the second derivative (acceleration) and Min2: minimum value of the second derivative (deacceleration). The aPTT second derivative curve (aSDC) is variable may be: single peak (SP), peak with shoulder (PSH), wide peak (WP) or double peak (DP).

Aims: To evaluate the utility of the aSDC and the parameters obtained from the curves in patients with HA and HAadq.

Methods: 63 samples of HA patients (17-80 years, 48 severe HA (SHA), 10 moderate HA (MoHA), 5 mild HA (MiHA). 13 samples from SHA and 17 from HAadq patients presented aFVIII Inhibitor (SHAInh: 12 high titre; 1 low titre, HAadq: 11 high titre; 6 low titre); 41 samples

TABLE 1 aSDC and the parameters obtained from patients with HA and HAadq

	Number of samples		aPTT (sec)	Max1 (dAbs/dt)	Max2 (dAbs/dt2)	Min2	aSDC
CS	41	Median / Range	26,7 / 23,5 - 31,9	287,0 / 210,0 - 383,8	841,6 / 596,5 - 1168,6	-344,7 / (-452,5)-(-233,9)	SP
SHA	48	Median / Range	87,2 / 62,4 - 127,0	48,3 / 15,3 - 148,4	40,5 / 16,8 - 109,1	-4,9 / (-37,6)-(-0,1)	DP
MoHA	10	Median / Range	52,5 / 47,4 - 59,7	119,4 / 75,4 - 167,5	153,4 / 105,7 - 217,6	-59,3 / (-93,7)-(-36,0)	DP
MiHA	5	Median / Range	40,0 / 33,5 - 43,8	207,7 / 139,6 - 290,9	370,3 / 327,2 - 642,3	-162,8 / (-261,2)-(-70,1)	DP, PSH
SHAInh	13	Median / Range	99,0 / 67,4 - 129,1	27,6 / 8,3 - 44,6	27,8 / 12,9 - 58,5	-5,6 / (-9,8) - (0,0)	DP
HAadq	17	Median / Range	81.8 / 41.4 - 127.1	102.8 / 28.0 - 535.2	51.3 / 13.61 - 527.1	-16.2 / (-356.8)-(-0.2)	WP and/or DP

(CS) from patients with no clinical manifestations and normal PT and APTT were used as controls. aPTT (APTT-SP), ACL TOP 300 Instrumentation Laboratory. GraphPad InStat.

Results: Significantly lower values of Max 1, Max 2 and Min 2 are observed in patients with SHA than with MoHA (p:< 0.0001), and also Lower values of Max1, Max 2 and Min2 in patients with MoHA than MiHA (p:0.0027, p:0.0007, p:0.0080 respectively). Additionally, lower values of the three parameters are found in SHAInh than HAadq (p:< 0.0001, p:0.0132, p:0.0087, respectively). SHA Vs SHAInh (p:0.0019, p:0.0510, p: 0.8258 respectively). aSDC were DP in SHA and MoHA, and DP, WP or PSH in MiHA and HAadq , See table 1.

Conclusions: These preliminary study shows that by using the parameters of the aPTT reaction curve it could be possible to differentiate HA severity and also SHAInh from HAadq. In addition, Max1 could be used to differentiate between SHAInh and SHA. However, these findings would require a more data to be confirmed.

PB 1811 | Secondary and Tertiary Prophylaxis in Patients Haemophiliacs, Update from Algiers Experience about One Center

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Background: Primary prophylaxis is the gold standard for preserving joint function of care for boys with severe haemophilia A and B, indications in adulthood are less well definided ,is effective in reducing the number of bleeding episodes and prevention of arthropathy. Adoption of this approach has varied from country to country.

Aims: Showing the benefits ,the clinical and psychological impact of secondary prophylaxis.

Methods: We follow up at our center 300 patients with Haemophilia. The population we studied is a cohort of 66 patients 42 adults 24 children . Their ages are between (2-55). Prophylaxis started at an age between (2 and 56) years old, since 7 years.

Results: The patients had higher numbers of joint bleed and of days of work-school lost, regimens starting prophylaxis with infusions one per week introduced ,we observed a remarkable reduction of bleeding frequency from a median of 198 bleeds per year during on demand treatment to a median of 15 bleeds per year during prophylaxis,The prophylaxis reduced the number of absentee days from 2 to 3 times per week to 0 to 1 day per year during the prophylaxis period.

Conclusions: It is unquestionable that prophylaxis at any age reduces the number of joint bleeds and slowed, but did not stop the progression of haemophiliac arthropathy, and in parallel reduces, the patients physical and psychological restrictions, being able to radically transform the lives of severe haemophyliacs.

PB 1812 | Personalized Treatment in Hemophilic Patients in Argentina

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Background: Prophylaxis treatment for adults with Severe Haemophilia A must be approached as a group with therapeutic goals of it is own, primarily focusing in improving quality of life and social inclusion of the patient. Although tertiary prophylaxis is controversial regarding cost effectiveness, since most patients have damage in several joints, therefore prophylaxis is focused in this group of patients in delaying progression of joint damage. In Argentina the access for prophylaxis treatment in adults is very limited and traditionally is based on patient weight.

Aims: Evaluate the myPKFiT software, in adults with severe haemophilia A in prophylaxis with Recombinant Coagulation Factor VIII (rFVIII).

Methods: Fifteen patients with severe hemophilia A in prophylaxis treated with rFVIII were studied. They were divided according the dose regimen in: low doses (< 20 IU / kg), standard doses (20 - 40 IU / kg) and high doses (> 40 IU / kg). Mean Age: 38 years old (19-57), Mean weight: 72 Kg (48-93). Mean dose of rFVIII: 2033 IU (2000 - 3000 IU). Dosage of FVIII was done by one-stage coagulometric method in Coagulometer ACL TOP 300 (Instrumentation Laboratories). A myPKFiT program was used to calculate customized pharmacokinetic (PK) curves for patients after the infusion of Octocog alfa rFVIII. The minimum level objective of PK was to maintain the FVIII above 1%, with dosage interval of 72 hours.

Results: When evaluating patients with the software, 9 patients who were in the standard dose category were maintained; the rest changed their dose category. See Table 1 y Table 2.

TABLE 1 Patients without dose change

Patient	Dosis administered IU/Kg	Dosis suggested myPKFiT IU/Kg
1	21.5	28.0
2	25.6	26.9
3	27.8	26.0
4	21.5	23.0
5	25.3	31.7
6	24.4	31.3
7	21.5	24.7
8	23.1	32.5
9	41.7	43.6

TABLE 2 Patients with dose change

Patient	Dosis administered IU/Kg	Dosis suggested myPKFiT IU/Kg
1	36.4	18.8
2	33.3	17.6
3	26.7	14.7
4	29.4	19.4
5	62.5	24.5
6	53.6	32.0

Conclusions: 40% of patients (6/15) changed the dose regimen to lower doses that were suggested by myPKFiT seeing on PK parameters. By these results we implemented in our institution dose regimen based on PK parameters in order to optimize the use of concentrates and getting the profilaxis for a cost effective treatment.

PB 1813 | Global Hemostatic Assay at Different Target Procoagulant Activity of Factor VIII and Factor IX

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Background: Based on reports addressing hemophilia B patients bleed less common and less intensively than hemophilia A, it has been expected that the hemostatic level of factor IX (FIX) activity can be lowered than that of factor VIII (FVIII) activity.

Aims: We compared the hemostatic efficacy of the different hemostatic level of FIX and FVIII activity using global hemostatic assay.

Methods: A total of 17 severe hemophilia patients without inhibitor, aged more than 15 years old were subjected; 12 hemophilia A patients and 7 hemophilia B patients. Factor concentrates were injected to reach the target activity of 60% in hemophilia A and 40% in hemophilia B which is given by Korean health insurance guideline. All patients were in non-bleeding state and kept the wash-out period of 3 days for hemophilia A and 5 days of hemophilia B. Before and on 15 minutes after injections, we conducted one-stage factor assay, thrombin generation assay (TGA), thromboelastography (TEG) and clot-wave form analysis (CWA).

Results: Median ages of hemophilia A and hemophilia B patients were 28 and 33 years old. Baseline FVIII:C and FIX:C were 0.6% and 1.8% and they rose after injection rose to 70.8% and 49.8%. The dosage of FVIII concentrates and recombinant FIX concentrates were 28.4 IU/kg and 50.7 IU/kg. In-vivo recovery (IVR) in hemophilia A and hemophilia B patients recorded 2.43 %/U/kg and 0.91 %/U/kg. Peak thrombin of FVIII and FIX were 451.3 nM and 376.6 nM (P=0.108, normal range, 458 nM±60). TEG index of FVIII and FIX were -1.60 and -3.77 (P=0.004, normal range, -2~+2). MIN2 of CWA of FVIII and FIX were 0.62 and 0.59 (P=1.000).

Conclusions: Global hemostatic assay indicates even though IVR of FVIII and FIX are normal, less amount of FIX is insufficient to normalize hemostatic parameters in comparison with FVIII.

PB 1814 | The Impact of Secondary and Tertiary Prophylaxis in the Reduction of Bleeding Events in Moderate or Severe Hemophilia A or B

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Background: The purpose of this paper is to identify the impact of prophylaxis in the reduction of bleeding events in severe or moderate hemophilia patients A or B assisted at the Nova Friburgo's Regional Blood Center of Nova Friburgo, Rio de Janeiro, from 2009 to 2015.

Aims: To study the incidence of bleeding events before and after the beginning of the secondary and tertiary prophylaxis treatment, comparing their frequency and types.

Methods: This is a Quasi-experimental pre-post test intervention study which inclusion criteria was the severe or moderate hemophilia A or B patients without inhibitor registered at Regional Blood Center of Nova Friburgo.

Data was extracted from the medical chart and/or electronic medical record from Hemovida Coagulopatia Web, from the Brazilian Health Ministry.

The project was approved by the Ethics and Research Committee of Instituto Estadual Arthur de Siqueira Cavalcante - Hemorio before its beginning and with the patient free consent term.

Results: Most patients presented the severe form of hemophilia A;

A reduction of 89,1% in the total number of bleeding complications after the beginning of secondary/ tertiary prophylaxis (from 614 to 67 complications) was noticed, being similar in hemophilia A or B and in the severe or moderate forms;

There was a reduction in the incidence of bleeding events in all situations after intervention, with a significant statistic ($p < 0,05$) for knees, elbows and shoulders;

Observing the quantity of events, the average number of annual complications diminished from 9,1 before intervention to 1,3 after prophylaxis ($p=0,001$); the reduction was statistically significant in all areas ($p < 0,05$), except in muscle bruises.

Conclusions: We can conclude that the secondary/tertiary prophylaxis is efficient in the reduction of bleeding events in severe or moderate hemophilia patients A or B.

PB 1815 | New Methods of Physiotherapy in Patients with Advanced Haemophilic Arthropathy

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Background: Advanced arthropathy occurs even in young adults with haemophilia, if they have not been properly treated. It has been proved that physical training increases isometric muscular strength and proprioceptive performance in haemophilia patients. Some new methods of physiotherapy, introduced recently, might improve the results of haemophilic arthropathy treatment.

Aims: The aim of this study was to present the new methods of physiotherapy process and their effects on patients with advanced haemophilic arthropathy.

Methods: Five subjects, aged from 32 to 42 years, with severe haemophilia A or B attended physiotherapy in an outpatient ambulatory setting over a 3-month period. The following treatment modalities were performed: walking on AlterG anti-gravity treadmill, deep penetrating electromagnetic stimulation (Salus Talent), manual physical therapy, mobilization and manipulation techniques, active muscle-strengthening exercises, post isometric relaxation (PIR) muscle energy techniques, as well as exercises for improvement of the coordination, postural equilibrium and proprioception exercises using sensorimotor discs. The HJHS (Haemophilia Joint Health Score) has been used to assess the effectiveness of the treatment, VAS scale (Visual Analog Scale) to assess level of pain, TUG test (Timed Up and Go) to assess mobility as well as dynamic and static balance.

Results: Strength of the muscles acting on the joints improved, swelling of joints diminished and the level of pain decreased. An improvement of the dynamic and static balance was found as well. The range of motion did not change. Physiotherapy process did not provoked bleeding episodes in patients with haemophilic arthropathy included to the study.

Conclusions: New methods of physiotherapy including walking on AlterG anti-gravity treadmill, and deep penetrating electromagnetic stimulation (Salus Talent) are an interesting option, which might improve the results of physical training in patients with haemophilic artropathy.

PB 1816 | High Adherence in Adult and Pediatric Patients with Hemophilia B Receiving Prophylaxis with rIX-FP

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Background: rIX-FP is a fusion protein genetically linking recombinant human coagulation Factor IX with recombinant human albumin. In clinical trials, 7-, 10- or 14-day rIX-FP prophylaxis in adults (≥12 years) and 7-day prophylaxis in pediatric patients (< 12 years) achieved median annualized spontaneous bleed rates of 0.00.

Aims: This analysis evaluated adherence to different regimens in two clinical trials of patients with hemophilia B (FIX ≤2%).

Methods: Adults with hemophilia B received either on-demand treatment for 6 months then prophylaxis every 7 days (on-demand arm; n=23) or 7-day prophylaxis for 6 months then, if eligible, prophylaxis once every 10 or 14 days (prophylaxis arm; n=40). Pediatric patients (n=27) received prophylaxis every 7 days. Dose, dosing frequency and rIX-FP consumption was recorded in an e-diary; reported adherence was reconciled with the number of used vials returned at each study visit. Adherence to prophylaxis was determined in terms of schedule (defined as a compliance rate ≥80%; adults and pediatrics) and prescribed dose (adults only).

Results: In the adult study, 94.9% of subjects were adherent to their prophylaxis schedule. Mean prophylaxis compliance rate for the 7-day regimen was similar between on-demand and prophylaxis arms (95.5% and 94.7%, respectively). Mean compliance rates with 10- and 14-day regimens was 90.7% and 97.2%, respectively. Overall, 85.7% of adult patients were dose compliant (within 10% of prescribed dose ≥80% of the time). In the pediatric study, all patients were adherent with the weekly prophylaxis schedule; mean overall compliance rate was 97.9% and was similar between those aged 1-5 years and those aged 6-11 years.

Conclusions: Adherence to prophylaxis schedule is essential for bleed prevention in patients with hemophilia. Data show that 7-, 10- and 14-day rIX-FP prophylaxis regimens result in high rates of compliance and very low bleeding rates in both adult and pediatric patient populations.

PB 1818 | PROTECT VIII: Can Patient Characteristics Predict Eligibility for Less-frequent Prophylaxis Dosing Regimens?

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Background: Efficacy of prophylaxis with BAY 94-9027, a prolonged-half-life recombinant factor VIII product, was shown using individually tailored dosing regimens (2x/wk, every 5 or 7 days) in PROTECT VIII, a phase 2/3 study in which previously treated adolescents and adults with severe hemophilia A received BAY 94-9027 for 36 weeks on demand or as prophylaxis (*J Thromb Haemost* 2016:Epub). Prophylaxis intervals were determined after a 10-week run-in period of 25 IU/kg 2x/wk BAY 94-9027 prophylaxis for preselection of patients suitable for less-frequent dosing. Patients with ≤1 spontaneous joint or muscle bleed during the run-in were eligible for randomization to prophylaxis every 5 days (45–60 IU/kg) or every 7 days (60 IU/kg); patients with >1 spontaneous bleed were ineligible for randomization and subsequently received 30–40 IU/kg 2x/wk.

Aims: To identify baseline characteristics of PROTECT VIII patients that are potentially predictive of patients with hemophilia A who may

be considered for every-5th-day or every-7th-day prophylaxis with BAY 94-9027.

Methods: In this post hoc analysis, baseline characteristics were compared for patients who were eligible vs ineligible for randomization to less-frequent dosing regimens in PROTECT VIII.

Results: Based on bleeds in the run-in period, 97/110 patients (88.2%; median age, 34 y) were eligible and 13 patients (median age, 32 y) were ineligible for randomization to less-frequent prophylaxis dosing. Patients eligible vs ineligible for randomization had fewer median total bleeds and joint bleeds in the previous 12 months (5.0 vs 15.0 [total]; 2.0 vs 10.0 [joint]) and fewer median target joints (1.0 vs 2.0; **Table**). A higher proportion of patients eligible vs ineligible for randomization received prophylaxis before the study (81.4% vs 69.2%).

TABLE. 1 Baseline Characteristics of Patients Eligible vs Ineligible for Randomization to Less-Frequent BAY 94-9027 Dosing Regimens

	Eligible for Randomization (n=97)*	Not Eligible for Randomization (n=13)
Age, y, median (Q1; Q3)	34 (26, 46)	32 (24, 37)
Number of total bleeds in previous 12 months, median (Q1; Q3)	5.0 (1.0; 15.0)†	15.0 (9.0; 25.0)
Number of joint bleeds in previous 12 months, median (Q1; Q3)	2.0 (0.0; 11.0)†	10.0 (6.0; 19.0)
Target joint present, n (%)	69 (71.1)	11 (84.6)
Number of target joints per patient, median (Q1; Q3)	1.0 (0.0; 2.0)	2.0 (1.0; 2.0)
Previous prophylaxis treatment, n (%)	79 (81.4)	9 (69.2)

*11 eligible patients were not randomized because randomization arms were full.
†n=95.

Conclusions: Based on PROTECT VIII data, number of previous joint bleeds and target joints are relevant indicators for predicting suitability of patients for less-frequent BAY 94-9027 prophylactic dosing regimens.

PB 1819 | Is Intermediar Intensity Prophylaxis with Factor VIII Sufficient for Improvement of Overall Hemostasis Potential in Hemophilia A Patients?

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Background: Prophylaxis with Factor VIII (FVIII) concentrate prevents recurrent bleeding and hemophilic arhtropathy in patients with severe hemophilia A (hA) but it is not widely available due to the high costs.

Aims: To investigate the efficacy of different prophylactic regimens in comparison with on-demand treatment in patients with severe hA using Overall Hemostasis Potential (OHP) as a laboratory tool for estimation of global hemostatic capacity.

Methods: Five patients received FVIII concentrate in standard dose (20 IU/kg three times per week), five received intermediate dose (10-15 IU/kg three times per week), while seven patients received FVIII concentrate only on-demand. Blood samples were collected before the start of prophylaxis and after 3 months, before receiving next dose. OHP and its parameters: Overall Coagulation Potential (OCP) and Overall Fibrinolysis Potential (OFP) were analyzed. Study is improved by medical ethics committee and informed consent was obtained.

Results: OHP was significantly improved after 3 months of treatment with FVIII concentrate (0.99 vs 11.15 and 2.26 vs 9.60 for standard and intermediary dose respectively), while no improvement was observed in on-demand treated patients (2.28 vs 1.94). Similar results were observed for OCP, while no change neither difference between prophylaxis and on-demand treatment was observed for OFP.

Conclusions: Prophylactic treatment with FVIII concentrate improved global hemostasis in hA and this improvement was not directly and only associated with FVIII level. Intermediary dose was as good as standard dose prophylaxis in this context confirming that as it was previously described frequency rather than intensity is the most important for the treatment efficiency. This also indicates that sufficient and safe hemostasis may be achieved with lower cost of the treatment what is very important not only for developing but even countries with large healthcare budgets.

PB 1820 | Can Tertiary Prophylaxis Bring about Zero Bleed in Adult Persons with Hemophilia? A Single Center Observation Study in Japan

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Background: It has been reported that prophylactic infusions of factor concentrates (prophylaxis) were effective to suppress joint bleedings in persons with hemophilia (PWHs). However, it is unclear that prophylaxis contribute to zero bleed or not.

Aims: To clarify the efficacy of tertiary prophylaxis on decreasing annualized joint bleeding ratio (AJBR) in PWHs who received this treatment. And then, we also guess whether the prophylaxis accomplish zero bleed in PWHs' joints or not, by observing the change of their AJBRs.

Methods: Patient diaries from persons undergoing home infusion treatment have been collected from 2002 to 2016. Infusion days,

dose, reasons of infusion and joint bleeding sites in respective patients were identified with reference to their diaries.

Results: The subjects were 35 adult PWHs (A, n=29; B, n=6), who received prophylaxis for more than 2 years continuously and had good adherence to recording their infusion. Because 19 patients (A, n=14; B, n=5) also had the record of on-demand treatment, we could compare their AJBRs before and after introducing prophylaxis. AJBRs before it were statistically more than the first year after starting prophylaxis (11.2 vs 1.5, median, p< 0.001). Comparing AJBR in hemophilia A and in B, AJBR in hemophilia A was dramatically decreased (from 22.8 to 1.25, p< 0.001). On the other hand, in hemophilia B, the change was not so much (from 7 to 3, p< 0.05). Their AJBRs at the second year of prophylaxis were more decreased comparing with them at the first year (1.4 vs 0.5, median, p< 0.01). However, comparing AJBRs at the second year with them at the third year, there was not statistical different. Although 18 of the subjects accomplished zero bleed at the second year prophylaxis, there were 15 PWHs who accomplished it at third year by contrast.

Conclusions: Tertiary prophylaxis is effective to reduce ABR of adult hemophilic patients. However, all of them cannot necessarily achieve zero bleed.

PB 1821 | Personalized Treatment of Pediatric Patients with Severe Haemophilia A. Importance of Pharmacokinetics. Assessment Benefits from Kinetics of Factor VIII (Simoctocog Alpha)

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Background: It is now known that both primary and secondary prophylaxis should be adjusted (tailored prophylaxis) for each individual patient since the differences between patients are remarkable for severity, frequency, magnitude and location of bleedings. Simoctocog alpha is the first recombinant B domain deleted FVIII, produced in a human cell line, without chemical modification or protein fusion. Undergoing species-specific post translational modification confers to the product the lack of non-human epitopes as well as a high binding affinity to vWF .

Aims: The focus on our patients was to establish a personalized prophylaxis associated with ABR (Annualized Bleeding Rate) reduction of 96-97%, resulting in an improvement of treated subjects' quality of life.

Methods: Infusion and blood samples were managed at patient's home and sent to a central lab. Individual PK profile has been performed with blood samples collected before Simoctocog alpha infusion and at +0.5, +1, +3, +9, +24, +48, +72 h.

Results: In our center we performed 4 PK in 4 patients with haemophilia A and we decided to modify the prophylaxis according to the individual results. The average bleeding annual rate (ABR) of all patients before the personalized prophylaxis was 41. Preliminary results are confirming what was suggested by the study GENA 21. The kinetic predictions have been made considering a trough of 3% and 5%. On average, in our patients Simoctocog alpha half-life was long (24.51 h) enough that the trough of 1% determined very low values of Cmax and dose. Even with a trough of 3%, the interval of 48 hours with a 10.62 IU / kg dose led to a Cmax of 17%.

Conclusions: Our approach of prophylaxis optimization with Simoctocog alpha allowed us to establish a different dosing schedule for our patients, leading to a reduction of the ABR, reducing the number of weekly infusion, lowering product consumption.

PB 1822 | Splenectomy for Liver Cirrhosis Improved Systemic Condition in HIV/HCV Co-infected Patients with Hemophiliacs

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Background: HIV infection no longer became a fatal disease by virtue of an anti-retroviral therapy (ART), however liver cirrhosis (LC) due to HCV is still problematic because it has been the major cause of death in HIV/HCV co-infected patients with hemophiliacs. Once LC develops, liver function never turns back to a former state. Moreover, the risk of developing HCC is remained even if HCV was eradicated. A number of CD4 cells (CD4) in HIV/HCV co-infected patients with LC inclined to decrease due to their splenomegaly.

Aims: A decreased CD4 also might be one of risk factors for developing some opportunistic infections (OIs). We evaluated the efficacy of splenectomy in hemophiliacs with LC retrospectively.

Methods: Splenectomy was performed in five hemophiliacs co-infected HIV/HCV and had less counts of both CD4 and platelets (PLT) with splenomegaly from 1994 to 2015. CD4, PLT, and clinical condition were evaluated before and after splenectomy.

Results: At 6 months after splenectomy median PLT ($\times 10^9$/L) has changed to 205 from 38 and CD4 (/μL) to 529 from 141, respectively. The HIVRNA (VL) has reached to undetectable level in four patients and the rest one who would not have been responding by ART from the HAART era also decreased after splenomegaly. Esophageal varices disappeared in two patients and none showed any complications such as OIs or overwhelming post-splenectomy infections (OPSI). One with meld score13 who had underwent liver transplantation at 42 months after splenectomy still stays in shape.

Conclusions: Splenectomy lead to the elevation of both CD4 and PLT. Hereby their systemic condition was improved than before and further bleeding tendency was also prevented. Finally, whereas hyposplenic states were deeply concerned, these results suggested splenectomy may consider as one of therapeutic options for improving both their immune and hepatic condition in patients with an end stage of liver disease.

PB 1823 | Two Effective Cases of Immune Tolerance Induction Using Turoctocog Alfa Combined with Immunosuppressive Therapy in Non-severe Hemophilia A

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Background: Non-severe hemophilia A (NSHA) has a higher risk of developing inhibitors after 100 exposure days (ED); therefore, inhibitor development throughout a patient's lifetime might occur.
Aims: To improve the treatment outcome of NSHA with inhibitors.
Methods: Retrospective patient chart review and literature search.
Results:
Case 1: An 18-year-old man with NSHA. The inhibitor was triggered by therapy with a conventional recombinant factor VIII preparation (rFVIII) for left femoral compartment syndrome when he was 16 years old (100 ED or more), and the titer reached a maximum of 85 Bethesda units (BU). Although the titer decreased and disappeared after 4 doses of 375 mg/m^2 rituximab, it subsequently increased to 14 BU. Immune tolerance induction (ITI) was then initiated with about 80 IU/kg Turoctocog alfa three times a week. The titer was 0 BU at 2 weeks after the initiation of ITI without anamnestic responses. Although the reduction of recovery rate and shortening of half-life was prolonged, remission was obtained 22 weeks after starting ITI.
Case 2: A 42-year-old man with NSHA. The inhibitor was triggered by surgery for cervical myelopathy with rFVIII, and detected as 14 BU just before a single dose of rFVIII against spontaneous muscle bleeding, corresponding to 16 ED. Therefore, hemostasis management was switched to a bypassing agent. Subsequently, the titer increased to 32.8 BU. Thereafter, ITI was initiated with about 80 IU/kg Turoctocog alfa once a day, and the titer was reduced to about 10 BU in 1 week and that state continued. Methylprednisolone 1000 mg once a day for 3 days was added at 5 weeks after the introduction of ITI. The titer decreased to 0.2 BU at 19 weeks after starting ITI, and is currently maintained at this level.
Conclusions: We performed ITI using Turoctocog alfa combined with immunosuppressive therapy only in the two cases reported here. As a result, both patients are improving; therefore, this therapeutic strategy might be an option for the treatment of NSHA with inhibitors.

PB 1824 | Tailored Prophylaxis: A Bridge between QoL and Retrenchment. Our Experience with Simoctocog Alfa

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Background: Prophylaxis has become the gold standard approach in patients with severe Hemophilia A. The main goals of prophylaxis are converting severe to moderate disease, reducing number of bleeds, preventing or delaying arthropathy and improving Quality of life (QoL). Simoctocog Alfa (Nuwiq) is a new generation recombinant Factor VIII protein; clinical studies with Nuwiq in severe Hemophilia A showed relevant differences between patients with half-life ranging from 6.5 to 32h. These results suggest to tailor patients' treatment with a single dose individual Pk analysis.
Aims: We used Pk to optimize prophylaxis therapy with Nuwiq in an adult patient. He was a 48 years old male, 60 kg, history of high titer inhibitors eradicated, HJHS (Hemophilia Joint Health Score) 60. At the time of accrual, the patient was on prophylaxis with other rFVIII (3.000U x 3/w), maintaining a trough level 0.08 IU/dl with ABR 2 in the previous year.

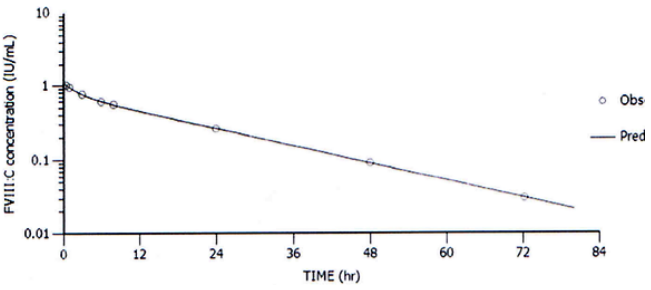

FIGURE 1 Result of individualized analysys of FVIII pharmacokinetics (Pk)

TABLE 1 Simulated Dosing Schemes

Weight (kg)	Nominal Dose (IU/kg)	T1/2 (hr)	C0 (IU/ml)	Target Trough level(IU/ml)	Tau (hr)	Est. dose (IU/Kg)	Est. Cmax (IU/ml)	Est. C0 (IU/ml)
60	50	15.44	1.079	0.08	24	15.2	0.31	0.33
					36	26.1	0.53	0.56
					42	34.1	0.69	0.64
					48	44.7	0.91	0.96
					56	64.0	1.30	1.38
					60	76.6	1.55	1.65
					72	131.2	2.66	2.83

Methods: Infusion and blood samples were performed at Hospital or at patient's home, using NuPreviq service, and sent to central and local lab. Blood samples were collected before Nuwiq 50U/Kg and +0.5, +1, +3, +6, +9, +24, +48 and + 72h after infusion. Demographic data and Pk timing were sent to Accovion with the desired Trough level (0.08 IU/kg). Pk data were analyzed with a 2-compartment model (2CP).

Results: Pk results demonstrated 15.44h half-life, recovery of 2.0 % and levels of 0.09 IU/dl at +48h.

The simulated dosing scheme suggested a 12% reduction dose to reach the tough levels of 0.08 IU/dl at +48h. Therefore, we proposed to our patient a prophylactic regimen of 2.500U x3/w. At 1 year follow-Up ABR was 0 with a good compliance. FVIII needs were reduced from 468.000U/y to 390.000U/y (from 318240€ to 253500€).

Conclusions: Our experience suggests that Pk could be a useful tool to optimize prophylactic therapy. The reduction of total IU of FVIII led to a relevant impact on public health cost containing and on patients' QoL.

PB 1825 | Therapeutic Efficacy of Low-dose Immune Tolerance Inductionin 5 Chinese Severe Hemophilia A Children with Inhibitor in Good Risk

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Background: Immune tolerance induced (ITI) is an effective way to eliminate the hemophilia inhibitor, but by economic constraints, high or medium dose ITI treatment in China is hard to carry out. Low dose ITI is economically feasible.

Aims: This paper aims to review low dose ITI treatment experience at our hemophilia treatment center by collecting information of hemophilia A patients with good risk and their response to ITI treatment, and to evaluate the feasibility of low dose ITI in China.

Methods: Retrospectively collected clinical information of 5 patients at our hemophilia clinic who accepted low dose ITI. Clinical information included the baseline data; Inhibitor found time, degree of peak, ITI bleeding before treatment; ITI treatment start time, application solutions, duration and treatment outcome; bleeding during ITI treatment, and descriptive analysis.

Results: Inhibitor occurred at 57(18-65)months.The peak titer was 13.6BU/ml (3.9-20.2 BU/ml). After the appearance of inhibitor, the average bleeding rate(ABR) was 6.31times/person/month. ITI treatment started at 64 month-old (28-91), and interval between inhibitor appearance to ITI treatment was 20 months (range, 7-26). Low dose ITI was 25 to 50 u/kgQod. For 4 successful cases, inhibitor turned negative at 13 months, 4 months, 8 months and 9 months respectively and remain negative till now.The other case suspended ITI because of inhibitor titer increasing after 3 months. Only 2 cases bleed during ITI treatment, the ABR was 0.21.

Conclusions: Low dose ITI benefits hemophilia A patients with inhibitor and was effective to eliminate inhibitor and control hemorrhage.

PB 1826 | Efficiency of Prophylaxis in an Adult with Hemophilia after Dose Adjustment Using a Bayesian Model FVIII Pharmacokinetics Estimation

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Background: We describe the case of a 32-year-old patient with severe hemophilia A (HA; FVIII level 0.25%) diagnosed at 12 months of age, serology negative for infectious diseases and no history of inhibitors. His HA clinical history is summarized in Table 1.

TABLE 1 Patient's hemophilia history

Age	Clinical Event	Management and Comments
12 months	Severe hemophilia A diagnosis, serology negative for infectious diseases and no history of inhibitors	Treatment on-demand
10 years	Patient presents target joint (right ankle)	Standard prophylaxis initiated: 20-40 IU/kg 3 × week Right ankle becomes target joint
15 years	Self-administration of factor	Good adherence
16 years	Synoviorthesis of the right ankle	
21 years	Patient suspends prophylaxis	Office work and cycling on occasion
21-29 years	Joint bleeds frequent (15-20 per year) Left ankle becomes new target joint	
29 years	Both ankles present severe functional impairment and pain that hinder walking Advanced arthropathy on MRI	Daily infusions for joint pain, so the patient reinitiates prophylaxis (40 IU/kg 3 × week) and receives physical rehabilitation
29 years	Clinical improvement after 4 months	Infusions are spaced to 2 × week
32 years	Arthropathy pain in the ankles worsens	Prophylaxis adjusted to 28 IU/kg 48 h, and NSAIDs taken on a regular basis. Pain decreased, and mobility was recovered. Pharmacokinetic study performed after 4 months (see methods)

Aims: To describe the experience of prophylaxis adjustment after pharmacokinetic (PK) study performed with web-based software using a published population PK model together with a Bayesian

algorithm, designed for patients receiving octocog alfa, antihemophilic factor (plasma/albumin free method).

Methods: The web-based software myPKFiT® V1.1 (www.mypkfit.com, Baxalta) was used for PK study. The patient had received octocog alfa (Advate, Baxalta) 2000 IU/48 h (28 IU/Kg) for 4 months and 2 samples taken at 4 and 28 h were analyzed (Figure 1-Top). FVIII half-life was 13 h, clearance rate 0.031 dl/h/kg, volume in steady state 0.5 dl/kg, and time to 1% above baseline 68 h. The regimen was adjusted for body weight (77 kg) and a desired trough level of 2% (due to lifestyle and arthropathy; Figure 1-Bottom).

Figure 1. Top: Bayesian pharmacokinetic estimation based on the patient information provided by the web-based software (www.mypkfit.com). The dots represent the two samples taken. Bottom: Simulation of dosage calculation based on desired trough level of factor VIII

Results: The dose in the adjusted regimen was 1343 IU (17.4 IU/kg) every 48 h, thus reducing factor consumption by 38%. The patient has not presented any bleeding event in the past year, and has recovered mobility in both ankles.

Conclusions: myPKFiT® may be useful for designing an adjusted prophylaxis regimen with Advate and, even in a regimen with trough level 2%, factor consumption and costs may be reduced, compared to unadjusted prophylaxis.

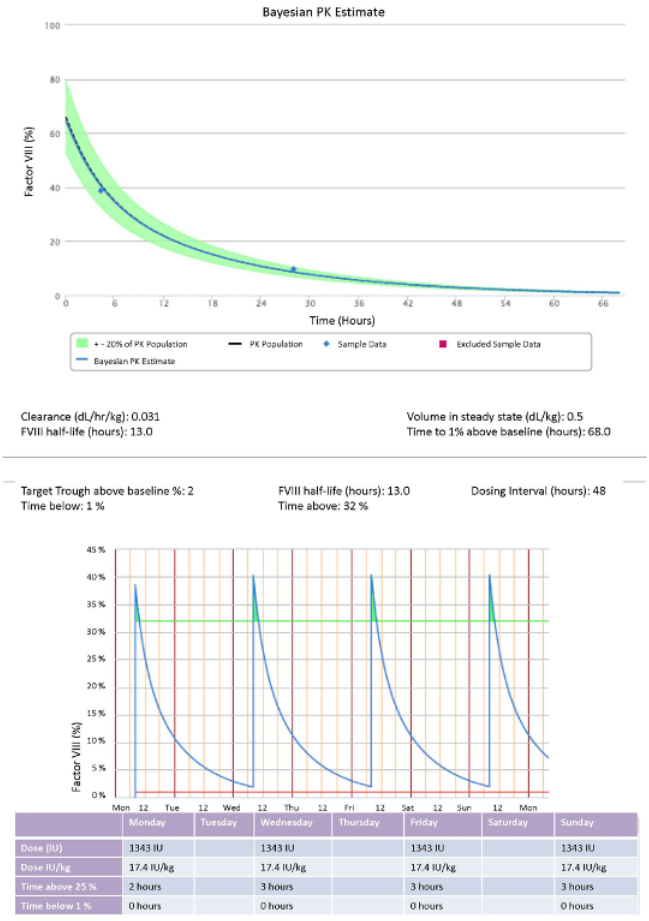

FIGURE 1 Bayesian pharmacokinetic estimation based on the patient information provided by the web-based software (www.mypkfit.com) and simulation

Note: *Labeled use of Advate for prophylaxis in severe HA is 20-40 IU/kg every 2-3 days. myPKFiT® v1.1 requires ≥2 samples taken approx. 3 h apart, 24-32 h after infusion.*

PB 1827 | Evaluating the Frequency of Haemophilia and Bleeding Parameters of Persons with Bleeding Disorder in South East, Nigeria

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Background: Haemophilia is under reported and under diagnosed in most developing countries like Nigeria Paucity of data, Increased childhood mortality , diease associated morbidity, poor access to factor replacement therapy,comprehensive care and non commitment of government and policy makers towards haemophilia care and management may be contributory.

Aims: To evaluate the frequency of haemophilia among those with bleeding disorders in South East Nigeria.

Methods: A pilot study of fifty consecutive consenting persons with bleeding disorders were convienently recruited from the four tertiary hospitals in South East Nigeria. Blood samples collected in EDTA for platelet count using haematology analyser Mythic 22, platelet poor plasma was used to run Prothrombin time (PT), Activated partial thromboplastin time (APTT), mixing studies and Bioassays for factor VIII and IX assays using the 1-stage APTT based assays . Data was analyzed using the graph pad prism version 6.0.

Results: The study showed 2% of subjects with bleeding disorders had haemophilia. It also showed that 68% of subjects were thrombocytopenic which was the commonest cause of bleeding disorder. The most common bleeding

symptoms was gastrointestinal bleeding (23.4%).Majority of subjects (32.4%) had a bleeding score of four based on MCMDM1VWD bleeding assessment questionnaire

Conclusions: Haemophilia should be considered as a differential diagnosis in patients with bleeding disorders. Thrombocytopenic bleeding are the commonest cause of bleeding disorders and larger multicenter studies are needed to determine prevalence of haemophilia and the likely causes of thrombocytopaenic bleeding in Nigeria.

PB 1828 | Personalized Approach to Treatment due to Pharmacokinetic Study: Description of One Case of Moderate Haemophilia A with Severe Bleeding Phenotype after Switch to Simoctocog Alfa

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Background: Clinical studies with simoctocog alfa in adults affected by severe haemophilia A revealed half-lives ranging from 6.5 to 32 hours. In order to assess In-Vivo-Recovery and elimination Half-Life, we proposed a pharmacokinetic study to a patient with moderate haemophilia A and severe phenotype -annual bleeding rate (ABR) > 4 despite regural prophylaxis with octocog alfa.

Aims: The study of pharmacokinetic (PK) was proposed in order to reduce spontaneous bleeding that occurred during previous prophylaxis scheme; furthermore we needed to avoid more than two infusions per week, for difficult venous access. We ipothesized a trough level of 0.03 IU/mL.

Methods: The patient has FVIII residual activity of 0.02 IU/mL with severe bleeding phenotype. He is 46 years old, he has active life. He presents severe haemophilic arthropathy of both ankles and elbows. He was treated on demand until 2013 when he started prophylaxis with octocog alfa 40 U/kg two days per week; in 2014 we had to increase dosage to 50 IU/Kg two days per week because of ABR > 4. In April 2016 we switched to simoctocog alfa and we studied PK after infusion of 4000 U simoctocog alfa (52 IU/Kg), exploiting the NuPreviq program.

Results: The In-Vivo-Recovery was 1.86 % per IU/Kg, elimination half-life was 20.10 hrs. Considering an ideal trough level of 0.03 IU/mL and trying to mantain two per week infusions, we adopted the dosage of 40 IU/Kg every 84 hours. The patient has experienced only two spontaneous joint bleeding during the last nine months. The actual dosage of simoctocog is 2000 UI/week lower than the previous scheme, with a favourable reduction of bleeding rate.

Conclusions: Individualized PK analysis permits tailoring the best dosage in order to optimize the control of spontaneous heamorrhages in patients on prophylaxis. In this case we could mantain the frequency of infusions and also decrease the dosage of infused FVIII, obtaining a significative reduction in bleeding manifestations.

PB 1829 | Haemophilia and Acute Coronary Syndrome (ACS)

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Background: Haemophilia A doesn´t protect from cardiovascular (CV) risk: haemophilic population (HP) can present thrombotic problems related to age and risk factors. Acute CV management and secondary prevention in HP presents an increased bleeding risk caused by drugs active on the coagulation pathway need.

Aims: Epidemiologic studies evaluated the prevalence of cardiologic disorders in HP related to age. Below 30 years coronary syndromes have a prevalence of 0,05%, after 60 it rises to 15,2%. In spite of increased CV disease in HP, due to a rise in their life expectancy, there is no standard treatment.

Methods: We describe the case of an acute coronary syndrome (ACS) in a 39 y.o. patient with mild A haemophilia (FVIII 2%, no inhibitor) and with a rare bleeding tendency. He had several CV risk factors: abdominal obesity, hypertension, hypercolesterolemia and smoke. He was treated with FVIII infusion (30 U/Kg), coronarography and bare metal stenting for a proximal IVA stenosis. He was then given unfractionated heparin followed by Enoxaparin at a subtherapeutic dose (6000 U bid , 90 Kg) and duble antiplatelets (DAPT). FVIII infusion was continued for 5 days (2000 U/day) with serum levels 20-60%. DAPT was then continued for a mouth, keeping FVIII prophylaxis at a reduced dose (2000 U every other day with FVIII level of 10%) in the absence of bleeding. When Clopidogrel was stopped we interrupted FVIII too.

Results: At 6 mounths the patient does´t present haemorrhages and he has no inhibitors.

Conclusions: ACS treatment in HP is feasible thanks to a useful collaboration between different specialists. DAPT has a pivotal role in HP, with an acceptable bleeding risk. Further studies are needed to evaluate wich FVIII are best to protect HP both in the revascularization phase and during DAPT period. As a matter of fact HP, erroneously considered to be at low CV risk, should be educated to better control CV risk factors and to reduce this risk of events, expecially in the young.

PB 1830 | The Diagnostic Value of Rotational Thromboelastometry in Patients with Non-severe Hemophilia A

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Background: Factor VIII activity (FVIII:C) assay in citrated plasma is usually used to diagnose hemophilia A. This test just study the initiation of clot formation, but hemostasis consists of complicated interactions between blood cells and proteins and endothelial. Recently, global assays like Thromboelastometry which study hemostasis in whole blood has become a useful way to evaluate coagulation disorders.

Aims: The aim of this study is to determine the diagnostic value of rotational thromboelestometry in Iranian patients with non-severe hemophilia A and its relation with factor VIII level.

Methods: Rotational Thromboelastometry was performed for 73 patients with mild/moderate hemophilia A by use of INTEM reagent and ROTEG device. FVIII:C was also measured by one-stage clotting and chromogenic assays.

Results: From 73 cases, 45 were classified as mild and 28 as moderate hemophilia A by on-stage FVIII:C assay. The results of ROTEM were not acceptable in 7 patients due to technical errors so they were removed from the study. ROTEM results for 7 patients (6 mild and 1 moderate ones) were normal. In our patients, parameters of the Rotational Thromboelastometry such as coagulation time (CT), clot formation time (CFT), maximum clot firmness (MCF), Alpha angel and

TABLE 1 Results of Rotational Thromboelastometry

	Categorization of the patients according to relation of FVIII:C results by one-stage and chromogenic methods								
ROTEM INTEM Parameters	Non-Discrepant n=26			Discrepant (Lower chromogenic F VIII:C) n=38			Reverse Discrepant (Lower one-stage FVIII:C) n=2		
	Mean	SD	No. of Abnormals	Mean	SD	No. of Abnormals	Mean	SD	No. of Abnormals
CT(S)	258.35	56	19	302.33	64.91	36	234	22.62	1
CFT(s)	81.44	22.2	2	93.41	14.47	10	90	25.45	0
AAngle	73.61	3.95	3	60	72.82	14	72	5.56	1
MCF (mm)	63	5.28	1	72.82	60	5	58	0	0

A5 had a meaningful statistical relation with F VIII:C in the plasma (P value for most of parameters was 0.01). CT (and after that A5) had the most relation with factor VIII level. CT increased in 76% of patients and Alpha angel decreased in 25% of patients.

Conclusions: Increase of CT and decrease of Alpha angel indicate decrease of clot formation rate and clot firmness in hemophilia A patients. CT parameter had a 68 % sensitivity in distinguishing mild hemophilia A patients from moderate ones in our study. Due to the meaningful statistical relation of Rotational Thromboelastometry parameters with F VIII:C level and disease severity it can help physicians as a complementary test to categorize patients as mild or moderate hemophilia A.

PB 1832 | Hemophilia Care in Pakistan

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Background: Hemophilia is a rare congenital disorder characterized by prolonged bleeding, either spontaneously, or after injury. In the developing world,where majority of the hemophiliacs live, awareness of this disease and its management is poorly done.

Aims: Aim was to determine the frequency, clinical picture and treatment information of hemophilia patients in Pakistan.

Methods: A cross sectional, observational study was carried out. Adult and pediatric hemophilia A (HA) and hemophilia B (HB) patients were included. Demographic and management history of patients were recorded and analyzed. Descriptive statistics was applied by using SPSS.

Results: A total of 102 male patients diagnosed as HA(n=69) and HB(n=33) were evaluated. Mean age was 15.34±4.75 years. Age at diagnosis ranged from birth to 5 years. History of consanguinity was present in 91% of cases and significant family history of bleeding in 69% of patients. 46(45%) were severe and moderate types respectively. Hemarthrosis and hematoma were more frequent symptoms in these patients. Surgical history including circumcision was done in 55% patients while 4 patients had major surgeries (hip& femur bones fracture, extensive nasal septum, and head surgery). 29(28%) of patients had transfusion-transmitted infections in which HCV 19(66%) was most prevalent followed by HBV 6(21%) and HIV 4(13%). 14 HA patients (20%) were found to have positive results for inhibitors and none in HB. Treatment included tranexamic acid, fresh frozen plasma, cryoprecipitate, cryosupernatant and factor concentrates on demand.

Conclusions: Hemophilia A and B are common among congenital bleeding disorders. Availability of poor diagnostic facilities and lack of proper management for this group of patients, often leads to wrong diagnosis and inadequate treatment. Rate of transfusion-transmitted diseases, particularly hepatitis C infection, has gained a huge proportion. Comprehensive hemophilia care center with multidisciplinary approach needs to be established in Pakistan.

PB 1833 | Risk Management of Pelvic Pseudotumor in a Patient with Inhibitors and Severe Hemophilia

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Background: Management of hemophilia patients with inhibitors can be very challenging. On top of that if that patient develops a pelvic pseudotumor in the iliopsoas muscle could be incapacitating in the short-term and can have serious complications in the long term.

Aims: Our aim is to report a successful experience in managing pelvic pseudotumor in patient with inhibitors.

Methods: A 49-year-old patient with severe hemophilia A with inhibitors was admitted in the ER with a clinical picture of about 4 weeks of pain in the lumbar region and left hypochondrium that was exacerbated and associated with deformity in hip flexion and paresthesia of the anterior thigh face. Physical examination revealed a hard mass in the left hypochondrium with hip flexion deformity of 20 degrees. Rx and pelvic MRI revealed a large mass at the level of the left Psoasilliac muscle with defined edges and heterogeneous content with alternating images of hypo and hyper intensity. Psoasilliac Hemophilic Pseudotumor was diagnosed with left femoral-cutaneous nerve lesion. Treatment was started with a bypass agent (FEIBA) since the patient had high response inhibitors. Patient continued with pain and functional limitation of the hip, in addition control images showed that the pseudotumor was getting in contact with the internal cortex of the iliac crest, so it was decided to perform surgical treatment since there was imminent risk of bone erosion due to growth of the pseudotumor.

Results: The patient was taken to surgery service by performing drainage of the pseudotumor, curettage of the walls and filled with a combination of fibrin sealant, demineralized bone matrix and absorbable gel sponge. Successful postoperative status was achieved with the disappearance of pain, improvement of the arches and elimination of the risk of osteolytic erosion of the cortical sheet of the iliac bone.

Conclusions: Our experience shows that pseudotumors in patients with high response inhibitors could correctly be treated with elective surgery and a bypass agent.

PB 1834 | A Study of Home Therapy in Hemophilia Patients

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Background: Home therapy has merits, enabling rapid treatment thereby reducing complications,
but difficulty with venous access is a barrier to treatment. There is a paucity of data on this.

Aims: This study investigated the current status of home therapy for patients with hemophilia, and examined problems and potential solutions.

Methods: Patients and their families who were performing home therapy and who attended the camp of the Korea Hemophilia Association in 2014 were asked to complete questionnaires. The questionnaires pertained to complications of infusion and whether (or not) factor infusion was properly performed. Responses were scored on a scale from 1-5, with a high frequency of complications and adequate performance of infusion being allocated relatively higher scores.

Results: The mean score of complications arising from infusion was 1.56±0.46. This was relatively low and was not correlated with the factor infusion training method. The performance of home therapy obtained a relatively high score: 4.46±0.56. The performance score was significantly higher for patients who had practiced infusion with medical personnel, an injection simulator or a video clip.

Conclusions: Although most patients properly performed home therapy, further improvement is needed in training of infusion and keeping records of bleeds. It is essential to establish guidelines on home therapy, develop a standardized patient and family training program, and reinforce the role of hemophilia treatment centers in educating patients and their families.

PB 1835 | Are we Close to International Standards for Hemophilia Care in Tunisia?

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Background: Hemophilia is a genetic bleeding disorder due on defect in the X chromosome, that's why it is more common in males than females and affects roughly 1 in 5,000 males each year. According to the WFH, approximately 70% of PWHA are under diagnosed and untreated, mostly in developing countries.

Aims: We try to evaluate our efforts for hemophilia care in AOHTC in relation to international data.

Methods: For the cross-sectional evaluation we extract data from the AOHTC for the (PWHA) number and from the NIS for the male population number (census 2014). Three indicators are calculated: the percentage difference between the observed and expected hemophilia incidence, the percentage of the total number of PWHA with severe disease calculated and the ratio of adults to children among PWHA standardized to the ratio of adults to children for males in the general population.

Results: Statistical analysis demonstrates the following data:
The percentage difference between the observed and expected hemophilia incidence demonstrates a negative value - 4.37%.
The percentage of the total number of PWHA with severe disease is 48.9%.
The ratio of adults to children among PWHA standardized to the ratio of adults to children for males in the general population is 0.46.

Conclusions: Our results are quite satisfactory demonstrate that a lot of effort was done in the last years in our AOHTC.
The value of percentage difference between the observed and expected hemophilia A incidence (-4.37) it is a negative value but not so important.
The percentage of the total number of PWHA with severe disease in our AOHTC is of 48.6% showing that our health care system reaches an advanced stage of maturity by identifying more mild and moderate than severe PWHA.
The ratio of adults to children among PWHA standardized to the ratio of adults to children for males in the general population is of 0.46, which is near to 1. These results reflect the impact of the support of WFH and the involvement of multidisciplinary teams.

PB 1836 | TAURUS: A Multinational Phase 4 Study Evaluating Real-world Treatment Patterns in Previously Treated Persons with Hemophilia A Receiving BAY 81-8973 for Routine Prophylaxis

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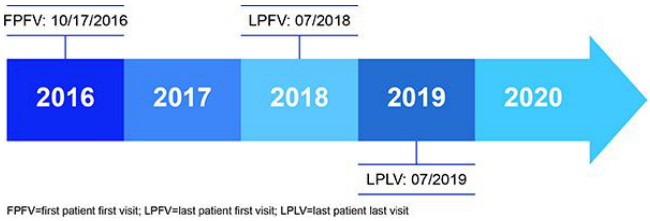

FIGURE 1 TAURUS: Planned Timeline

Background: Routine prophylaxis with BAY 81-8973 using 2x/week and 3x/week dosing was efficacious in the LEOPOLD trials in persons with severe hemophilia A. Observed treatment patterns and outcomes in clinical trials may not adequately represent patient outcomes for treatment outside clinical trials; thus, there is value from a clinical and pharmacoeconomic perspective in supplementing pivotal clinical trial evidence with real-world data.

Aims: To investigate weekly prophylaxis dosing regimens with BAY 81-8973 used in standard clinical practice.

Methods: TAURUS, a multinational, open-label, prospective, noninterventional, single-arm phase 4 study (ClinicalTrials.gov: NCT02830477) includes previously treated males of any age with moderate to severe hemophilia A (≤5% factor VIII [FVIII]:C), ≥50 FVIII exposure days, and no history of inhibitors who are receiving BAY 81-8973 for routine prophylaxis. The primary objective is to investigate the weekly prophylaxis dosing regimens with BAY 81-8973 used in standard clinical practice. Determinants of treatment decisions will be assessed as a secondary objective; other secondary objectives include assessment (in a real-world setting) of the effectiveness of routine prophylaxis with BAY 81-8973, dosing regimens in different age groups and countries, FVIII consumption, changes in treatment satisfaction and adherence, safety, and pharmacokinetics. Data sources include patient medical records, routine clinical visits, patient event and prophylaxis injection diaries, and electronic data capture.

Results: Planned sample size is 350 patients to be enrolled from 9 countries. The study has recently started enrollment; 19 patients from Germany, the Netherlands, and the United States have been enrolled as of January 5, 2017. The anticipated recruitment period is 2 years, with a planned observation period of 1 year **(Figure)**.

Conclusions: TAURUS is designed to obtain real-world data on treatment patterns of routine prophylaxis with BAY 81-8973 to supplement the LEOPOLD clinical trial results.

PB 1931 | Intracranial Haemorrhage Associated with during the Course of Extracorporeal Membrane Oxygenation for Severe Respiratory Failure

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Background: Intracranial haemorrhage (ICH) is a serious complication in patients supported with veno-venous extracorporeal membrane oxygenation (VV-ECMO), and is associated with a high mortality. It is unknown whether ICH is a consequence of the VV-ECMO or of the underlying respiratory pathology.

Aims: To determine the incidence of ICH at initiation and during the course of ECMO, the associated mortality and predictive markers of ICH in patients with severe respiratory failure

Methods: Data were collected from all 165 patients who received VV-ECMO over a 5 year period from Jan 2011 to Dec 2016 in a single tertiary centre in the UK. Only the patients who had brain CT within 24hrs of initiation of VV-ECMO (total 149) were included for further analysis.

Results: The incidence of ICH was 10% (15/149) within 24hrs of initiation of ECMO compared to 4% (7/149; p=0.02) during the course of ECMO. The baseline characteristics of patients with and without ICH on admission are shown in the Table 1.

TABLE 1 The baseline characteristics of patients with and without ICH on admission for VV-ECMO

Characteristics	With ICH (n=16)	Without ICH (n=132)	p value
Age (years) mean (SD)	48.8 (13.4)	45.3 (14.8)	0.37
Sex Male N (%) Female	11 (68.75) 5 (31.25)	77 (58.78) 54 (41.22)	0.44
Haemoglobin (g/L) median (IQR)	98.0 (88.5, 107.5)	100 (91, 111)	0.43
Platelets (109/L) median (IQR)	115 (85-140)	180 (102-218)	0.03
Prothrombin time (seconds): Median (IQR)	14.6 (11.4, 16.8)	14.0 (12.0, 16.0)	0.67
Activated partial thromboplastin time (seconds): Median (IQR)	41.6 (30.7, 43.0)	33.7 (29.5, 41.0)	0.07
Fibrinogen (g/L) :Median (IQR)	4.3 (2.5-5.1)	4.8 (2.1-5.3)	0.7
CrCL (mL/min) median (CI)	53.5 (20.5-62.4)	111.0 (73-120)	0.0002

Conclusions: Reduced CrCL and low platelet count were strongly associated with ICH within 24hrs of initiation of ECMO.Thrombocytopenia associated with ICH is in keeping with previous studies. Acute kidney injury is a frequent finding in ECMO patients, but its association with ICH within 24 hours of initiation of ECMO has not previously been documented. The higher incidence of ICH within 24hrs of the initiation of VV-ECMO suggests that ICH may be related to the clinical severity of the underlying lung injury rather than the intervention of VV-ECMO. Patients with ICH at the initiation of VV-ECMO had a significantly worse outcome.

PB 1932 | Classic Pathways of Fibrinolytic Activation Are Secondary in Non-traumatic Hyperfibrinolysis

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Background: Hyperfibrinolysis (HF) is a severe manifestation of coagulopathy associated with major trauma and out-of-hospital cardiac arrest (OHCA). The activated protein C (APC) has been linked to both conditions. Neutrophil Extracellular Traps (NETs) are suspected to interfere with hemostasis by reciprocally amplifying fibrinolytic activation.

Aims: We aimed to investigate how APC was regulated in hyperfibrinolysis in the absence of a traumatic injury and whether its inhibition was associated with features of NET formation.

Methods: Blood samples of 41 patients who suffered from non-traumatic OHCA were drawn on scene by an emergency physician. HF was diagnosed with a thromboelastometry (ROTEM) maximum lysis (ML) >15%. Plasma levels of APC-PCI, PMN elastase, histonylated (h) DNA fragments (indicator for NETs), sTM (marker of endothelial damage) t-PA, PAI-1, and t-PA-PAI-1-complex were determined and correlated with HF (ML>15%).

Results: Based on ROTEM measurements, 15 patients met the criteria for HF and were compared with 26 patients with a ML< 15 (non-HF). APC-PCI levels were significantly higher (p=0.03) in HF patients. There was a strong correlation (r=0.7, p< 0.001) between neutrophil elastase and hDNA levels (indicative of NETs formation) in all patients. Neutrophil elastase correlated also with sTM levels (r=0.37, p=0.015). T-PA activity (p=0.001) but not antigen levels (p=0.22) were significantly higher in HF patients. None of the other fibrinolytic mediators were elevated in HF compared to non- HF.

Conclusions: Data suggest that a) Inhibition of APC by PCI is associated with non-traumatic HF b) APC correlates with fibrinolytic activation and NETosis, c) NETosis is associated with endothelial cell damage, and d) elevated t-PA activity but not antigen level is associated with HF in OHCA. Apparently, the classic plasmin pathway of fibrinolysis appears secondary in non-traumatic HF.

PB 1933 | Soluble Fibrin Causes an Acquired Platelet GPVI-deficiency in Response to Convulxin, Collagen-related Peptide and Collagen: Implications for Trauma Induced Coagulopathy

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Background: Trauma can cause systemic thrombin generation as well as platelet dysfunction. About half of trauma patients display a defect in platelet deposition on collagen in a microfluidic assay.

Aims: We investigated causes of platelet dysfunction during trauma when blood may be exposed to low levels of wound-mediated release of tissue factor.

Methods: Using calcium-dye -loaded platelets, the effect of thrombin exposure and soluble fibrin generation on subsequent platelet GPVI activation was investigated.

Results: Exposure of apixaban-treated platelet-rich plasma (12% PRP) to thrombin (1-10 nM) for 500 seconds, but not ADP or thromboxane mimetic U46619 exposure, dramatically blocked subsequent GPVI activation by convulxin. Thrombin exposure also resulted in GPVI-insensitivity to collagen-related peptide. Consistent with soluble fibrin binding GPVI, the onset of convulxin-insensitivity required 200 to 500 seconds of thrombin exposure, was not mimicked by exposure to PAR-1/4 activating peptides, and was blocked by the fibrin polymerization inhibitor, GPRP. Consistent with the ADP and U46619 results, PAR-1 signaling through G_q was not required since vorapaxar blocked thrombin-induced calcium mobilization but had no effect on the ability of thrombin to cause GPVI-deficiency. Convulxin-insensitivity was unaffected by the ADAM10-inhibitor GM6001, indicating a negligible role for GPVI shedding. Thrombin treatment of washed platelets in purified fibrinogen also produced convulxin-insensitivity that was prevented by GPRP. Exposure of apixaban/PPACK-treated whole blood to thrombin-treated fibrinogen (±GPRP and then PPACK-inhibited) resulted in >50 % decrease in platelet deposition in a collagen microfluidic assay (wall shear rate, 200 s^{-1}) when fibrin was allowed to assemble (no GPRP).

Conclusions: Since only 1% conversion of plasma fibrinogen generates 90 nM soluble fibrin, which greatly exceeds GPVI levels in blood, circulating platelets in trauma patients may display an acquired GPVI-deficiency.

PB 1934 | Acquired Hemophilia A and Concomitant Factor XIII Consumption: A Case Series

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Background: Acquired hemophilia A (AHA) is a rare bleeding disorder caused by inhibitors (INH) against factor VIII (FVIII). AHA's effect on hemostasis apart from FVIII depletion is largely unknown. Factor XIII (FXIII) is a coagulation protein that promotes clot stability by crosslinking fibrin strands, and promoting wound healing. Here we report three cases of AHA with ongoing bleeding despite sequential bypassing agent and recombinant porcine-FVIII (rp-FVIII) therapy, found to have secondary FXIII consumption.

Aims: To describe our experience with acquired FXIII deficiency and effective FXIII replacement in three complicated cases of AHA.

Methods: Three cases were retrospectively reviewed at St. Michael's Hospital, Toronto, Canada from 2015 to 2016. Patients with FVIII INH (without congenital hemophilia) and low FXIII antigen were included in the study. FVIII activity was measured using a one-stage-aPTT-based assay, FXIII antigen using a latex immunoassay for subunit a, FVIII INH using the modified Nijmegen Bethesda assay and FXIII inhibition was assessed using a chromogenic assay.

Results: At time of diagnosis of AHA, the FVIII activity was 0.08, < 0.01 and < 0.01 U/ml, and the FVIII INH titer at 4.5, 346, and 42 BU/ml in cases 1, 2 and 3, respectively. FXIII deficiency was identified at median day 3 of hospital admission (case 1-0.08, 2-0.32, 3-0.07 U/ml). FXIII INH testing was negative for case 1 and was not pursued for cases 2, 3. A median of 3 (range 2, 9) doses of plasma-derived-FXIII (pd-FXIII) were given (see Figure 1). All patients achieved hemostasis once pd-FXIII was used in conjunction with conventional AHA therapy. All cases received corticosteroid and rituximab for INH eradication.

Conclusions: Our findings demonstrate that AHA can be complicated by FXIII consumption and that FXIII replacement improves the bleeding phenotype. To our knowledge, this is the first case series describing this association. This preliminary data supports a novel approach to patients with AHA and refractory bleeding.

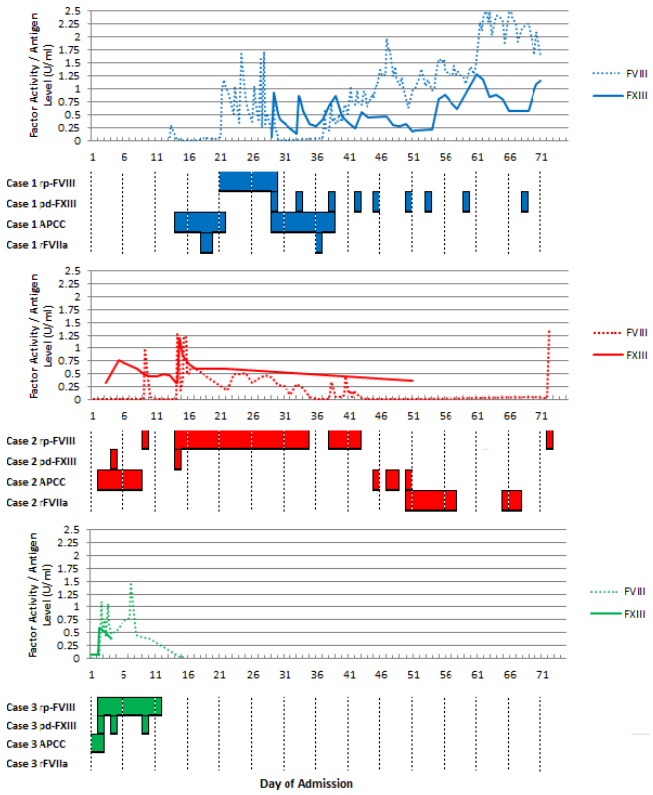

FIGURE 1 Factor Activity, Antigen and Product Support by Time

PB 1935 | Von Willebrand Factor and ADAMTS13 in Bothrops jararaca Envenomation: Involvement of Snake Venom Metalloproteinases and Botrocetin

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Background: Patients bitten by *Bothrops* snakes in South America manifest a bleeding tendency. Systemic clinical manifestations, such as mucous bleeding and thrombotic microangiopathy, share similarities with symptoms of von Willebrand disease and thrombotic thrombocytopenic purpura.

Aims: To study: (a) whether *Bothrops jararaca* venom (BjV) disturbs the steady state of von Willebrand factor (vWF) and ADAMTS13, and to investigate their relationship with BjV-induced thrombocytopenia; (b) the participation of snake venom metalloproteinases (SVMP) and botrocetin, a BjV cofactor for vWF-induced platelet agglutination, in hemostatic disorders.

Methods: Crude BjV was incubated with saline, a metalloproteinase inhibitor (Na_2-EDTA), anti-botrocetin polyclonal antibodies, or glycerol (the vehicle for antibodies). Saline was used as negative controls. Pre-treated-BjV was administered s.c. into Wistar rats and blood samples were collected at 3, 6 and 24h thereafter. Platelet count, vWF antigen quantification (Ag) and vWF collagen-binding (CB) activity, CB/Ag ratio, ADAMTS13 activity, vWF multimer distribution, and factor VIII (FVIII) coagulant activity were analyzed.

Results: BjV-treated rats exhibited intense thrombocytopenia, with the nadir at 6h. There was no statistical significant difference in vWF parameters, although variation was observed. FVIII decreased in rats treated with saline-BjV and botrocetin-inhibited-BjV at 3 and 6h. A drop in ADAMTS13 activity was also observed in saline-BjV treated rats at 3 and 6h. Botrocetin had no important participation in the thrombocytopenia and FVIII consumption evoked by BjV, but SVMP inhibition protected rats from FVIII consumption.

Conclusions: Although proteins that modify vWF function are present in BjV, apparently they showed a minor role in hemostatic disturbances. On the other hand, we show for the first time that BjV causes a drop in ADAMTS13 activity, but its clinical and hemostatic consequences needs further investigation. Financial support by FAPESP and CNPq.

PB 1936 | Efficacy and Safety of Combined Use of aPCC and Antifibrinolytics in Acquired Hemophilia A: Results from the FAIR Registry

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Background: Antifibrinolytics combined with aPCC are not routinely administered to patients (pts) with acquired hemophilia A (AHA) due to increased thrombotic risk, but the few reported and published data on this topic seem to show good tolerance, safety and efficacy.
Aims: To evaluate efficacy and safety of combined use of aPCC and antifibrynolytics in the treatment of AHA bleeding and to assess the difference in treatment between a retrospective group and a prospective one.
Methods: 59 pts, 34 retrospective (from Jan 2003 to Dec 2012) and 25 prospective (from Jan 2013 to Dec 2015), with AHA and treated with aPCC were initially enrolled in the FAIR Registry, a study that collected data from 12 Italian Hemophilia Centers. 3 retrospective pts were subsequently excluded cause of a protocol deviation and statistical analyses were carried out in the remaining 56 pts.
Results: 31 retrospective and 25 prospective pts were evaluated. A total of 101 acute bleeds were treated with aPCC, 65.3% of which in the retrospective group. The combined use of aPCC and antifibrinolytics was reported in the treatment of 19/35 bleeding events (54.3%) in the prospective group and of 21/66 bleeds (31.8%) in the retrospective one, showing a statistically significant difference (p=0.034). 20/25 prospective pts (80%) had only one bleed, while 48.4% of retrospective pts had two or more bleeding events (p< 0.05). In all bleeds treated with combined therapy (40/101) the treatment duration was reduced (mean reduction 16.3%) up to a median of 7 days (IQR 1-48). Good tolerance to combined therapy and no thromboembolic events were reported during the study.
Conclusions: The combined use of aPCC and antifibrinolytics in the treatment of AHA proved to be safe and efficacious. In the FAIR Registry prospective pts, many of whom treated with combined therapy, had a statistically significant reduction in bleeds, but further studies are needed to assess whether this association can be routinely used in case of AHA.

PB 1937 | Prediction of Outcome in Acquired Hemophilia A

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Background: Acquired Hemophilia (AH) is a rare bleeding disorder caused by antibodies that neutralize FVIII procoagulant activity (inhibitors). The neutralizing antibodies are detected by the Bethesda assay (BA) however this assay underestimates their level due to type II reaction kinetics and it does not measure non-neutralizing antibodies. Therefore the total antibody response in AH is poorly characterized by this method. As a result inhibitor levels are poor predictors of patient outcome. An alternative to inhibitor levels are anti-FVIII isotypes and IgG subclasses. A recent report showed that the presence at baseline of anti-FVIII IgA antibodies, but not anti-FVIII IgG antibodies, predict recurrence and poor out come in patients with AH.
Aims: Determine the IgA and IgG subclasses of FVIII-specific antibodies in patients with AH at diagnosis and at or about 1 year following diagnosis and relate the findings to their outcome.
Methods: Anti-FVIII IgA and anti-FVIII IgG subclasses (IgG1, IgG2, IgG4) were determined by ELISA and inhibitor levels by BA after IRB approval and informed consent.
Results: In 18 patients with AH the most prevalent anti-FVIII antibody at diagnosis was IgG4 (94%) followed by IgG1 (72%), and IgG2 (67%). IgA was the least prevalent anti-FVIII antibody (38%). We found that there was no association with the presence of anti-FVIII IgA antibodies, nor any of the anti-FVIII IgG subclasses analyzed at diagnosis, with poor or good patient outcome (p=0.3) using Fisher's exact test. 9/18 AH patients had good outcome (complete response to treatment or spontaneous remission). 4/9 patients with good outcome had anti-FVIII IgA antibodies, 1/9 had detectable IgG1 and IgG4 anti-FVIII antibodies at or about 1 year after diagnosis despite the BA being ≤0.6 BU/ml and FVIII activity ≥100%.
Conclusions: Our results did not support a recent report of anti-FVIII IgA antibodies predicting poor outcome for AH patients and found that anti-FVIII IgA and IgG antibodies persist in patients with good outcome.

PB 1938 | Severe Bleeding Diatheses in an Elderly Patient due to Autoantibody against Factor XIII A Subunit; Novel Approach to the Diagnosis and Classification of Anti-FXIII Antibodies

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Background: Acquired factor XIII (FXIII) deficiency due to autoantibody is a rare, life threatening bleeding diathesis. Its laboratory diagnosis and classification represents a difficult task.
Aims: Introduction of novel approaches into the diagnosis and characterization of anti-FXIII autoantibody and demonstration their use in the diagnosis of a patient with autoimmune FXIII deficiency.
Methods: FXIII activity, FXIII antigen levels and the titer of anti-FXIII-A antibody were monitored throughout the course of the disease. FXIII activity was measured by ammonia release assay; FXIII-A_2B_2 complex, total and free FXIII-B concentrations were determined by ELISAs. The inhibitory capacity of patient's IgG was expressed as the concentration exerting 50% inhibition of FXIII activation/activity (IC50). The binding constants for the interaction of the autoantibody

with recombinant FXIII-A_2 (rFXIII-A_2) and FXIII-A_2B_2 were determined by surface plasmon resonance (SPR). The truncation of FXIII-A by thrombin was monitored by Western blotting. The inhibition of Ca^{2+} induced FXIII activation and active FXIII (FXIIIa) were assessed by FXIII activity measurement.

Results: The antibody bound to rFXIII-A_2 and FXIII-A_2B_2 with high affinity, the affinity constants (Ka) were $2.66x10^8$ M^{-1} and $1.65x10^8$ M^{-1}, respectively. The autoantibody accelerated the decay of supplemented FXIII concentrate. An IC50 value of 170.1 µg IgG mL^{-1} indicated effective FXIII neutralization. The main neutralizing effect of the autoantibody was the inhibition of FXIIIa. After two months, due to combined therapeutic modalities the autoantibody disappeared and FXIII activity significantly elevated.

Conclusions: The anti-FXIII-A autoantibody exerted a combined effect including inhibition of FXIIIa and acceleration of FXIII decay in the plasma. IC50 and binding constant determinations added important information to the characterization of the autoantibody.

PB 1939 | Usefulness of Anti-factor VIII IgG Antibodies Detection by ELISA in the Management of Patient with Acquired Hemophilia A

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Background: Acquired haemophilia A (AHA) is a rare life-threatening bleeding disorder whose diagnosis must be performed in emergency. Laboratory diagnosis is based on evidence of the association of FVIII deficiency and neutralizing anti-FVIII antibody usually detected by Bethesda assay (BA). A recent publication reports the interesting performance of a recently marketed ELISA for IgG anti-FVIII (Zymutest, Hyphen BioMed)[1].

Aims: To compare the performance of a FVIII ELISA for detection of IgG anti-FVIII antibodies with the BA.

Methods: We retrospectively quantified neutralizing anti-FVIII antibody in 7 patients with AHA followed in our center. ELISA assays were performed on citrate plasma samples frozen at the time of diagnosis during the follow-up (period ranged from 230 to 3300 days). Complete remission was obtained in five patients and partial remission in the other two patients. Three patients relapsed. Twenty-four plasma samples were tested: 7 obtained at the initial diagnosis and 17 during the follow-up of 6 patients. FVIII ELISA is considered positive when OD>0.3.

Results: At the initial diagnosis, the new FVIII ELISA is positive in all 7 patients (BA extreme values: 2-155 UB, ELISA OD: 1.7-2.79, respective positivity limits: 0.6 BU and OD 0.3) with a good correlation to BA. During follow-up, concordant results were observed for 11 samples: positive for 4 (titer BA 1.2-1.5 UB vs DO ELISA 0.4-1.3) and negative for 7. For the remaining 6 samples discrepancies were observed. For 5 of them, the BA titer was 1 BU while the ELISA is negative. Conversely, the BA titer was < 0.6 UB and the OD ELISA was 0.44 for the 6th sample. The discrepancies observed only concerned results at positivity limit.

Conclusions: Although the BA is the reference standard for demonstrating neutralizing antibodies, the detection of FVIII-binding antibodies by ELISA seems a good approach for high titer of neutralizing anti-FVIII antibody but further larger studies are necessary in particular for patients with low titer of anti-FVIII IgG.

PB 1940 | Pulsatility as a Modulator of Circulatory Support-related Acquired VWF Defect

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Background: Continuous-flow ventricular assist devices (CF-VAD) are associated with a high incidence of bleeding complications. CF-VAD deliver a high shear blood flow inducing an acquired loss of von Willebrand factor (VWF) high molecular weight multimer (HMW-multimers) that may partially explain the bleeding pattern. CF-VAD also decrease the arterial pulsatility (AP) to a various extent. The residual level of AP has been also reported to directly influence the bleeding risk under CF-VAD. We raised the hypothesis that the intensity of the acquired VWF defect could be modulated by the level of AP under CF-VAD.

Aims: To investigate the effect of AP on the intensity of VWF defect under CF-VAD.

Methods: We investigated both in a mock circulatory loop in vitro and in an experimental swine model the time course of the acquired VWF defect with two clinically implanted Impella CF-VAD. A first swine model was designed to investigate three degrees of AP (low, n=6; intermediate, n=6 or normal, n=6) depending on the combination: maximum flow rate/Impella localization. A second swine cross-over model was used to assess the effect on VWF defect of acute variations of AP under constant high shear conditions. VWF:CB/VWF:Ag ratio and VWF HMW-multimers were used as markers of acquired VWF defect. The assessment of AP *in vivo* was based on the invasive measurement of pulse-pressure (difference between systolic and diastolic arterial pressure).

Results: A similar VWF defect was observed with both pumps in vitro in the absence of vascular bed. In vivo, we observed an inverse relationship between the level of AP and the intensity of the VWF HMW-multimers defect. This relationship was further confirmed in a cross-over swine model demonstrating an endothelial release of VWF modulated by the level of AP (Figure).

Conclusions: We experimentally demonstrated that arterial pulsatility could modulate the HMW-multimers defect observed under CF-VAD.

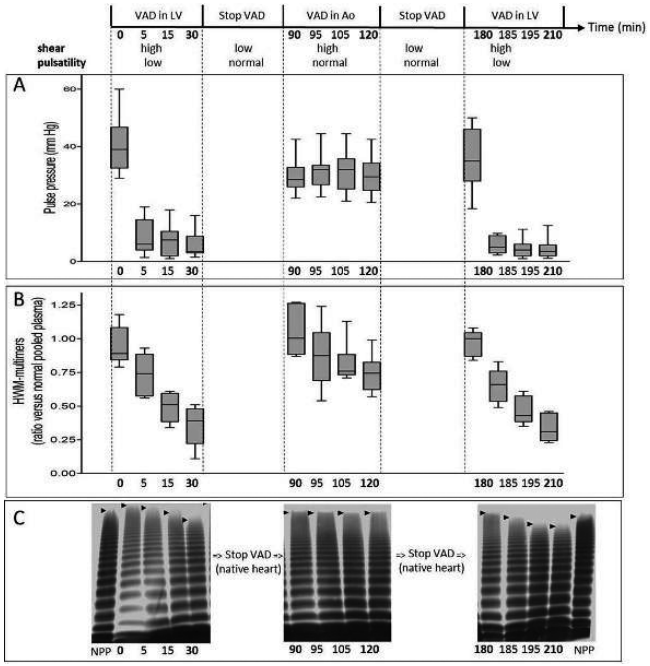

FIGURE 1 Cross-over swine study: Time course of pulse pressure (A) and VWF defect (B: HMW-multimer ratio; C: VWF multimeric profile) under CF-VAD support

PB 1941 | Acquired von Willebrand Syndrome in Infants with Aortopulmonary Shunt

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Background: The acquired von Willebrand syndrome (aVWS) was first described in 1968 by Simone et al in patients with autoimmune diseases. The aVWS is rare in children, most frequently being described in connection with congenital heart defects including aortic stenosis, PDA, VSD and pulmonary hypertension.

Aims: The aVWS often results in increased bleeding tendency such as mucosal-, gastrointestinal- or surgical bleeding. Until now, there are no reports describing aVWS in infants with aortopulmonary shunts.

Methods: Between 07/15-07/16 we evaluated 11 infants < 3 months with univentricular hearts and aortopulmonary shunt (9x BTS, 1x ZAPS, 1x Sano-Shunt) and tested for avWD. The shunt operation was performed between day 5.-180., the blood samples were collected between days 18-260 after surgery. In all these 11 patients we identified avWD with a reduction/loss of the largest VWF multimers.

Results: Despite the limited number of patients, we can presume that nearly 100% of the patients with aortopulmonary shunt present aVWS. Its pathogenesis is explained by the increased activation of the VWF under the influence of the turbulent flow within the shunt. The activated VWF is bound to its specific receptors located on the platelets and on the activated endothelial cells, and undergoes an ADAMTS 13 mediated proteolysis, which leads to the loss of large multimeres. First results show that the VWF swiftly normalizes shortly after suppression of the shunt dependent lung perfusion and switching to a cavopulmonary (Glenn) connection.

Conclusions: So far none of our patients demonstrated an increased bleeding tendency in everyday life. However, we must consider this anomaly as a potential cause of increased blood loss during cardiac catheterizations and operations. Knowledge of the existence of an avWD is therefore necessary for introduction of the replacement therapy with FVIII/VWF products.

PB 1943 | Successful Inhibitor Eradication with Ofatumumab in a Patient with Acquired Hemophilia A

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Background: Acquired hemophilia (AH) is a rare autoimmune disorder characterized by the development of neutralizing autoantibodies against clotting factors, mainly factor VIII, leading to various bleeding patterns up to severe and life-threatening bleedings in patients with a negative personal and family history of hemorrhagic disorders. The management of AH focuses on the goals: control and prevention of bleeding (if present or significant), inhibitor eradication, and treatment of the underlying disease (if applicable). Bleeding control can be achieved with recombinant activated factor VII (rFVIIa or NovoSeven® RT), activated prothrombin complex concentrate (aPCC or FEIBA®), or recombinant porcine factor VIII (OBIZUR).

Aims: First-line therapy in inhibitor eradication is prednisolone or cyclophosphamide, second-line is rituximab. Is Ofatumumab an alternative therapy in treatment of acquired hemophilia A?

Methods: Case report: 73-year-old man with Morbus Waldenström and acquired hemophilia (factor VIII < 1%, max. inhibitor titer 36 BU). First-line treatment with prednisolone and cyclophosphamide did not lead to inhibitor reduction and application of mycophenolic acid in exchange for cyclophosphamide was not successful either. Due to an anaphylactic reaction to rituximab in the past which was given for treatment of the M. Waldenström ofatumumab was given alternatively (4 cycles).

Results: This lead to a successful inhibitor eradication.

Conclusions: Ofatumumab and rituximab are specific human monoclonal IgG1 antibodies. Both bind to the CD20 antigen which is expressed on almost all B-cells and eliminates B-cells through several mechanisms, including complement-dependent cytotoxicity. The binding sites of ofatumumab and rituximab to the B-lymphocytes differ. Our case shows that ofatumumab can be applied as an alternative therapy for inhibitor eradication in acquired hemophilia.

PB 1944 | Individualization of Treatment with Recombinant Porcine FVIII (rpFVIII) Depending on Inhibitor Titres in a Patient with Acquired Hemophilia A (AHA)

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Background: Due to the novelty of treatment with rpFVIII experience in regards of optimal dosing and targeted FVIII plasma levels in clinical practice is limited. The pivotal study protocol intended an initial dose of 200 U/kg BW irrespective of the baseline FVIII inhibitor levels. Here we report on the management of a 76 year old patient with AHA who presented with a major gastrointestinal haemorrhage (FVIII level < 1%, human FVIII inhibitor titre 450 BU).

Aims: The aim was to dose rpFVIII respective of the baseline FVIII inhibitor levels and the clinical condition of the patient.

Methods: Treatment with rpFVIII was individualized according to anti-pFVIII inhibitor titres (baseline cross reactivity), FVIII levels and clinical response.

Results: The initial immunosuppressive regimen included both prednisolone and rituximab in standard doses. Despite insufficient bleeding control with rFVIIa the patient experienced a TIA. rFVIIa was discontinued and rpFVIII (50 U/kg BW) was administered without clinical response and FVIII increment

(FVIII inhibitor titre 231 BU, anti-pFVIII inhibitor titre 100 BU). Therefore, treatment with rFVIIa was resumed. Two cycles of immunoadsorption as well as six cycles of plasmapheresis were performed due to persistently high inhibitor titres. Afterwards the patient was retreated with 100 U/kg BW rpFVIII for uncontrollable diffuse bleeding (FVIII-level 8%, FVIII inhibitor titre 7 BU, anti-pFVIII inhibitor titre 19 BU). The 30-min postinfusion FVIII-level increased to 114% with satisfactory hemostasis within 24 hours. Subsequent doses were targeted to reach FVIII levels of approximately >30% and treatment was continued till FVIII levels normalized in week 12 (FVIII level 324%).

Conclusions: Insufficient FVIII increase after initial rpFVIII dosage due to a high baseline cross reactivity of anti human FVIII inhibitors could be altered by immunoadsorption and plasmapheresis. We conclude that readministration of rpFVIII resulted in rapid bleeding cessation at anti-pFVIII inhibitor titres < 20 BU.

PB 1946 | Thrombin Generation in Healthy Controls and Patients with Cirrhosis of the Liver: Comparison between Platelet-rich and Platelet-poor Plasma

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Background: Thrombin generation displays the hemostatic potential of the individual. Platelets play a central role in this physiological process and this may have a compensatory effect in hepatic dysfunction.

Aims: To compare thrombin generation variables from platelet-rich and platelet-poor plasma.

Methods: The study was approved by the institutional ethics board. Healthy controls and patients with cirrhosis of the liver stratified by the Child-Pugh grading were included after an informed consent. Thrombin generation was assessed using the Technothrombin TGA kit and the RC high reagent in platelet-rich plasma (PRP) and in platelet-poor plasma (PPP). Data for lag time, time to thrombin peak and peak thrombin were analyzed and a ratio was calculated by dividing the data from PRP by those from PPP in order to quantify the contribution of platelets.

Results: A total of 68 controls, 34 patients with Child A, 26 with Child B and 12 with Child C liver cirrhosis were included in the analysis. The ratios for lag time and time to thrombin peak were significantly lower in patients than in healthy controls ($p < 0.05$), while the ratio for peak thrombin did not show any significant intergroup difference.

Conclusions: The lower ratios for lag time and time to thrombin peak in patients with cirrhosis of the liver may be a sign of the compensatory effect of platelets on the process of thrombin generation in the face of reduced plasmatic coagulation. These findings underscore the significant role of platelets during thrombin generation in disease processes and measurements in PPP alone may not be sufficient.

PB 1947 | Acquired von Willebrand Syndrome (AVWS): Diagnostic and Treatment, a Single Centre Experience

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Background: AVWS is a probably under-diagnosed condition, similar to congenital von Willebrand disease (VWD), generally associated with other conditions as: cardiovascular diseases (CVD), monoclonal gammapathy of undetermined significance (MGUS), myeloproliferative syndromes (MS), Immunological diseases, tumors, or other. Treatment of primary condition is the first approach, but acute bleeds therapy and hemorrhage prevention in invasive procedures are needed.

Aims: Incidence of AVWS in patients with different pathologies and bleeding. Analysis of von Willebrand factor (VWF) profile. Analysis of therapy employed and impact on the AVWS.

Methods: Haemostasis study including factor VIII (FVIII:C), VWF antigen (VWF:Ag), VWF ristocetin cofactor (VWF:RCo), VWF collagen binding (VWF:CB) and ratios, and VWF multimeric distribution (SDS-agarose gel) was assessed at initial diagnosis and after different

TABLE 1 Baseline characteristics of all patients and comparison between patients with confirmed diagnostic of AVWS and no AVWS

	All patients	AVWS	No AVWS	p-value	Range of normal values
n (%)	81 (100)	46 (56,8)	35 (43,2)		
Age, years (mean ± SD)	68,7 ± 13,2	67,9 ± 12,9	69,8 ±13,8	0,525	
Male, n (%)	44 (54,3)	26 (56,5)	18 (51,4)		
Bleeding score (mean ± SD)	1,68 ± 2,5	2,37 ± 2,89	0,77 ± 1,55	0,009	
VWF:Ag (UI/dL) (mean ± SD)	177,4 ± 87,4	177,4 ± 93,3	177,4 ± 80,5	0,998	56-158
VWF:RCo (UI/dL) (mean ± SD)	135,4 ± 76,1	117,7 ± 68,4	158,8 ± 80,2	0,016	75-102
VWF:CB (UI/dL) (mean ± SD)	146,6 ± 93,5	120,9 ± 69,8	180,5 ± 109,9	0,004	50-150
VWF:RCo/VWF:Ag (mean ± SD)	0,78 ± 0,24	0,68 ± 0,25	0,9 ± 0,17	0,000	>0,7
Multimeric analysis		46 Abnormal	35 Normal		

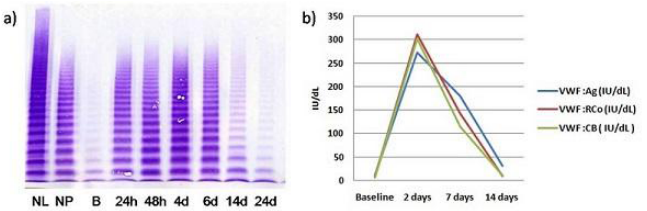
treatments employed (corrective surgery, intravenous immunoglobulins (IgIV), cytorreduction, FVIII rich in VWF concentrates (FVIII/VWF), desmopresin).

Results: We enrolled 81 subjects with suspicion of AVWS: 4 with MGUS, 68 with CVD, 7 with MS, 2 with tumor. AVWS was confirmed in 46 subjects.

3 with MGUS: 1 was diagnosed of myeloma. Severity of hemorrhage was unrelated with the malignancy of the disease. All answered poorly to desmopresin and FVIII/VWF. Answer to IVIg was acceptable in these patients.

Mixed plasma tests did not evidenced antibodies; 37 with CVD: 17 were hospitalized due to haemorragic episodes needing transfusions. 26 were submitted to corrective surgery. After surgery AVWS was resolved; 4 with MS: AVWS resolved after treatment with hidroxicarbamide or anagrelide; 2 with tumor: 1 with prostate cancer resolved AVWS after treatment of cancer. Other with colon cancer presented severe gastrointestinal bleed needing prophylaxis with FVIII/VWF.

Conclusions: Treatment of the underlying disorder resolves the AVWS, but patients face high risk haemostatic challenges, and surgical complications.



a) Multimeric analysis of VWF in SDS-agarose gel (1%) of platelet lysate and plasma of a normal subject (NL and NP), and of subject with AVWS and MGUS at baseline (B) and after IgIV administration at 24 and 48 hours, at 4,6,14 and 24 days (24h, 48h, 4d, 6d, 14d, 24d). b) Representative graphics of the results of VWF:Ag, VWF:RCo and VWF:CB, at baseline and after administration of IgIV.

FIGURE 1 Multimeric analysis of VWF in plasma and representative graphics of answer to IgIV administration in a patient with MGUS and AVWS

PB 1948 | Heparin-Like Substance in Patients with Dengue Infection and Prolonged Activated Partial Thromboplastin Time

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Background: Dengue infection (DI) can cause severe bleeding, shock and fatal outcome. Thrombocytopenia and coagulopathy contributed to bleeding problem. Previous small study demonstrated a heparin-like substance (HLS) in a few patients with isolated prolonged activated partial thromboplastin time.

Aims: To figure out the cause of prolonged APTT (pAPTT) in patients with dengue infection and its clinical significance.

Methods: Adult inpatients with DI from May 2014 to Oct 2016 were enrolled prospectively. Patients with known coagulopathy or liver disease were excluded. DI was categorized into dengue fever (DF) and dengue hemorrhagic fever (DHF) according to WHO 1997 criteria. All cases were tested for complete blood count, coagulogram, thrombin time (TT), and fibrinogen activity. Plasma samples with pAPTT were further tested for mixing study and assay of intrinsic factors (F.XII, F.XI, F.IX, and F.VIII). Plasma samples with prolonged TT were tested for HLS with protamine titration assay.

Results: A total of 183 patients (78 men) with a median age of 29 years (range 18-77) were diagnosed as DF in 153 (83.6%) and DHF in 30 (16.4%). Clinical bleeding in mild, moderate, and severe forms was found in 0.5%, 35%, and 21% of cases, respectively. Prolonged APTT, prolonged prothrombin time, isolated pAPTT, prolonged TT, and hypofibrinogenemia was found in 50.3%, 6.6%, 45.4%, 21.1% and 21.5%. Mixing study of pAPTT showed correctable in 89%. Median (range) levels of F.XII, F.XI, F.IX, and F.VIII were 56.7% (22.2-217.5), 80.0% (30.0-185.9), 69.7% (29.5-215.6), and 73.5% (31.5-308.1). In multivariate analysis, pAPTT was significantly associated with prolonged TT (P< 0.001). Heparin-like substance was found in all samples with prolonged TT with a median concentration of 1.11 U/mL (range 0.53-3.33). pAPTT was not associated with severe bleeding.

Conclusions: APTT prolongation in dengue infection is associated with the presence of a heparin-like substance in the plasma but not associated with severe bleeding.

PB 1949 | Acquired Hemophilia: Initial Presentation, Management and Outcome. Experience of a Single Hemophilia Centre

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Background: The clinical picture of AH is characterized by acute onset of severe bleeding in individuals who had no previously history of bleeding diathesis. Patients generally present with mucocutaneous or soft tissue bleeding including extensive ecchymoses and hematomas. Joint and muscle bleeding, common in patients with congenital hemophilia A, is rare. Treatment aims to stop acute bleeding and eliminate inhibitor.

Aims: Our aim was to perform a retrospective analysis, to assess initial presentation, management and outcome of 9 AH patients treated in our hemophilia centre over the last 15 years.

Methods: Patients diagnosed with AH had a median age of 71 (range:20-85), 6 male and 3 female. At diagnosis, the mean value of FVIII was < 1% (range:< 1%-6.8%), and inhibitor titer 87 BU (range:10-550). Underlying diseases included: postpartum (22.2%), malignancy (22.2%) and 55.6% were considered idiopathic.

Eight patients had bleeding episodes but only 5 patients required hemostatic therapy: bypassing agents (2 FVIIa, 2 aPCC) and the first AH diagnosed patient was treated with high doses of FVIII concentrate (before bypass agent's availability). One patient was diagnosed after a study of a prolonged aPTT on routine coagulation tests and had no bleeding manifestations.

Results: All 9 patients had corticoid therapy (CT) included in initial therapeutic scheme: 3 in monotherapy, 3 with associated immunoglobulin (IG), 2 with associated cyclophosphamide and 1 with CT + IG + cyclophosphamide. A complete remission was achieved in 8 patients (1 died) and 2 patients had relapsed. One patient (with multiple myeloma) only achieved a complete remission when bortezomib was introduced in therapeutic scheme.

Conclusions: Early diagnosis, effective control of bleeding and inhibitor eradication are gold standards to achieve good outcomes. Despite this small cohort of patients, they achieved a high complete remission and low mortality rates. Underlying conditions and relapses were in agreement with published literature.

PB 1950 | Two Patient Cases of Acquired Hemophilia Treated with Recombinant Porcine Sequence FVIII (rpFVIII, OBIZUR®)

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Background: Acquired haemophilia is a rare, often severe bleeding disorder with autoantibody formation against FVIII. The cause is unknown in half of the cases or secondary to underlying autoimmune disorders, malignancy, drugs or post-partum state. Treatment consists of control of bleeding and eradication of the antibody by immunosuppression. Traditionally by-passing agents (aPCC or rFVIIa) are used to control bleeding, but laboratory methods to follow response are lacking. Recombinant porcine sequence FVIII (rpFVIII, OBIZUR®) is a new treatment option allowing precise dosing based on FVIII:C measurements.

Aims: To describe the first two cases in Finland of acquired haemophilia, where bleeding was controlled by rpFVIII replacement therapy using FVIII:C measurements to tailor dosing.

Methods: Two patients were referred to our comprehensive care centre by local hematologists. Recombinant pFVIII was provided by Baxalta for compassionate use as the product was not yet available in our country. FVIII:C was measured by one-stage-clotting assay.

Results: The first patient was a 47-year-old male with a history of skin bruising and painful lower extremity soft tissue hematoma. Prolonged APTT raised suspicion of acquired hemophilia. Bleeding was effectively controlled with three doses (200IU/kg and 2x100IU/kg) of rpFVIII adjusted by FVIII:C levels. The second patient, a 74-year-old male had recurrent thigh and soft tissue bleeds uncontrolled with aPCC. Compared with the first patient, he required higher and more frequent rpFVIII dosing (150IU/kg every 3 hours) for clinical and FVIII:C response.

Conclusions: Our initial experience in controlling bleeding with rpFVIII proved clinically effective and allowed tailored coagulation factor control with individual dosing. On-site around the clock coagulation specialist and laboratory services are required for prompt FVIII:C measurements and interpretation. Anti-porcine antibody testing and evaluation of cost-effectiveness may also be needed to have this treatment option available.

PB 1951 | Acquired von Willebrand Syndrome Associated with Monoclonal Gammopathy: Importance of Hemostasis Screening in the Preoperative Management of Three Case Studies

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Background: Acquired von Willebrand Syndrom (AvWS) is a rare heterogeneous bleeding disorder with a percentage varying from 0.04 to 0.13 %. It is occurring in patients with out of family history of bleeding disorders and it is most frequently observed in association with lymphoproliferative, myeloproliferative, immunological and other disorders.

Aims: We describe three cases of AvWS associated with IgG-MGUS in patients scheduled to undergo surgery.

Methods: We found AvWS in three men aged 67, 70 and 79 years known to have IgG-MGUS by a preoperative hemostasis test for a surgery intervention (colposcopy, hip prosthesis and kidney biopsy). None of the cases had a history of bleeding disorders.

Results: The preoperative laboratory findings for these three patients were as follows: prolonged aPTT, normal PT, prolonged platelet plugging time. Plasma levels of FVIII, vWF antigen (vWF:Ag) and activity (vWF:RCo) were low: patient 1 (FVIII=45%, vWF:Ag=45%, vWF:RCo< 10%), patient 2 (FVIII=31%, vWF:Ag=12%, vWF:RCo=15%) and patient 3 (FVIII=18%, vWF:Ag=14%, vWF:RCo=10%). The ratio of vWF propeptide to vWF was elevated in the three cases. None of the three patients had experienced bleeding episodes in the past. The three patients had a kappa IgG-MGUS, with monoclonal pic of 2.2, 1.7 and 2.9 g/L for patient 1, 2 and 3, respectively.

High-dose immunoglobulin infusions were used for the three patients. This treatment normalized their hemostatic tests and they were operated on safely without any bleeding episodes.

Conclusions: In the presence of an associated underlying disease, such as MGUS, investigation of hemostasis is important in the view of the potential gravity of AvWS particularly for patients undergoing surgery. Management includes high doses of immunoglobulin or other treatment strategies to increase the level of associated vWF, in addition to management of the associated pathogenetic disorder.

PB 1952 | Study of Clinical Characteristics, Laboratory Findings and Response to the Treatment of Patient with Acquired Hemophilia in National Institute of Hematology and Blood Transfusion of Vietnam

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Background: Acquired haemophilia have been well described by many studies in all over the world while in Vietnam there are no studies that work on this subject.

Aims: Study of clinical characteristics, laboratory findings and response to the treatment of patients with acquired haemophilia in the National Institut of Hematology and Blood Transfusion.

Methods:

Subjects: 25 patients with acquired hemophilia diagnosed in the National Institut of Hematology and Blood Transfusion from January 2012 to May 2016.

Methods: Cross-sectional description study with retrospective and prospective.

Results: *Clinical characteristics*: Patients are mostly elderly, found in both men and women. Severe hemorrhage is the prominent symptom, occurs in multiple locations. The three most common bleeding sites are: muscle hemorrhage (68%), purpura (60%), and visceral hemorrhage (20%). *Laboratory findings*: All patients had prolonged APTT, mostly acquired hemophilia A, with a high titre of factor VIII's inhibitor. *About treatment*: Most patients stop bleeding with factor VIII (84%). There are 3 patients who do not respond to the factor VIII but stop bleeding with factor VIIa; Most patients respond to the elimination of the inhibitors by immuno suppression (68%). The average time of remission 13.23 ± 25.5 weeks. Recurrence rate was 17.6%.

Conclusions: Patients are mostly elderly, found in both men and women. Severe hemorrhage is the prominent symptom, occurs in multiple locations. The three most common bleeding sites are: muscle hemorrhage (68%), purpura (60%), and visceral hemorrhage (20%). Most patients have acquired hemophilia A, with a high titre of factor VIII's inhibitor. Most patients stop bleeding with the treatment of high dose of factor VIII concentrate. Most patients respond to the elimination of the inhibitors by immuno suppression . The average time of remission 13.23 ± 25.5 weeks. Recurrence rate was 17.6%.

PB 1953 | Acquired Hemophilia A in China: A Result of a Multicenter, Prosepective Study in China

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Background: Acquired hemophilia A (AHA) is a rare bleeding disorder caused by autoantibodies to coagulation FVIII. Bleeding episodes at presentation are spontaneous and severe in most cases. Optimal choice of hemostatic therapy is controversial, and there is little available data from prospective study.

Aims: To assess the factor influencing the severity of bleeding and the effectiveness of the different hemostatic therapies among 112 registered patients.

Methods: This is a multicenter, prospective study.We got back the data from participating centers on paper. All analyses were performed using SPSS 17.0 for Windows.

Results: There are 55 males and 57 females in our study, and there is no difference in incidence between sex.Most patients, 96 of 112, had spontaneous bleeding,while 8 patients had Peri- or post-surgery bleeding, 6 other cause and 5 had no data.The most common bleeding cites are cutaneous (75.7%) and muscle (44.3%). The time from bleeding to first hospital visit ranges from 1day to 1500days (mean 20days), and the mean time from first hospital visit to definite diagnosis is 18days.Immune suppression therapeutic regimens include: steroid alone (16%), steroid+Cyclophosphamide (29%), steroid+Rituximab (22%), steroid+Cyclophosphamide+ cyclosporine/Immunoglobulin/Plasmaexchange/Azathiopine(15%), rituximab alone (1%), steroid+ Cyclophosphamide+cyclosporine+Immunoglobulin+Plasma exchange+Azathiopine, (8%), no data (9%).

Conclusions: Some underlying disorders, such as pemphigoid, autoimmune disease, pregnancy, infections and malignancies are related with AHA. Delay of dignosis is related to the severity of bleeding. Bleeding control was significantly higher in patients treated with bypassing agents versus FVIII/DDAVP (95.7% vs 61.2%; P <0.05). Bleeding control was similar between rFVIIa and aPCC (100% vs 94% p<0.05).Immune suppression therapy (steroid or Cyclophosphamide) has a higher remission rate than other choice.

PB 1954 | Clinical Importance of Vascular Wall Antithrombotic Activity Disorder Detection in the Course of Hormonal Replacement Therapy (HRT) and its Pharmacological Correction

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Background: The vascular wall plays an important role in regulation of blood clotting potential.

It´s beyond the question that only pathogenic therapy of prevention, elimination and restriction of every patient condition caused by extensive coagulation can effectively prevent thromboembolic conditions. Complementary treatment intended for vascular wall function improvement greatlu decreases risk of thrombosis episodes. Recently researchers have been placing high emphasis on ω-3 polyunsaturated fatty acids (PUFAs) influence on hemostasis and vitamins influence on homocysteine serum level decrease.

Aims: comparative study of PUFAs and B group vitamins and folic acid complex effect on vascular wall antithrombotic activity in women taking OC and HRT.

Methods: We studied 60 perimenopausal women without bad habits and examined carefully for organ disorders. In the 1st group were included 30 perimenopausal women taking HRT - Femoston 2/10. All women in this group have been given PUFA at 1 g/day and B group vitamins, folic acid at 200 µg/day 1 month before and 2 months in the course of HRT.Other 30 perimenopausal women were included in the second, control group who were given only HRT-Femoston 2\10. In order to estimate vascular wall antithrombotic activity we used application of tonometer cuff on patient arm for short-term (5 min) local ischemia and applying a pressure10 mm Hg.

Results: 3 months PUFAs administration caused decrease of collgen-induced platelet aggregation while ADP-induced aggregation was not affected. After 2 weeks of drug administration t-PA activity reduced from 109,6 ± 7,2% to 71,3 ± 5,0%. In the 1st group there was no homocysteine level increase while in the 2nd group moderate hyperhomocysteinemia was revealed in 5 patients.

Conclusions: Antithrombotic activity of PUFAs is quite sufficient for prevention of procoagulation activity before HRT administration. These results suppose PUFAs to maintain antithrombotic activity of vascular wall.

PB 1955 | Some Haemostatic Parameters and International Normalize Ratio (INR) Values of Patients with Snakebite Envenomation Before, During and After Treatment with Antivenom Snakebite Research, Training and Treatment Centre, General Hospital, Kaltungo, Gombe State, North-Eastern Nigeria

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Background: Snakebite envenomation is a global problem affecting mostly the rural Communities living in the tropics, where basic health facilities are poor.

Aims: To evaluate the effects of envenomation and antivenom on some haemostatic parameters among patients with snakebite envenomation.

Methods: In this observational study, 296 participants comprising of 141 patients with snakebite were treated with Anti-snake-Venom (ASV) and 155 apparently healthy subjects as control. We evaluated platelet, PT, aPTT and INR using Standard Manual Method.

Results: The result showed deranged PT, aPTT, platelet and INR values of the study group on admission, after 6th hour and 24th hour interventions, subjects had their envenomation resolved indicated by 80% PT, 95% aPTT and a normal INR value (0.5-4.0). The mean±SD of PT and aPTT of the controls when compared with the study population were statistically significant (p≤0.05). Age groups that were maximally affected by Snakebite were 18-30 years (55.9%) and the least affected were 0-10 years (3%) and 40 - 70 years (10%). Farming and cattle rearing were the occupations with high snakebite incidence (42% & 30%) respectively. This study also showed that the incidence of snake bite in the study population due to carpet Viper is 83% while that of cobra is 5.7% and unidentified was 11.3% respectively. Rural dwellers were the mostly affected with 82% and 18% of the study population were Urban dwellers.

Conclusions: We concluded that the deranged platelet, PT and aPTT and INR were normalized on intervention with ASV within 24hours. However, we suggested an additional attention of the Federal and State Government in establishing a nearby Health Centres in the rural Communities for easy accessibility by the rural dwellers since they were the most vulnerable to envenomation. Although, further research is required.

PB 1957 | Utility of Measurement of Plasma Thrombin Generation Using Calibrated Automated Thrombography (CAT) in the Management of Acquired Haemophilia

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Background: Bleeding risk in acquired haemophilia(AH) is poorly correlated with partial thromboplastin time or inhibitor level. Strategizing pre-emptive by-passing agent use during critical bleeding is highly challenging.

Aims: To determine the role of global coagulation testing using CAT measurement to guide therapy in AH.

Methods: Plasma thrombin generation (TG) data using standard parameters obtained during the course of treatment (non-protocolised) from bleeding AH patients were analysed with clinical features and by-passing agent infusion to determine its value in guiding by-passing agent therapy.

Results: Two AH patients with serious bleeding events were studied. A 44 year-old man (inhibitors >700BU) suffered a spontaneous large subdural haemorrhage which was managed without surgery. Baseline CAT parameters indicated reduced TG. FEIBA 100iu/kg was initially infused 12-hourly. CAT parameters measured using standard methods were beyond measurable ranges suggesting overcorrection of thrombin generation potential. FEIBA was then titrated to 75iu/kg and 50iu/kg 12-hourly over the subsequent 1 week keeping peak/trough CAT parameter within normal ranges. TG potential improved with inhibitor elimination allowing stoppage of FEIBA over the subsequent 2 weeks. He recovered fully with no further bleeding or thrombosis. The second patient, a 70 year-old man (inhibitor 21 BU) had a retroperitoneal bleed and was treated with 50iu/kg FEIBA given 12-hourly. He suffered further bleeding when trough TG measures were below normal. FEIBA was increased to 75 iu/kg 12-hourly and subsequently reduced over 3 weeks with corresponding improvement of CAT parameters and no further bleeding or thrombosis.

Conclusions: Plasma TG measured using CAT and kept within normal range has potential value in guiding by-passing agent dosing and infusion frequency in AH patients with serious bleeding, reducing risk of rebleeds. Optimal target range and frequency of monitoring remain unknown.

PB 1958 | Acquired Haemophilia Patients' Journey from the Exhaustive French Nationwide Database Collecting the Activity in Hospital (Programme of Medicalisation of the Systems of Information - PMSI)

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Background: Epidemiological data on rare bleeding disorders is difficult to collect. Acquired haemophilia (AH) is a rare disease occurring in 1.48/million/year[1].

Aims: To establish if PMSI that could be a supplementary for current registries to define the patients with AH.

Methods: International versions of ICD10* may differ and this used in France by PMSI has no specific code for AH. The whole AH population could not be diagnosed, the authors decided to identify a new population based on elderly patients (≥65yo) treated with by-passing agents (BPA) (rFVIIa/aPCC) for bleedings coding: D66 „Hereditary FVIII deficiency"/D68.4 „Acquired deficiency in clotting factor"/D68.3 „Bleeding disorders due to circulating anticoagulants". The analysis was on 5 years (2010-14).

Results: The population represented 206 patients: 118 for D66; 67 for D68.4 and 21 for D68.3. 67% were treated with rFVIIa. Perhaps some patients belonging to this population didn't have AH but they were certainly few due to the criteria of selection. According to FranceCoag registry[2], since Jan the 1st of 2009, ~76 patients with congenital haemophilia, irrespective of their age, are treated with BPA. Moreover, other elderly patients treated with BPA are very rare.To validate this methodology, the incidence of this population was compared to those of the published registries[3]. The present data were consistent with this of registries over 5 years (~200 patients).

Conclusions: PMSI as an alternative to the registries for characterizing the journey of all AH patients was not possible. However, an elderly (≥65) subpopulation treated with BPA has been identified. A national standardization of the coding for AH should be considered for an optimal epidemiologic and medico-economic use of the PMSI. These results will allow starting the 2nd phase of the project, a detailed analysis of the patients' journey which will help to refine also the population.

**10th International Classification of Diseases*

1 Collins *et al.* Blood 2007

2 www.francecoag.org

3 Baudo Blood 2012

PB 1959 | A Report of Clinicians' Attitude Survey of Acquired Hemophilia A for Improving its Prognosis in Japan

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Background: We previously analyzed a present state and clinical problems of long term outcome of patients with acquired hemophilia A (AHA) in our hospital to know better treatment or management essential for clinical practice. The study revealed mean period to APTT normalization, period to disappearance of inhibitor, all treatment period, and admission period were 42, 61, 96, and 62 days, respectively. High age patients with severe complicated diseases hardly return to society. It is important to treat earlier and comprehensively with making sure long outcome or treatment goal by adequate evaluation of patient or its complicated disease.

Aims: A questionnaire survey for clinicians' attitude of AHA was performed in order to improve its prognosis and to understand situations in 8 hospitals in Niigata Prefecture in Japan.

Methods: It was performed in 2 big base hospitals of wide area, 3 middle core hospitals and 3 small remote place hospitals. One hundred and eithty-five answers were available, contained from 45% of phisicians, 42% of surgeons, and 8% of residents.

Results: Degree of recognition of AHA was about 60%, which was higher in foundation hospitals. Main knowledge source of AHA was daily clinic in foundation hospital and books in core hospitals and remote place hospitals. Seventy to eighty percent of doctors who did not know AHA wanted to know AHA. Ten to twenty percent of doctors encountered AHA, frequently encountered by physicians and orthopedists in that order. Only 5 percent of doctors had a treatment experience of AHA. About 80 percent of doctors wanted to introduce AHA patients to hematologist. About 60 percent of doctors wanted to introduce AHA patients to hemophilia center like hospital which has hematologists.

Conclusions: It is important to know the consultation departments or hospitals in order to improve the medical cooperation system to the department of hematology where treatments are done, which leads to the improvement of prognosis of AHA.

PB 1960 | An Unusually High Incidence of Acquired Haemophilia A in Two Provinces in North-Eastern Italy in the Winters 2014 through 2016

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Background: Acquired Haemophilia A (AHA) is associated with the onset of autoantibodies targeting coagulation FVIII activity in subjects with no personal or family history of bleeding. The reported incidence is 1-4 cases per million population per year with increasing frequency in elderly people. Our Center is a reference coagulation laboratory for the provinces of Trieste and Gorizia with a catchment area of 375.000. In this district the incidence of AHA was negligible before 2014.

Aims: To report a series of consecutive AHA diagnosed in our district from 2014 to 2016 and calculate incidence rates.

Methods: In patients with unexplained elevated aPTT and bleeding symptoms, mixing studies were performed and Lupus anticoagulant was excluded. Presumed AHA cases were immediately notified to clinicians, FVIII level was estimated and the Bethesda assay performed.

Results: From 2014 to 2016, 8 patients (4 males and 4 females, mean age 78.6 years) who were admitted to the Emergency Room for spontaneous bleeding with no history of haemostasis disorders, were diagnosed with AHA. Patients had severe bleeding with inibitor titre in the range 4-210 BU/ml. Their characteristics are summarized in the Table: 7 out of 8 patients were treated in the hospitals of the district with bypassing agents and immunosuppression. Calculated incidence rates of AHA in our area are: 5.33 per million in 2014 and in 2016 and 10.66 per million in 2015.

TABLE 1 Characteristics of patients

ID	Month and Year of diagnosis	Sex	Age	Symptoms	APTT ratio	FVIII level %	Inhibitor titre BU/ml	Underlying disease
1	January 2014	F	76	Subcutaneous bleed	3.19	< 0.5	7.5	Diabetes Mellitus, Hypertension
2	January 2014	F	56	Haematuria, Retroperitoneum bleed	4.11	< 0.5	130	Sjogren´s syndrome Connective tissue disease
3	January 2015	F	75	Haematuria	4.43	1.7	5.7	Breast cancer (in the past)
4	February 2015	M	85	Subcutaneous bleed	2.92	1.6	210	Prostate cancer, chronic renal failure, arterial hypertension, rheumatoid arthritis, dyslipidemia
5	February 2015	M	85	Haematuria	1.86	5.1	4	Chronic renal failure
6	November 2015	M	93	Subcutaneous bleed	3.8	< 0.5	179	none
7	December 2015	M	80	Subcutaneous bleed	3.21	< 0.5	127	Prostate and bladder cancer
8	December 2015	F	79	Subcutaneous bleed	2.19	4.7	5.8	Connective tissue disease, cryoglobulins, parossistic atrial fibrillation

Conclusions: Our data show an unexpected increase in the number of AHA cases in this area starting from 2014, with significantly higher incidence rates compared with data reported in literature. All cases were diagnosed in the coldest months, an unusual finding not reported in previous studies. As no common trait in the clinical history of these patients was found so far, we speculate that infections (possibly of viral origin) might be the reason of the AHA cases recorded in our district. We can also figure out that the real incidence of AHA may be underestimated.

PB 1961 | Acquired Haemophilia: Overcoming Practical Challenges to Deliver Optimal Nursing Care

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Background: Acquired Haemophilia (AH) is rare. It may present where clinical staff have limited experience. Bleeds may be caused by healthcare professionals delivering basic care, due to a lack of understanding of bleeding risk. Delays liaising with hospitals with experienced Haemophilia teams result in worsening clinical condition, and poor outcomes.

Aims: To identify key themes in nursing people with AH.

Methods: A meeting of Haemophilia Nurse Specialists took place to exchange experiences of nursing AH. A follow up meeting discussed the key themes previously identified.

Results: Raising awareness of treatment and management of AH was identified (preserve life and limb, control bleeding, treat any underlying condition, and clinically evaluate the patient's progress) The delivery of optimal care for AH patients is essential, minimal delay in diagnosis, prompt treatment to stop bleeding, and liaison with (and transfer to) a Centre of excellence. Preventing further bleeding from accidental trauma and invasive procedures is key in nursing management. Considerations for the choice of bypassing agent, include efficacy in controlling bleeds, venous access, volume and frequency of infusions and staff resources available to administer them.

Discussion: The complex nature of AH requires multidisciplinary, expert care. Non-specialist nursing staff should be supported to identify and report early warning signs of bleeding. Practical guidance is needed for nursing care that avoids provoking further bleeding.

Conclusions: The education of non-specialist staff is important. Two main paths are recommended: development of educational materials to disseminate best practice among non-specialists, and publication of the core principles of nursing care of AH in general nursing journals. We welcome input from colleagues, in order to ensure the materials are evidence-based, effective and robust, optimizing outcomes for AH patients in the future.

Sources of support: Medical writing was supported by an educational grant from Novo Nordisk Ltd.

PB 1962 | Pulmonary Thromboembolism due to Lead Poisoning in Opium Addict Patients

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Background: Lead poisoning is a major public health problem. Recently, there have been reports of opioid adulteration with lead in Iran. Lead is a toxic metal that affects many organ systems in humans. The following case series is the first of its kind in that pulmonary thromboembolism (PTE) due to lead toxicity has been described.

Aims: Lead is available in the environment widely and affects major organ systems in the body including hematopoietic, respiratory, renal, nervous and cardiovascular systems, mainly through increased oxidative stress, ionic mechanism and apoptosis. Exposure may result from ingestion or inhalation of lead compounds. Diagnosis of lead poisoning depend on a high index of suspicion and a through patient history. Lead is sometimes deliberately added to opium by the smugglers or salesman to increase its weight during trading.

Methods: We report four patients with lead poisoning in Iran, all of whom presented with leg pain and dyspnea. A history of opium ingestion or inhalation was present in each of these patients. None of the patients reported known occupational exposure to toxin and any risk factor for PTE. Clinical finding including the swelling and erythema in lower leg , elevated of jugular venous pressure and decrease O2 saturation. Color doppler sonography In all patients showed deep vein thrombosis. Chest CT were in favour of massive PTE. Lead level were higher than normal.

Results: All patients underwent medical treatment and discharge with good condition.

Conclusions: Opium is still one of the most frequently abuse drugs. Although the amount of lead in opium is usually small, when taken in large amounts opium adulterated with lead can produce toxicity. Lead toxicity should always be consider in cases of PTE with an unknown etiology, especially in a setting were opium abuse is common.

PB 1963 | Hemostatic Alterations in Hematological Disorders Rather than Inherited Coagulopathies

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Background: Incidence of haemostatic and thrombotic complications in hematological malignancies are reported in few literature but not much more documented in other hematological disorders like thalassemia, dengue, malignancies and etc. It's a big challenge for hematologist in terms of diagnosis as well as in management of these complications in hematological disorders rather than inherited Coagulopathies.

Aims: To observe and deter mine the frequency of hemostatic abnormalities in all requested investigations from hematology clinic to the department of Thrombosis and Hemostasis in NIBD.

Methods: This cross sectional study was initiated 2 years ago after approval of institutional IRB /ethics committee and written informed consent of patients. Data was documented by clinical research associate in structured questionnaire. Data was analyzed by using simple descriptive tools.

Results: A total of 800 patient's requests were received in the hemostasis department for the haemostatic workup in hematological disorders rather than inherited Coagulopathies. ITP 200(25%) cases with thrombocytopenia. Significant increased levels of PT, APTT, Von Willebrand antigen, fibrinogen, D.Dimer and factor VIII were found in 250(31.2%) pretreated patients of AML, ALL, multiple myeloma and diffuse large B cell lymphoma. Prolonged APTT with positive Lupus anticoagulant in 20 (16.53%) and Cardiolipin Antibody were present in 11(9.09%) 31(3.8%) patients of thalassemia. Marked hematological and hemostatic abnormalities were noted in 319(39.8%) cases of dengue hemorrhagic or dengue like fever.

Conclusions: The present investigations indicate that impaired coagulation parameters in dengue infection and hematological malignancies are more common in our setting. Early detection of haemostatic defects will help to cope up the severity of the disease and reducing morbidity and mortality rates. Early diagnosis and active intervention will help in reducing the bleeding complications and provide better survival with good quality of life.

PB 1964 | Acquired Factor XIII Deficiency in the Inpatient Setting: Retrospective Case Series

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Background: Disproportionate surgery related bleeding has been reported in association with acquired factor XIII (FXIII) deficiency in patients with normal coagulation screening tests.

Aims: To describe the clinical characteristics and outcome of patients with acquired FXIII deficiency and to estimate the frequency of this deficiency in patients with disproportionate bleeding.

Methods: Retrospective case series. All patients studied for FXIII deficiency due to disproportionate or unexplained bleeds between January 2014 and December 2016 were included. An immunotubidimetric FXIIIA subunit test (Instrumentation Laboratory) was performed in real time. FXIIIA < 50% was considered deficiency. Patients less than 2 years old or with suspicion of congenital FXIIIA deficiency were excluded. Descriptive statistics were used. Normal or deficient FXIIIA populations were compared by using Fisher and Mann-Whitney tests (Stata13 software).

Results: 43 patients met inclusion and exclusion criteria, 26 studied because of disproportionate surgery related and 17 because of spontaneous bleeding. Sixteen had FXIIIA less than 50%. Statistically significant differences between groups were found in hematocrit points drop and red blood cell units transfused (table 1).

TABLE 1 Patients ,characteristics Comparison between Groups with or without FXIIIA Deficiency

	FXIIIA deficient	FXIIIA Normal	p
Patients, n	16	27	
Gender, Female/male	14/2	17/10	0.158
Age (years), median,(IQR)	38 (26-55)	46 (18-69)	0.841
Charlson Comorbidity Index, Median (IQR)	2 (1-4)	1 (0-2)	0.11
Surgical bleeding, n (%)	12 (75)	14 (52)	0.199
Spontaneous bleeding, n (%)	4 (25)	13 (48)	0.199
Hematocrit fall, points Median (IQR	5 (3-8,5)	2 (0-4)	0.01
RBC Transfusions, units Median (IQR)	3 (2-4,5)	0 (0-2)	0.001
FXIIIA, % median(IQR)	39 (27,5-43,5)	72 (61-91)	0.0001

Type of bleeding of FXIIIA deficient patients are described in table 2.

TABLE 2 Bleeding manifestations of patients with acquired FXIIIA deficiency

Patients, n		16
Surgical Bleeding, n(%)		12 (75)
	Kidney transplant, n	3
	abdominal surgeries, n	3
	Femoral venipuncture, n	2
	Other surgeries, n	4
Spontaneous Bleeding, n(%)		4 (25)
	Bruises on limbs, n	2
	CNS bleeding, n	2

Plasma derived FXIII concentrate was used in five patients to treat persistent or life-threatening bleeding, stopping it in < 24 hours. Four patients died, none because of bleeding.

Conclusions: In our study, 37% of patients studied because of disproportionate or unexplained bleeds had factor XIIIA deficiency. It is possible that this entity is underdiagnosed in clinical practice. In our experience, most cases were related to postsurgical bleeding, FXIIIA deficient patients bled significantly more, as it was stated by a greater fall in hematocrit and increased transfusion requirement, and in some of them factor XIII replacement therapy helped to stop bleeding.

PB 1965 | Bleeding Manifestations as First Clinical Presentation of Systemic Lupus Erithematosus: A Case of Acquired von Willebrand Syndrome (AVWS)

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Background: AVWS is a rare bleeding disorder often associated with underlying diseases.

Differential laboratory diagnosis between acquired and congenital von Willebrand disease (vWD) could be difficult and a crucial aspect is the personal and family anamnesis.

Aims: We describe a case of this rare disease.

Methods: Clinical and laboratory data were collected on medical record.

Results: *A 39*-year-old woman showed since 2 months recurrent nosebleeds, spontaneous cutaneous hematoma and menorrhagia. In anamnesis she reported 1 pre-term delivery by caesarean section, hypotiroidism treated with tiroxin, Raynaud Syndrome since 1 year, no previous bleedings. Laboratory tests showed microcytic anemia (Hb 9.5 gr/dL), leukopenia and neutropenia, normal platelet count, thyroid profile, liver/renal function and prothrombin time (PT), prolonged activated partial thromboplastin time (PTT ratio=1.73) partially corrected with normal pooled plasma, PFA100 very prolonged. Second level coagulation tests revealed the significant reduction of FVIII activity (FVIII:C=4%) and von Willebrand factor (VWF:Ag=4%, VWF:RCo=2%) and the absence of ristocetin platelet agglutination. Family history was negative for bleeding disorders. Neutralizing FVIII/VWF inhibitor was confirmed by mixing assay. Test for autoimmunity confirmed systemic lupus erithematosus disease. Patient was treated with oral prednisone 1 mg/Kg + hydroxychloroquine 200 mg/die and oral tranexamic acid. We observed the resolution of bleeding manifestations and complete normalization of haemostatic parameters after a month.

Conclusions: AVWS is often unrecognized or misdiagnosed as inherited VWD and its diagnosis is often difficult and delayed. Differently from acquired hemophilia the antibodies in AVWS cannot easily be demonstrated by routine assay and inhibitor activity against FVIII/VWF using coagulative method based on mixing tests is found only in few cases. Our experience demonstrates.the necessity to rapidly identify the bleeding disorder and search for the underlying diseases.

PB 1966 | IPS Derived Vascular Endothelial Cells (vECs) from HA-patients with Nonsense Mutations Are a Potential Cell-model to Study the MHC-driven Risk for Inhibitor Development

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Background: In about 20% of patients with severe Haemophilia A (HA), treatment with replacement FVIII is complicated due to the development of inhibitory antibodies against FVIII. F8 null mutations, like nonsense mutations, have a higher risk for inhibitor formation than other mutation types. Furthermore the location of a specific nonsense mutation influences the risk for inhibitor development. We established patient-specific IPS cells to differentiate into vECs. As endothelial cells have the ability to act as non-hematopoietic antigen-presenting cells (nhAPC), we think these cells are a potential cell-model to study the mutational effect on inhibitor development.

Aims: We differentiated IPS cells from three HA-patients into vECs to localize for intracellular FVIII. Furthermore we wanted to ascertain, if IPS derived vECs have the capability to express MHC-I and MHC-II.

Methods: IPS cells from three HA-patients (R363X, R431X, R1941X) were differentiated into vECs and MACS-separated using CD144 Microbeads. vECs were immunostained with domain-specific antibodies against FVIII and stimulated with IFNγ, to analyze MHC-I and MHC-II expression by FACS.

Results: IPS derived vECs from all three patients present intracellular FVIII and specific endothelial marker like vWF, CD31 and CD144. vECs with a nonsense mutation in the heavy chain show normal intracellular trafficking of FVIII. However, vECs harboring the high-risk inhibitor mutation R1941X, located in the light chain, represent

abnormal intracellular trafficking, when stained with an antibody against the A2 domain of FVIII. After IFNγ stimulation, patient-derived vECs are able to express significant amounts of MHC-I. Low levels of MHC-II were only detectable on IFNγ stimulated HUVEC, but not on IPS derived vECs.

Conclusions: These HA-patient specific vECs enable us to track endogenous mutant FVIII and to study the MHC-I presented FVIII peptide repertoire for each patient in correlation to their risk for inhibitor development.

PB 1967 | Investigation of an F8 Exon 25 Deletion Suggests an Unusual Mutation Mechanism

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Background: Large deletions or insertions in the FVIII gene (*F8*) underlie severe haemophilia A in approximately 5% of patients. In the few deletion breakpoints characterised to date, a clear transition from 5' to 3' flanking DNA sequence is apparent, proposed to arise from repeat mediated non-allelic homologous recombination (NAHR) or non-homologous end joining (NHEJ).

Aims: We aimed to characterise the breakpoints within *F8* DNA from a boy with severe haemophilia A arising from a deletion of *F8* exon 25.

Methods: A standard primer walking strategy with DNA sequencing was used, followed by an NCBI ‚BLAST' search to aid sequence analysis. PCR was used to determine carrier status in the family.

Results: An approximately 5.5kb deletion was identified including exon 25. The breakpoint sequence included an insertion of approximately 300bp, derived from *F8* intron 22, with a 9bp sequence of unknown origin. The absence of repetitive elements and the presence of inserted sequence at the breakpoint preclude NAHR and NHEJ as mutation mechanisms. Microhomology-mediated break-induced replication (MMBIR) may underlie the observed deletion. The presence of low copy number repeat sequences in intron 22, which form the basis of the *F8* gene inversion causal of about 45% of severe haemophilia A, may predispose the formation of secondary structures such as cruciforms and stimulate MMBIR events. Identification of the breakpoint in the index patient enabled the development of a mutation-specific assay to confirm previous Multiplex ligation-dependent probe amplification (MLPA) based carrier diagnosis in family members.

Conclusions: The present investigation suggests an unusual mutation mechanism as the cause of severe haemophilia A in an index patient. It has enabled confirmation of carrier status in family members, and additionally provides insight into the distinct mechanisms responsible for copy number variation in the human genome.

PB 1968 | Stoichiometry of Immunodominant Factor VIII Immune Complexes

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Background: Inhibitory antibodies (inhibitors) to factor VIII (fVIII) represent the most significant complication in patients with congenital hemophilia A. FVIII also is the most frequently targeted coagulation factor in autoimmunity. Antibodies recognizing immunodominant epitopes in the fVIII A2 and C2 domains are present in most inhibitor patients. Anti-A2 monoclonal antibody (MAb) 4A4 and anti-C2 MAb 3D12 recognize human inhibitor epitopes. Additionally, 3D12 inhibits the binding of fVIII to von Willebrand factor (VWF). Thus, fVIII complexes formed by 4A4 and 3D12 may be representative of VWF-free immune complexes in human inhibitor plasmas.

Aims: The goal of this study was to determine the stoichiometry of fVIII immune complexes formed by MAbs 4A4 and 3D12.

Methods: Sedimentation velocity of immune complexes formed by varying ratios of 4A4 and 3D12 with a high-expression fVIII construct designated ET3 was conducted at 55,000g and 20 °C by measuring protein absorbance at 280 nm in a Beckman XL-I analytical ultracentrifuge. Sedimentation coefficient ($s_{20,w}$) distributions of fVIII, MAbs and immune complexes were determined using SEDFIT.

Results: The sedimentation coefficients of fVIII in the absence of MAbs and of the MAbs in the absence of fVIII were 7.7 S and 6.4 S, respectively. Singly-ligated, 10.3 S, and doubly ligated MAb, 11.9 S, complexes were identified under conditions of excess MAb and excess fVIII, respectively. A mixture containing equimolar fVIII and 4A4/3D12 MAb binding sites produced a dominant 14.0 S species and an estimated molecular weight of 610 kDa, consistent with a stoichiometry of two fVIII: one 4A4: one 3D12.

Conclusions: These results demonstrate that a biclonal anti-fVIII antibody population can form higher-order immune complexes. These complexes may be a driving factor in the immune response to fVIII by promoting B cell activation and/or antigen presentation. Additionally, these results indicate that analytical ultracentrifugation is a useful tool to characterize fVIII immune complexes.

PB 1969 | Analysis of VWF-FVIII Interaction in Natural pdFVIII/VWF Complex Compared with rFVIII+VWF Complex through a Novel Flow Cytometry Technique

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Background: The interaction between VWF and FVIII is crucial for FVIII function and clearance. It has been reported that the binding of VWF of hemophilic patients to the isolated FVIII of certain therapeutic

products may not be complete (Lin Y et al. 2004; Grancha S et al. 2012; Ofosu FA et al, 2012).

Aims: To compare, by a new flow cytometry technique, the natural pdFVIII/VWF complex and preformed rFVIII+VWF complex, regarding the resulting free-FVIII fraction.

Methods: rFVIII and pdVWF were incubated in a ratio 1:1 IU (100 ng FVIII : 10 mg VWF; i.e., conditions equivalent to those of the plasmatic natural pdFVIII/VWF) at 25°C for 15 min. Samples of the obtained rFVIII+VWF and natural pdFVIII/VWF were incubated (25°C; 2 h) with CBA-ESH4 (Cytometric Bead Array with anti-light chain of FVIII), followed by incubation (25°C; 2 h) with Dyn-antiVWF (DynaMag™-2 beads with anti-VWF). The supernatant was incubated (25°C; 2 h) with antiA2-FITC (anti-A2 FVIII marked with Fluorescein). The fluorescent signal of the pellet containing the CBA-ESH4 beads and the antiA2-FITC was measured by FACS Calibur flow cytometer. By using this model, FVIII-bound VWF can be detected through specific fluoresce signal. The assay was performed in duplicate and with 6 concentrations of FVIII (0.1-50 ng) maintaining the 1:1 ratio with VWF.

Results: The fluorescent signal from CBA-ESH4 beads bound to pdFVIII/VWF was 21.7% higher than that from rFVIII+VWF. Measurement of free-FVIII bound to antiA2-FITC indicated that rFVIII+VWF complex contained higher levels of free-FVIII (28.4%) than pdFVIII/VWF (8.7%), regardless the FVIII concentration assayed. Globally the results showed a 19.7% free FVIII in the FVIII+VWF complex, not present in the pdFVIII/VWF.

Conclusions: In agreement with previous reports, formation of the rFVIII+VWF complex resulted in a larger amount of VWF free rFVIII (≈20%) in the rFVIII+VWF complex than in the natural pdFVIII/VWF. The impact of this difference in FVIII products in vivo deserves future research.

PB 1970 | FVIII-vWF Complex Displays Greater Efficiency than Vwf-Free Preparations in Restoring Thrombin Generation in Hemophilic Plasma with Inhibitor under Conditions of Physiological Relevance

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Background: Development of factor (F)VIII inhibitor is a serious complication of hemophilia A (HA) treatment.

Aims: To compare the efficacy of three FVIII preparations (FVIIIP) in restoring thrombin generation (TG) in hemophilic plasma with inhibitor, under different experimental conditions.

Methods: FVIIIP were recombinant FVIII (Kogenate), plasma FVIII-vWF complex (Fanhdi) and vWF-free plasma FVIII (Beriate). TG was evaluated by calibrated automated thrombography; thrombin activatable fibrinolysis inhibitor (TAFI) activation by functional assay. Anti-FVIII IgG (FVIII-inh) were purified from a patient with high inhibitor titer. Plasma was obtained from patients with severe HA (FVIII< 1%).

Results: In plasma from a HA patient without inhibitor, the three FVIIIP (1 U/ml) displayed similar efficiency. On the contrary, upon addition of FVIII-inh (2.5 and 5 BU/ml), Fanhdi caused a stronger enhancement of TG and TAFI activation. Similar results were obtained when FVIIIP were incubated in FVIII-inh plasma for 2 h at 37°C or when plasma was supplemented with thrombomodulin (TM, 4 nM). In a model containing endothelial cells (EA-hy926, $625x10^3$/ml) and washed platelets ($250x10^6$/ml), Fanhdi enhanced TG more than Kogenate and Beriate both in the absence (peak increase: 1631, 695 and 1174%, respectively) and in the presence of FVIII-inh (541, 207 and 322%, respectively with 2.5 BU/ml). The effect of Fandhi and Kogenate on TG was also tested in plasma of 12 patients with HA, 6 with inhibitor (1-12 BU/ml) and 6 without. In samples with inhibitor, Fanhdi proved more efficient than Kogenate (both tested at half BU level) in enhancing thrombin generation, median (range) peak increase amounting to 355 (249-1876) and 185 (78-1028)%, respectively (P=0.03). The superiority of Fandhi was also evident in the presence of activated protein C (0.1 μg/mL).

Conclusions: FVIII-vWF complex shows greater hemostatic efficiency in HA plasma with inhibitor as compared to vWF-free preparations, especially under conditions of physiologic relevance.

PB 1971 | 40 kDa PEG of N9-GP is Eliminated from Tissues and Will Reach Steady State after Chronic Dosing

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Background: PEG in human tissues has not been measured during clinical development of N9-GP (40 kDa PEGylated rFIX). Data from a rat distribution study with N9-GP was used to estimate terminal elimination half-life ($t_{½}$) and time to steady state (SS) in toxicity studies as well as in clinical trials. To support the robustness of the N9-GP assessment, distribution data from other 40 kDa PEGylated compounds are included.

Aims: To estimate elimination $t_{½}$ and time to SS of PEG in plasma, kidney, liver and choroid plexus in rat and humans using single dose N9-GP distribution data from rat.

Methods: Tissue distribution studies were conducted in rats (n=1/time point) followed for up to 12 weeks post dose after a single i.v. dose of N9-GP[1] and other 40 kDa PEG compounds (same study design and tritium label in PEG). Terminal elimination $t_{½}$ were estimated by linear regression. Applying simple allometric scaling of $t_{½}$, time to SS of PEG was estimated in rat and man for N9-GP.

Results: Consistent decrease of PEG levels in rat tissues was shown for all compounds (Figure 1), with similar tissue elimination $t_{½}$ across

TABLE 1

Table 1 Estimated $t_{½}$ of PEG and time to SS after repeated N9-GP dosing

	Plasma	Kidney	Liver	Choroid plexus
Rat ($t_{½}$ days)	15	16	16	49
Human ($t_{½}$ days)*	59	63	63	192
Rat SS (weeks)**	7	8	8	23
Human SS (month)**	6	7	7	21

* Human $t_{½}$= rat $t_{½}$ x $(BW_{Human}/BW_{rat})^{0.25}$; BW - Body weight; BW_{Human}: 70 kg; BW_{rat} 0.3 kg[2]
** 3.3 x $t_{½}$ (90% steady-state, considered clinically relevant)[2]

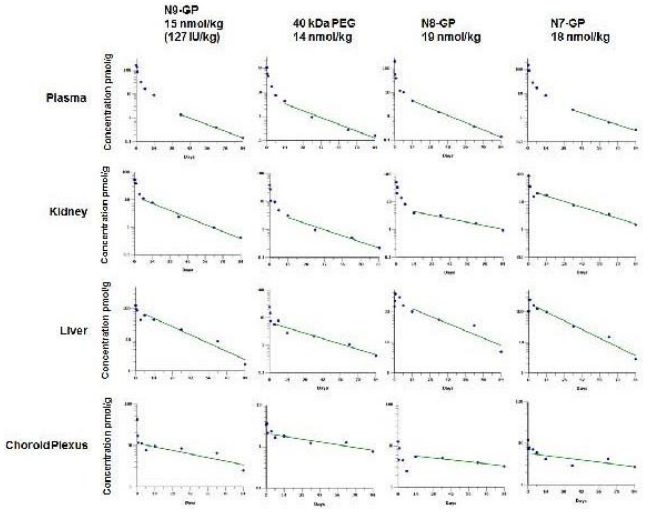

FIGURE 1

compounds. The predicted time to PEG SS in rat is within 7-23 weeks and in humans within 0.5-2 years after repeat N9-GP dosing (Table 1).

Conclusions: Animal distribution studies have shown that 40 kDa PEG is eliminated from tissues over time. For N9-GP, PEG SS is predicted to have been reached in 26-week rat chronic toxicity studies as well as in clinical trials, with no unexpected safety concerns.

Figure 1 Consistent decrease of PEG concentrations in tissues over time.

Blue dots observed data, green lines regression lines used to estimate $t_{½}$.

N8-GP and N7-GP: 40 kDa PEGylated rFVIII and rFVIIa

References:

1) Sternebring O et al. Eur J Pharm Sci. 2016;92:163-72

2) Rowland M, Tozer TN. Clinical Pharmacokinetics and Pharmacodynamics, Concept and Applications, 4th edition, 2011

PB 1972 | Spectrum of F8 Gene Mutations with FVIII Assay Discrepancies in an Irish Patient Cohort with Mild/Moderate Haemophilia A

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Background: FVIII assays can exhibit discrepancies in haemophilia A (HA) patients by FVIII one-stage (FVIII:C) and two-stage (FVIII:Chr) methods. Mutations within the *F8* gene have been associated with discrepancies but many remain undocumented. The coagulation and molecular laboratories at our centre work together to determine the legitimacy of assay discrepancies comparing FVIII assays ratios with known genetic data. However, using the standard ratio cut-off of 0.6 is too stringent, and data within and between families is not always as expected leaving.

Aims: We aim to analyse the FVIII:C, FVIII:Chr, and *F8* genetic data in patients with mild and moderate HA to determine patterns within our patient group.

Methods: Informed consent was obtained and data retrieved from electronic patient records and familial pedigrees. FVIII assays were performed using the HemosIL method and *F8* gene mutations using direct sequencing.

Results: FVIII:C and FVIII:Chr levels were retrieved for 110 patients from 63 families. 22 missense mutations and one splice site mutation (c-219C>T) were detected. The common p.Arg550Cys/His variant was found in 28 patients with mild HA from 9 families (mean ratio of 0.4). p.Met681Ile was found in four unrelated families, and previously not associated with assay discrepancies (mean ratio 0.2). This variant re-assigns the severity from mild to moderate with the lowest FVIII:Chr reduced to 0.01IU/ml. p.Arg1985Gln was found in both discrepant and borderline cases (ratio´s 0.58 vs. 0.72). Inverse discrepancy was detected in two unrelated patients with p.Tyr365His/Cys (ratio´s 0.41 and 0.57) and one with p.Arg1708His (ratio 0.38). Five families had ratio's that were borderline and differing within families (mean ratio 0.70).

Conclusions: The standard ratio cut-off of 0.60 is too stringent to elimiate the presence of a FVIII assay discrepancy. This data suggests that FVIII assay ratio's alone are insufficient and should be analysed alongside genetic data considering familial patterns.

PB 1973 | Identification of High-molecular Weight Species in Commercial Recombinant Factor VIII Products

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Background: The development of inhibitory antibodies to factor VIII (fVIII) is the most significant complication in management of patients with hemophilia A. Recent clinical studies have provided evidence that recombinant fVIII products are more immunogenic than plasma-derived fVIII products. Additionally, some clinical evidence suggests that Kogenate is the most immunogenic recombinant fVIII product. High molecular contaminants, aggregates, or viral-lke particles can increase the immunogenicity of biotherapeutics.

Aims: The goal of this study was to identify and quantitate all macromolecular species present in the recombinant fVIII products Kogenate, Helixate and Advate.

Methods: Vials of Kogenate, Helixate and Advate were reconstituted according to instructions provided by the manufacturers. The solutions were subjected to sedimentation velocity analysis at 105,000 *g* in a Beckman

XL-I analytical ultracentrifuge scanning at 280 nm. Sedimentation coefficient (s-value) distributions were calculated using SEDFIT.

Results: Kogenate and Helixate produced similar results, consistent with their identical manufacturing process. A broad peak at ~ 8 S was present in Kogenate representing 78% of the sedimenting species. Additional species were identified between 12 - 100 S, representing 22% of the product (Fig. 1).

Advate produced a major peak at $s_{20,w}$ 7.8 S representing 77% of the product. Additional species were identified at 5.2 and 11.5 S. There was a broad distribution between 12-20 S representing 7% of the product and negligible material > 20 S (Fig. 2).

Conclusions: Kogenate, Helixate and Advate contain large s-value, high molecular weight species that are significantly larger than the ~8 S species corresponding to full-length, monomeric fVIII. Species up to 100 S were identified Kogenate and Helixate. The amount of high molecular weight material was greater in Kogenate and Helixate than Advate. The high molecular weight material identified in these products may contribute to the immunogenicity of fVIII.

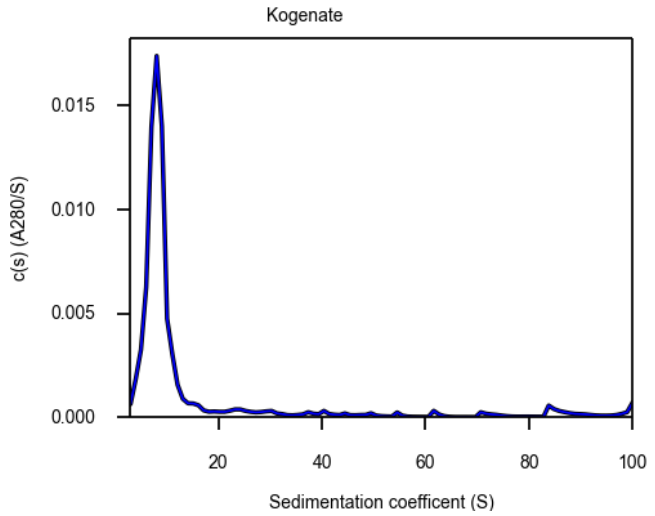

FIGURE 1 Sedimentation coefficient distribution of Kogenate

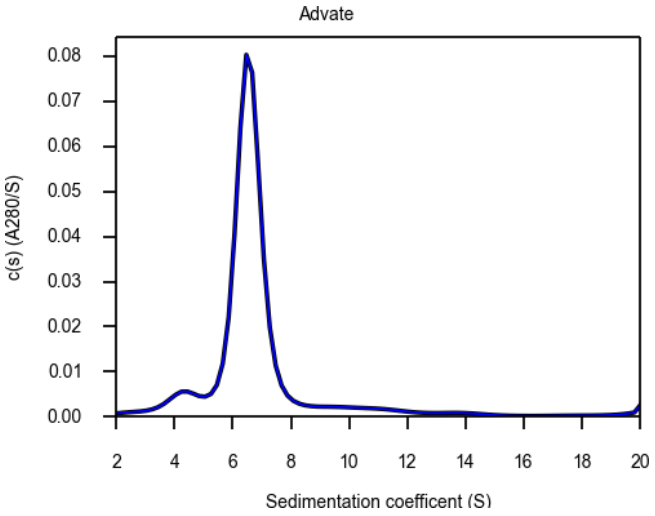

FIGURE 2 Sedimentation coefficient distribution of Advate

PB 1974 | Pharmacokinetics (PK) of N8-GP (Turoctocog Alfa Pegol) Dosed Subcutaneously (SC) to Non-human Primates (NHPs) and Prediction of Human PK Profile

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Background: Haemophilia A (HA) treatment requires intravenous (IV) factor VIII (FVIII) administration. N8-GP SC could eliminate the need for IV injections for routine prophylaxis and provide dosing regimens that achieve higher minimum plasma FVIII activities with smaller fluctuations than current IV FVIII therapy.

Aims: To evaluate N8-GP SC absorption in NHPs and develop a human PK prediction model of N8-GP dosed SC to patients with HA.

Methods: Six NHPs received daily doses of N8-GP, 100 IU/kg SC for 3 days, and 5 NHPs received 1 dose of N8-GP IV; 100 IU/kg (n=2) or 250 IU/kg (n=3). Blood samples, drawn over a 96-hour period after first dose, were analysed for FVIII activity with a glycoPEGylated FVIII-specific chromogenic assay. NHP IV and SC plasma FVIII activity-time data were simultaneously modelled using a 2-compartment non-linear mixed-effects PK model for estimation of absorption rate and SC bioavailability.

For human PK predictions following SC administration, a PK model was developed using a 1-compartment model, with population PK parameters of the clearance and volume of distribution from the pathfinder™1 trial in which patients with HA received N8-GP IV (data on file). N8-GP SC absorption PK in patients were assumed similar to NHPs, in line with data with recombinant (r)FVIIa (NovoSeven®), glycoPEGylated rFVIIa (data on file) and rFIX.[1]

Results: The estimated first-order absorption rate constant and SC bioavailability of N8-GP in NHPs were 0.35 h^{-1} and 24%, respectively. The human PK model predicted that daily SC doses of N8-GP 1.9-28 IU/kg would provide FVIII activity trough levels of 1-15%.

Conclusions: This NHP study is the first evaluation of N8-GP PK after SC administration. N8-GP bioavailability in NHPs was higher than that published for rFVIII.[2] The data indicate that human daily SC doses of 1.9-28 IU/kg should achieve FVIII activity trough levels of 1-15%.

Reference:

1. McCarthy, *et al.Thromb Haemost* 2002;87(5):824-830

2. Liu, *et al. Haemophilia* 2016;22(Suppl.4):17

PB 1975 | Molecular Characterisation of Variants on Non-coding RNA Genes in the X-inactivation Centre from Three Symptomatic Carriers with Severe Haemophilia A (HA) and Extremely Skewed X-chromosome Inactivation

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Background: Random X-chromosome inactivation (XCI) of one X-chromosome in females achieves dosage equivalency for X-linked genes with males. Non-coding RNA genes were described in the X-chromosome inactivation centre (XIC, Xq13) associated with the inactivation process: *XIST* (X-inactive specific transcript), *JPX* (just proximal to *XIST*) and *FTX* (five prime to *XIST*). We hypothesized that SNPs (single nucleotide polymorphism) may modify XIC gene expression or function impacting its allelic ability to inactivate.

Aims: Study the association between SNP variants on relevant sequences of *XIST*, *JPX* and *FTX* with extremely skewed XCI in three symptomatic carriers with severe HA.

Methods: An extensive genetic variant screening of *XIST*, *JPX* and *FTX* was performed in leukocyte-extracted DNA samples from three symptomatic carriers with severe HA and extremely skewed XCI (97-100%) and 11 controls with random XCI (50-55%) by CSGE (conformation sensitive gel electrophoresis) and Sanger sequencing. Symptomatic carriers were previously *F8* genotyped as heterozygous for severe HA mutations and the phenotypic expression resulted from their association *in cis* with the preferential X-inactive (Radic et al., JTH 2015,13:530-9).

Results: Analysis of the non-coding RNA genes on XIC from the three cases showed no deleterious mutations, but 12 SNP variants out of 161 annotated in public databases: 5/80 SNPs on *XIST*, 4/46 on *FTX* and 3/35 *JPX* in heterozygous status (Table 1). Despite this observation, these variants were also found in controls (Table 1).

Conclusions: This is the first study of *XIST*/*FTX*/*JPX* variants addressing their ability on XCI skewing performed thus far. The finding of SNPs in both groups (i.e., symptomatic carriers and controls) indicated no causal effect on XCI skewing. A suggestive absence of SNP heterocigosity in some DNA segments may indicate the involvement of large deletions that may hinder the ability to inactivate the X *in cis*.

PB 1976 | Clinical Trials and Registries in Rare Diseases: Opponents or Collaborators?

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Background: Hemophilia A (HA) is a rare disease with 1 in 5000 male births, which relates to ~250 newborns with severe HA in the EU annually. Due to the concurrent development of FVIII products the number of PUPs mandatory for CTs could be difficult to meet. Well-defined registries might be a potential source to improve knowledge on safety of FVIII products.

Aims: To analyze whether other data sources can complement to clinical knowledge especially on inhibitor development.

Methods: The CT database, established as part of the ABIRISK project and located at the Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, with 369 PUPs and minimally treated patients and the first cohort of the PedNet registry with 632 PUPs served as data sources. To analyze comparability of data collection systems, patient characteristics and parameters suspected for potential impact on inhibitor development as well as study design were investigated.

Results: Study design of already conducted CTs in PUPs are too various (e.g. study duration, study population, inhibitor testing) and patient numbers too limited to compare outcomes, whereas patient

TABLE 1 Molecular and biochemical characteristics of three Cases vs Controls (non-skewed X-chromosome inactivation)

	Case #15	Case #29	Case #31	Controls (n)
F8 genotype	Heterozygous c.4241C>A	Heterozygous c.4825dupA	Heterozygous c.325A>G	Carriers or Not
FVIII:C [IU/dl]	1.5	1.2	<1	-
XIP [%]1	100	97.3	100	50-55
XIST	rs41305409*G/C; rs6527*C/A; rs16992443*G/T; rs16992436*T/C; -	-; -; -; -; rs16992442*T/C	rs41305409*G/C; rs6527*C/A; rs16992443*G/T; rs16992436*T/C; -	(4); (2); (2); (1); (2)
FTX	rs174138*C/T; rs68124822*TT/-; rs146632102*A/T; -	-; -; -; rs187332478*A/G	rs174138*C/T; rs68124822*TT/-; rs146632102*A/T; -	(1); (1); (2); (5)
JPX	rs55824328*C/T; rs67649459*C/G;rs72278733*TAAAA/-	-;-;rs72278733*TAAAA/-	rs55824328*C/T; rs67649459*C/G;rs72278733*TAAAA/-	(2); (2); (7)

cohort and research parameters for all PedNet patients follow the same conditions. The comparison of country, race/ethnicity, genetic mutation, family history of hemophilia and inhibitor development shows that both sources collect valuable data with a quite similar distribution of risk factors and observed inhibitor incidences.

Conclusions: Available PUP-CT data does not allow for comparison of outcomes among different products. The FVIII-Guideline requires key parameters for PUP studies to harmonize research. There are currently 11 HA-PUP studies announced (clinicaltrials.gov), in need of 600 PUPs until 2024. Our results support the assumption that in a rare disease such as HA clinical trials and well conducted registries/cohort studies are potential collaborators, as all available information should be combined to contribute to the knowledge of clinical performance of HA products.

PB 1977 | In Haemophilic Patients, Individual Factor VIII or IX Level for the Correction of Thrombin Generation Is Predictable

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Background: The thrombin generation assay (TGA) assesses the individual clotting potential, which can differ greatly from one haemophilic patient to the other for the same level of factor VIII or IX. Normalization of thrombin generation (TG) is a valuable target for individualizing patient treatment. The factor VIII or IX levels to be reached in order to normalize TG are patient-specific.

Aims: To define in haemophilic patients the individual target levels of factor VIII or IX required to normalize their TG.

Methods: Plasmas from 16 haemophilic patients were spiked with increasing levels of the deficient coagulation factor and TG parameters were measured. The relationships between factor levels and TG parameters were determined by linear regression. The normal values were determined in 40 healthy volunteers.

Results: Despite inter-individual heterogeneity in basal TG and in responses to spiking, a true linear correlation, without any appearance of a plateau, was found between the measured fVIII or fIX levels and endogenous thrombin potential (ETP), peak (P) and velocity (VI) for each individual (median R^2 values: ETP: 0.96; P: 0.99; VI: 0.97). These relationships were characterized by straight lines. This means that two data points might be sufficient to predict individual patient response to antihaemophilic treatment. The slopes of these regression lines correspond to the rates of variation of TG parameters with increasing fVIII or fIX level. The slopes of ETP response to spiking in PPP from HB patients were much steeper than in PPP from HA patients ($p < 0.001$). Similar differences were observed for peak, and for velocity ($p < 0.001$). As a consequence, the target factor level required to normalize TG was significantly lower in PPP from HB patients than in PPP from HA patients ($p < 0.02$) .

Conclusions: Based on the individual response of patient plasmas to spiking, it is possible to define with only two points the target factor VIII or IX levels to be reached in order to normalize TG.

PB 1978 | Factor 8 Assay Discrepancy in a United States Non-severe Hemophilia A Cohort

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TABLE 1

Table 1. *F8* Mutations

		DISCREPANT F8 Phenotype		
cDNA	AA sequence	Present	Absent	Total
c.1660A>G	Ser554Gly	13	1	14
c.5096A>T	Tyr1699Phe	2	0	2
c.5822A>T	Asn194Ser	2	0	2
c.1948T>G	Leu631Val	2	0	2
c.1569G	Leu523Leu	1	0	1
c.1636C>T	Arg546Trp	1	0	1
c.1658C>T	Ser553Cys	1	0	1
c.1820G	Arg531	1	0	1
c.1834C>T	Arg612C	1	0	1
c.2043G>C	Met681Ile	1	0	1
c.219+3A>G	n/a	1	0	1
c.2320C>T	Arg696Trp	1	0	1
c.398A>G	Tyr133Cys	1	0	1
c.4339del	Val1447Serfs*18	0	1	1
c.5399G>A	Arg1800His	1	0	1
c.5423T>C	Leu1808Pro	1	0	1
c.5953C>G	Arg1985Gly	1	0	1
c.5954G>A	Arg1985Gln	1	0	1
c.6046C>T	Arg2016Trp	1	0	1
c.6089G>A	Ser2030Asn	0	1	1
c.6505C	Arg216	1	0	1
c.6506G>A	Arg2169His	0	1	1
c.6622C>G	Gln2208Glu	0	1	1
c.962A>T	Asp321Val	1	0	1
c.6370T	Tyr2124	1	0	1
Not available		11	4	15

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Background: Significant discrepancy (≥2-fold) between the 1-stage (1SF8) and chromogenic (CF8) assays has been described among patients with non-severe hemophilia A (NSHA).

Aims: To characterize F8 assay discrepancy in NSHA in a multi-ethnic US cohort.

Methods: In this retrospective cohort study, records of patients with NSHA at one hemophilia treatment center (HTC) were queried for 1SF8 and CF8 levels. Demographic data, *F8* genotype, and clinical history were retrieved. Data were analyzed using SPSS version 16.

Results: A total of 114 NSHA patients were identified; 56 (49.1%) had 1SF8 and CF8 performed on the same sample on ≥1 occasions. 47 of these 56 patients (85.7%) manifest discrepant test results; 46 had classical discrepancy while 1 subject had a reverse discrepant profile. Patients with a family history of hemophilia were more likely to demonstrate discrepant test results compared to those without such a history; BMI, ethnicity, HBV or HIV infection, presence of inhibitor, joint disease or therapy type were not significantly associated with discrepant results. Molecular findings demonstrated missense variants predominantly in the A2 domain, with other variants scattered throughout the A1, A3, C1 and C2 domains (Figure 1).

15 subjects had 1SF8 and CF8 assays done on >1 occasion, with 2 patients no longer discrepant and the single reverse discrepancy became a classical discrepancy. 5 subjects underwent a DDAVP trial with a tendency for the 1SF8:CF8 ratio to lessen, especially at 1H post infusion (Table 2).

Conclusions: While findings may be limited by selection bias, there is a high prevalence of F8 discrepancy among patients with NSHA in this US cohort. F8 mutations identified in patients with discrepant results appear similar to those reported previously. Repeat testing may lead to re-classification of patients as discrepant or non-discrepant. DDAVP tends to initially increase CF8 more than 1SF8 leading to a change in the magnitude of the assay discrepancy.

TABLE 2

Table 2. DDAVP Trial in 5 NSHA Patients

	1SF8:CF8 Ratios in Subjects on DDAVP				CV within each
Time	0 hr	1 hr	2hr	4hr	
Patient 1	3.33	2.00	NT	2.13	29.5%
Patient 2	2.00	1.69	NT	16.83	126.5%
Patient 3	4.00	2.43	NT	4.60	30.4%
Patient 4	2.60	1.75	1.61	1.24	31.9%
Patient 5	7.67	2.50	2.29	2.83	67.4%

Key: DDAVP= 1-deamino, 8-D-arginine vasopressin; NT= Not Tested

PB 1979 | Aberrant Inflammatory Cytokines, Skew T Helper Cells and Decreased Regulatory T Cell Numbers in Hemophilia A Patients with FVIII Inhibitor

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Background: Current therapy for hemophilia A (HA) is based on intravenous infusion of the FVIII. However, 20-30% of HA patients generate inhibitory antibodies against FVIII. The mechanism of FVIII inhibitor formation is complicated. Aberrant immunologic balance is considered important in the production of FVIII inhibitor.

Aims: The aim of this study was to explore the profile of IFN-γ/TNF-α,T helper (Th) cells and regulatory T cells (Treg) in HA patients.

Methods: Seventy one HA patients and 42 healthy controls (HC) were enrolled in this stuty. Serum IFN-γ as well as TNF-αconcentrations were measured by ELISA. The percentages of CD4+IFN-γ+ Th1, CD4+IL-4+ Th2, and CD4+CD25+Foxp3+ Tregs were analyzed by flow cytometry.

The immunosuppressive functions of CD4+CD25+ Tregs sorted by MACS were studied by mixed lymphocyte reaction.

Results: The serum IFN-γ and levels in HA patients with or without inhibitor (inhibitor+ or inhibitor-) were significantly higher than that of HC ($p< 0.01$), while IFN-γ in inhibitor+ group were significantly higher than that of inhibitor- group ($p< 0.05$). Both the inhibitor+ group and inhibitor- group had significant higher Th1/Th2 ratios than HC ($p< 0.01$). The Tregs in inhibitor+ group were significantly lower than that in the HC ($p< 0.01$) and inhibitor- group ($p< 0.01$), while Tregs in inhibitor- group were significantly higher than that in HC ($p< 0.01$). Sorted Tregs from inhibitor- or inhibitor+ patients with HA showed comparable immunosuppressive activity in vitro compared with HC ($p>0.05$).

Conclusions: The aberrant inflammatory cytokines and skew Th cells and decreased Treg numbers may play roles in FVIII inhibitor production in HA patients.

PB 1980 | Hemophilia A and the Role of Factor VIII in Balancing Pro- and Anticoagulant Processes

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Background: Hemophilia A is a genetic disorder resulting in the partial or complete dysfunction of FVIII. Hemophilia A patients are categorized into mild, moderate, or severe disease, and this severity is associated with the bleeding tendency. However, the optimal method to determine the threshold level of FVIII that is required to prevent severe bleeding is still unknown, and it is also unknown whether this threshold level differs between patients.

Aims: To study the dynamics of thrombin generation and coagulation factor levels in samples of mild, moderate, and severe hemophilia patients.

Methods: Healthy subjects (n=130) and hemophilia A patients (n=39) were enrolled in this study after informed consent was obtained. Thrombin generation (TG) was measured at 1 pM TF and plasma

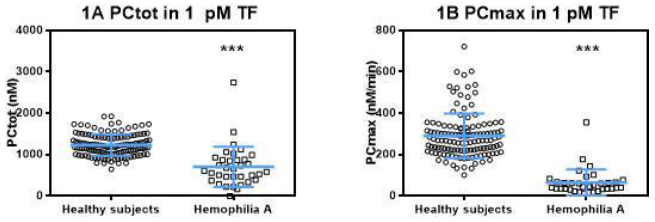

FIGURE 1 1A-B Total prothrombin conversion (PCtot), 1C-D maximum prothrombin conversion (PCmax) in hemophilia A patients (n=39) and healthy subjects (n=130)

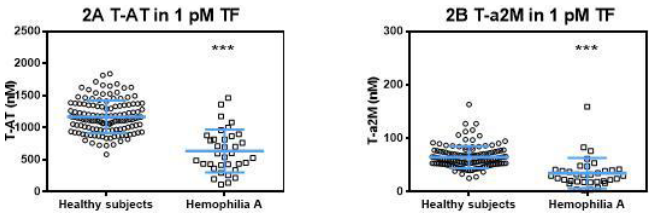

FIGURE 2 2A-B Thrombin-antithrombin (T-AT) complexes, 2C-D thrombin-α¬2M (T-a2M) complexes in hemophilia A patients (n=39) and healthy subjects (n=130)

prothrombin and FVIII levels were determined using one-stage coagulation assays. The dynamics of thrombin generation were studied computationally by quantifying the prothrombin conversion and thrombin inactivation.

Results: TG (ETP) was 55% lower in hemophilia patients at 1 pM TF compared to healthy subjects (p< 0.0001). No differences were observed in FII levels, but total FII conversion (PCtot) and maximum FII peak (PCmax) were significantly lower in hemophiliacs at 1 pM TF: PCtot and PCmax were reduced with 57% (p< 0.0001) and 22% (p< 0.0001), respectively (**figure 1**). The formation of thrombin-inhibitor complexes differed in hemophiliacs at 1 pM TF: thrombin-antithrombin complexes were 55% reduced (P< 0.0001) and thrombin-α_2-macroglobulin level was 54% reduced (p< 0.0001) (**figure 2**).

Conclusions: Even though FII levels are similar in hemophiliacs and healthy subjects, the prothrombin conversion is decreased in hemophilia patients, mainly due to an attenuation of the rate of prothrombin conversion. The reduced prothrombin conversion results in lower T-AT and T-α2M complex levels.

PB 1981 | Towards a Methylation Based Detection of F8 Rearrangements

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Background: Inter-chromosome homologous recombination between repetitive inverted identical repeats at Xp28 leads to the two inversion mutation hotspots (the known Intron1 and Intron 22 inversions) that cause severe hemophilia A. Several methods already exist to detect these inversions hotspot; this include Southern blot, RT-PCR, Long range-PCR and Inverse PCR. Each of these methods has it own limitations and advantages. However all are quite laborious and requires between 2 to 4 days time to be performed.

Aims: Heron we worked toward developing a faster method to detect any rearrangement in the F8 gene based on methylation epigenetic changes associated with position effects of DNA rearrangement.

Methods: We screened for methylation changes associated with F8 rearrangements (mainly intron 1 and intron 22 inversions) using enrichment of bisulfite treated DNA followed by massive parallel sequencing of DNA corresponding to the X chromosome. Additionally, we used pyrosequencing to screen for methylation changes at CpG islands and CpG rich region in the F8 gene regions (up to extra genic Intr1h and Intr22h repeats).

Results: The enrichment strategy together with the NGS detected differentially methylated region specific for each type of rearrangement; high discrimination power with both high sensitivity and high specificity were observed. Pyrosequencing data were also largely consisting with the enrichment-NGS data.

Conclusions: Methylation based detection of rearrangement within the F8 gene is a promising rapid and accurate method to detect the two inversion hotspots as well as other rare rearrangements in the F8 gene. An extensive multi-center validation of the method is still pending.

PB 1982 | The Combined Use of Bypassing Agents with Antithrombin Reduction in Plasma of Hemophilia A and B Patients with Inhibitors

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Background: An investigational RNAi therapeutic, fitusiran, targeting antithrombin (AT) to improve thrombin generation (TG) and promote hemostasis is in development in patients with hemophilia A (HA) and B (HB) with and without inhibitors. in vitro AT reduction has been shown to correct TG in plasma of patients with inhibitors.

Aims: As treatment with bypassing agents (BPA), rFVIIa and aPCC (FEIBA), is the standard therapy for patients with inhibitors, we aim to assess TG in plasma of patients with HA and HB with inhibitors in the presence of BPA and AT reduction.

Methods: Plasma samples from patients with severe HA and HB with high responding inhibitors were spiked with an anti-AT antibody to reduce AT levels by 80-90%. Plasma was spiked with rFVIIa (1.25 and 2. 5 µg/ml correlated to 45 and 90 µg/kg, respectively) or FEIBA (0.5 and 1U/ml correlated to 37.5 and 75 U/kg, respectively) either alone or in combination with AT reduction. TG was measured by calibrated automated TG assay using 1 pM tissue factor and 4 µM phospholipid.

Results: 11 plasma samples (8 HA; 3 HB) were investigated. Median baseline TG peak height was 16.1 nM (range 0.1-55.9) and 15.5 nM

(range 10.6-32.7) in plasma of patients with HA and HB, respectively, and 220.7 nM (range 136.4-326.6) in 24 healthy male volunteers. AT reduction improved TG peak in plasma of patients with HA and HB to 44.4 nM (range 9.7-83.5). Spiking of 90% AT-reduced plasma with 45 and 90 µg/kg rFVIIa induced an additive increase in TG peak to 76.6 nM (range 18.8-136.8) and 77.4 nM (range 25.0-161.5), respectively. Spiking of 90% AT-reduced plasmas with 37.5 and 75 u/kg FEIBA induced higher TG peak of 83.6 nM (20.7-151.4 nM) and 139.9 nM (range 40.4-228.8), respectively. No significant differences were observed between HA and HB patients.

Conclusions: Peak thrombin levels did not exceed the normal range in this study, suggesting that BPA may potentially be used safely in conjunction with AT reduction.

PB 1983 | Plasma Steady State PEG Concentrations Are Reached in Patients after Once Weekly Prophylactic Treatment with N9-GP

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Background: PEG concentration was not initially measured during clinical development of N9-GP (40 kDa PEGylated rFIX). Data from a rat distribution study with N9-GP (tritium radiolabelled in PEG)[1] have shown that PEG is eliminated from plasma and tissues. Using pharmacokinetic modelling and allometric scaling human plasma and tissue concentrations were predicted to reach steady state within 1-2 years. Here new data on human plasma PEG concentrations from ten paediatric patients from the paradigm™ 5 trial are presented.

Aims: To measure PEG concentrations in human plasma after > 2.5 years of once weekly N9-GP prophylactic treatment and to compare these with the predicted steady state levels.

Methods: Nuclear Magnetic Resonance (^{1}H-NMR) was used for quantitative determination of PEG levels (conjugated and/or free) in plasma. Plasma samples from 4-10 patients per time-point collected after ~2.5, ~3.5, and ~4.5 years were analysed. The patients were on once weekly prophylaxis with 40 IU/kg N9-GP (~230 µg/kg PEG).

Results: Human steady state PEG concentrations ranged from 3.3 - 7.3 µg/mL after up to 4.5 years of once weekly prophylactic treatment with 40 IU/kg N9-GP. This was within the range of 2 - 8 µg/mL predicted from the single dose rat distribution study.

Conclusions: The measured clinical PEG plasma concentration confirmed that steady state was reached. The measured PEG concentrations were within the predicted range from the single dose rat distribution study. As plasma PEG concentrations could be predicted from the rat, this indicates that human tissue concentrations may also be predicted from the rat distribution data.

PB 1984 | Thrombin Generation Assay as Additional Tool in Diagnosis of Mild/Moderate Haemophilia A Patients with Discrepancies in FVIII Activity Assays

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Background: Approximately 30% of mild/moderate hemophilia A (MHA) patients show discrepancies in factor VIII activity (FVIII:C) measured with one-stage clotting method (Os) *vs* chromogenic method (Chr). Current international guidelines do not recommend one specific type of assay to be used in the diagnosis of MHA.

Aims: To investigate whether thrombin generation assay (TGA) can provide additional information on the hemostatic potential of MHA patients.

Methods: The study group comprised 51 patients (aged 18-79; median 37) from 48 families with confirmed MHA and 21 healthy male controls (aged 21-63; median 35). FVIII:C-Os, FVIII:C-Chr and TGA were performed in all study subjects (MHA patients and controls). Assay discrepancy was defined as ratio of FVIII:C Os/Chr ≥2 or ≤0.5.

Results: Mean value of TGA peak thrombin (TGA-pt) was significantly lower in MHA patients than in healthy controls (43.06±14.79 *vs* 92.20±11.74, respectively). Discrepant FVIII:C results were reported for 25 (49%) patients. In 14/ 25 discrepant patients (54%) haemophilia severity (mild *vs* moderate) differed depending on the assay used. The mean value of TGA-pt (30.65±8.19; median 27.70) for those 14 patients was lower than in the whole MHA group (43.06±14.79; median 43.30).

Conclusions: The study shows a correlation between TGA-pt and factor VIII activity. Measurement with FVIII:C-Os or FVIII:C-Chr assay as well as TGA-pt allows to differentiate between mild hemophilia A patients and healthy individuals. TGA seems to be a useful laboratory tool in MHA diagnosis. Peak thrombin is a particularly useful parameter for confirmation or exclusion of mild hemophilia A, especially if FVIII:C oscillates around 50 IU/dl.

PB 1985 | Burden of Comorbidities in Persons Living with Haemophilia: Insight from the PROBE Phase 2b Study Database

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Background: Optimal care for haemophilia requires an integrated care approach (Pai, Haemophilia, 2016;22:6-16). Generating evidence on the burden of the comorbidities affecting persons living with haemophilia (PWH) will inform modulating the composition of the care team. To this scope we have analysed relevant data collected during the country-level test-retest reliability study of the PROBE questionnaire.
Aims: Investigate the burden of comorbid diseases in PWH as compared to controls.
Methods: PROBE is a 29 items questionnaire investigating patient reported outcome in haemophilia. Face and content validity and test-retest reliability has been previously assessed (Chai-Adisaksopha, HQLO, submitted), and the phase 2b study design and objectives described elsewhere (NCT02439710). With version 2 of the questionnaire we have introduced a standardized list to collect presence or absence of Hepatitis B, Hepatitis C, Stroke, High BP, Angina, Heart Attack, Heart Failure, Asthma, Liver Cancer, Other Cancers, Diabetes, Seizure, Arthritis, Gingivitis, HIV AIDS, Other Disease. Prevalence of each category in survey respondents with and without haemophilia, for severe HA, and for PWH with inhibitors was calculated; ORs were calculated stratifying by age.
Results: We collected 654 surveys in 6 countries. 392 were HA (61.2% severe), 69 HB (59.4% severe). The median age was 33 (IQR 24-56.5) for PWH and 34 (IQR 34-52) for controls. Statistics for the 4 more frequent comorbidities are reported in Table 1 and 2.
Conclusions: Compared to controls (individuals with no bleeding disorder), there is an increased prevalence of arthritis, gingivitis, hypertension and hepatitis C in PWH. Higher gingivitis rates highlight the importance of allied health professionals to supplement the core care team within haemophilia treatment centers. Analysis of PROBE data will allow an assessment of the association of oral health issues with other metrics e.g., upper extremity (elbow) joint heath. This is the first PROBE report of PWH with inhibitors.

PB 1986 | Cost-Effective Algorithm for Molecular Diagnosis of Families with Severe Haemophilia A in Argentina

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Background: Haemophilia A (HA), the commonest X-linked coagulopathy, is caused by defects in the factor VIII gene (*F8*). Due to *F8* size and complexity and mutational heterogeneity, gene testing in HA still represents a technical challenge. A half of severe HA (sHA) is caused by recurrent inversions disrupting *F8* at IVS22 (Inv22) and IVS1 (Inv1). The rest of sHAs are mostly caused by family-specific large or small del/ins and point mutations.
Aims: Present a cost-effective gene testing algorithm for families with sHA suitable for developing countries.
Methods: Leukocyte-extracted genomic DNA from patients are subjected to sequential *F8* genotyping protocols (Fig 1): Inv22/Inv1

TABLE 1 Burden of comorbidities in haemophilia (Arthritis and Gingivitis)

Comorbidity		Non haemophilia	All haemophilia	All haemophilia A	Severe haemophilia A	Haemophilia A with inhibitors
Arthritis	Prevalence (n/N)	21/157	125/365	109/309	86/224	3/14
	OR (Versus non-hemophilia)	Reference	5.41	6.40	6.81	3.72
	95% CI	--	3.11-9.40	3.57-11.47	3.72-12.46	0.83-16.66
Gingivitis	Prevalence (n/N)	16/161	111/382	92/326	65/236	9/16
	OR (Versus non-hemophilia)	Reference	3.85	3.82	3.38	10.59
	95% CI	--	2.16-6.87	2.10-6.95	1.83-6.28	3.18-35.21

TABLE 2 Burden of comorbidities in haemophilia (Hypertension & Hepatitis C)

Comorbidity		Non haemophilia	All haemophilia	All haemophilia A	Severe haemophilia A	Haemophilia A with inhibitors
Hypertension	Prevalence (n/N)	21/160	87/375	70/319	58/227	1/15
	OR (Versus non-hemophilia)	Reference	3.55	3.69	3.75	0.66
	95% CI	--	2.01-6.28	2.03-6.71	2.04-6.87	0.08-5.58
Hepatitis C	Prevalence (n/N)	0	98/378	79/322	64/231	3/15
	OR (Versus non-hemophilia)	Reference	54.25	50.97	59.40	38.75
	95% CI	--	7.49-392.80	6.94-365.91	8.14-433.37	3.74-401.52

diagnosis by inverse shifting PCR, ver.2016; PCR amplification targeting all relevant *F8* sequences in 38 products to detect large deletions in inversion negative cases (designing specific gap-PCR or qPCR approaches for carrier diagnosis); and conformation sensitive gel electrophoresis (CSGE) screening for small mutations on the *F8* amplimers in multiplex and characterisation of the anomalous CSGE product by Sanger sequencing. Genotype/Phenotype assignment of the observed variant is achieved by applying internationally accepted criteria.

Results: We characterised the causative mutation in 325 families with sHA, whereas 13 families remain uncharacterised (3.8%). We found 153 cases with the Inv22 (47.1%) (82% Inv22 type 1 and 18%, type 2), 4 Inv1 (1.2%), 18 large deletions (5.5%), 58 small ins/del (17.8%), 48 missense (14.8%), 32 nonsense (9.8%), and 12 splicing defects (3.7%).

Conclusions: The presented algorithm allowed characterisation of the sHA causative mutation in 96.2% of families in a relatively rapid and cost-effective way. The remnant 4% may be due to the intrinsic limitation of the CSGE screening (≈95%), the theoretical extent of the *F8* PCR amplification scheme to detect deep intronic splicing defects and other rare mutations, and the failure of gene dosage analyses to detect duplications.

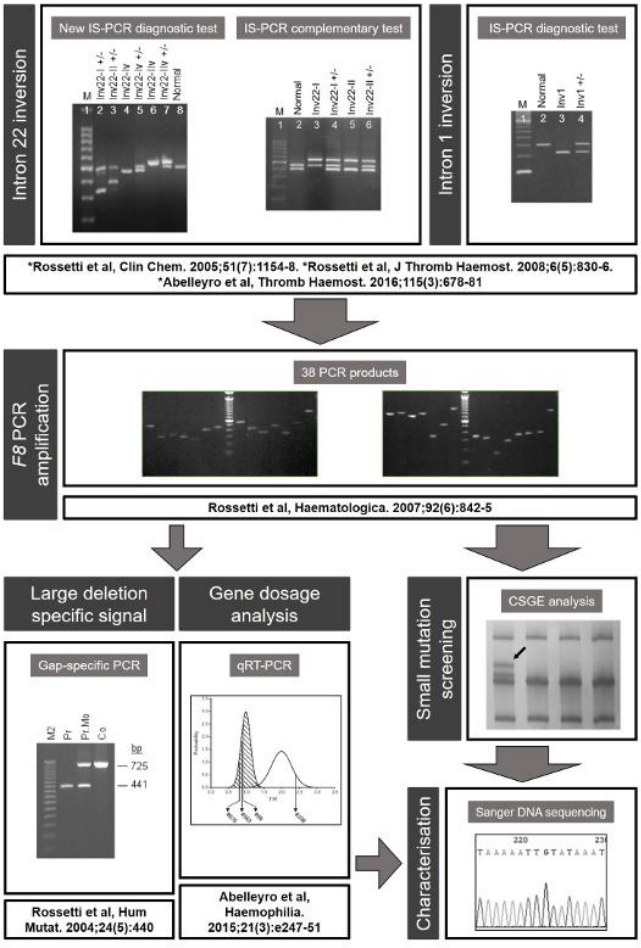

FIGURE 1 Laboratory algorithm for mutational analysis in F8

PB 1987 | Tissue Factor and Phospholipid-activated Thrombin Generation Assays for Detection of Factor VIIa Activity, Dose and Duration of Effect in Mice

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Background: Recombinant factor VIIa (rFVIIa) is used for treatment of hemophilia patients with inhibitors. Frequent infusions and high doses are often needed to achieve and sustain hemostasis in patients. Traditional laboratory assays have not been useful in guiding FVIIa therapy but the thrombin generation test (TGT) was proposed for this purpose.

Aims: We used mice to study the relationship between plasma levels of FVIIa activity and its pharmacodynamics as measured by the phospholipids (PL)- and tissue factor (TF)-activated TGT assays *ex vivo*.

Methods: Blood was collected from normal CD1 mice 5 minutes after treatment with 0.125 to 5 mg/kg (a dose escalation study) or 5 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr and 24 hr after administration of 2.5 mg/kg of FVIIa (a pharmacokinetics [PK] study). FVIIa activity was measured by two commercially available assays, a soluble TF (sTF)-based clotting StaClot (Stago) and an ELISA-based chromogenic assay (AssayPro). TGT was studied using four activation conditions: PL, PL and sTF, and human full length TF (FLTF) with and without PL.

Results: FVIIa activity correlated well with the PL-, PL/FLTF- and PL/sTF-TGT assays (Pearson's r>0.79) and poorly with the FLTF- TGT (r =0.32). The PL/sTF-TGT showed superior sensitivity to FVIIa activity in plasma (~ 1 nM FVIIa), FVIIa dose (0.125 mg/kg), and duration of FVIIa effect (8 hr). The PL- and PL/FLTF-TGT were sensitive to medium doses (1 mg/kg) and effect duration (4 hr). The FLTF-TGT showed poor sensitivity to FVIIa activity (100 nM), dose (1 mg/kg) and effect duration (2 hr).

Conclusions: PL but not FLTF are required for detection of TGT responses to FVIIa in mice. Because sTF reagent dramatically improves TGT assay's sensitivity to FVIIa, an sTF variant of TGT may be more suitable for the optimization of FVIIa dose and dosing intervals.

PB 1988 | Assessment of *F9* Mutation Associated with Inhibitor Development in Japanese Hemophilia B Patients

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Background: Hemophilia B (HB) is an X chromosome-linked hereditary bleeding disorder, which shows a quantitative or qualitative reduction of coagulation factor IX (FIX) due to genetic abnormality of FIX gene (*F9*). In case of HB, FIX replacement therapy is exclusive way to treat hemophiliacs. However, inhibitors against FIX replacement agents can neutralize the therapeutic effect, which is a serious problem in the treatment of HB.

Aims: Japan Hemophilia Inhibitor Study (J-HIS) has launched to investigate the inhibitor developing risk factor in HB. Here, we present a relationship between inhibitor development and *F9* genetic mutation in Japanese HB patients.

Methods: Genomic DNA was extracted from leukocytes peripheral blood. Mutation analysis was performed by direct DNA sequencing using *F9*-specific PCR primer sets. In cases of *F9* large deletions, we identified their breakpoints. The study was approved by the institutional committees for research ethics.

Results: Forty five HB patients including 10 inhibitor positive cases were registered in the J-HIS. We detected causative genetic defects in all HB cases tested, including 37 point mutations (20 missense,
7 nonsense, 8 splice-junction and 2 promoter region mutations) and 8 other genetic abnormalities
(2 insertions, 2 small deletions and 4 gross deletions). All patients carrying inhibitor were severe HB, in which detected *F9* mutations were 5 nonsense mutations, 4 deletions and 1 insertion, leading to frameshift or immature translation.

Conclusions: *F9* mutations of inhibitor positive cases in the J-HIS registered HB patients were all null mutations. This was consistent with the previous report that a severe phenotype of FIX deficiency was the most important factor to expect inhibitor developments. On the other hand, a few *F9* missense mutations have also been found to be responsible for the development of inhibitor. In order to strictly assess the risk factors for developing inhibitor against FIX among Japanese HB patients, further examination is necessary.

PB 1989 | Homogenous Reactivity of Recombinant FVIII against Inhibitors in the Presence of VWF Regardless Origin of Cell Line or Sequence Integrity

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Background: In the circulation, FVIII is included in a complex (FVIII/VWF), composed 98% in mass by VWF. In contrast to plasma-derived FVIII antihemophilic products with physiologically-bound VWF (pdFVIII/VWF), purified FVIII products are obtained from plasma (pdFVIII) or are prepared by recombinant DNA technology (rFVIII) using transfected cells from animal or human origin. It is known that VWF from the patient binds to infused VWF-lacking purified FVIII, although it is currently under debate if this bond is comparable to the way FVIII is protected in the natural FVIII/VWF complex.

Aims: To determine reactivity to inhibitors in samples of different commercially available brands of FVIII concentrates, previously allowed to bind VWF.

Methods: Commercial FVIII products, grouped according to VWF content and FVIII origin, were assessed: A to D: pdFVIII/VWF with VWF≥1 (normal plasma: VWF=1); E to G: purified FVIII with VWF=0–0.5 (pdFVIII); H to J: rFVIII produced in Chinese hamster ovary cells; K: rFVIII produced in human embryonic kidney 293F cells. pdVWF and FVIII (2 IU/mL each) were incubated (2 h, 37°C) and then serial dilutions of inhibitor IgG (commercial pool) were added in equal volume. Residual FVIII:C was determined using a chromogenic assay. The inhibitor titer (BU/mL) was determined, tested for FVIII group homogeneity and analyzed by ANOVA.

Results: Titers against products A-D (pdFVIII/VWF) were homogeneous and ranged from 9.1±1.0 BU/mL to 12.3±2.0 BU/mL, comparable to 12.0±0.06 BU/mL of normal plasma (product:plasma ratio: 0.8–1.0). Conversely, titers and product:plasma ratio observed in products E-K containing no or little VWF were significantly above the values of normal plasma (Table 1).

TABLE 1 Ratio product/plasma and inhibitor titer in concentrates of isolated FVIII after incubation with VWF

Purified FVIII origin	Product	BU/mL (range)	Ratio product/plasma(range)	P value vs plasma
Plasmatic	E, F, G	15.2±1.6 to 25.2±3.9	1.3 to 2.1	<0.05 to <0.0001
Recombinant - Animal cells	H, I, J	15.8±2.2 to 21.9±3.9	1.3 to 1.8	<0.01 to <0.0001
Recombinant - Human cells	K	21.2±1.4	1.8	<0.0001

Conclusions: Therapeutic products containing the natural FVIII/VWF complex showed lower reactivity to inhibitors than products of isolated FVIII incubated with isolated VWF, regardless the origin of FVIII: plasmatic or recombinant obtained from cells of animal or human origin.

PB 1990 | Validation of the Factor IX One-stage aPTT Clot Assay Calibrated with Pooled Normal Plasma for the Measurement of N9-GP in Patient Plasma

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Background: Nonacog beta pegol (N9-GP) is a 40-kDa, glycoPEGylated, recombinant, human coagulation factor IX (FIX) currently in clinical development.

Aims: To validate the FIX one-stage clot assay using HemosIL® SynthAFax activated partial thromboplastin time (aPTT) reagent on a Siemens BCS®XP analyzer for the quantitative measurement of FIX activity in patient plasma containing N9-GP using a pooled normal plasma calibrator.

Methods: The FIX one-stage clot assay was calibrated using pooled normal plasma [CRYOcheck™ Normal Reference Plasma (Precision BioLogic Inc.)] or N9-GP. High (0.1-1.5 IU/mL) and low (0.005-0.1 IU/mL) calibration curves were generated for each assay system. HEPES buffer containing 1% BSA, used as sample diluent on the N9-GP high calibration curve, was replaced with immuno-depleted FIX-deficient plasma to improve accuracy for N9-GP when using pooled normal plasma calibration. Results obtained for 100 N9-GP-treated patient samples when tested in the two assay systems (i.e., N9-GP and pooled normal plasma calibration) were compared.

Results: Acceptable accuracy, precision and linearity were demonstrated for N9-GP, across a quantitation range of 0.010-2.800 IU/mL, when using normal pooled plasma calibration. No significant difference in reported FIX activity was observed when using pooled normal plasma compared to product-specific N9-GP calibration.

Conclusions: The FIX one-stage clot assay with the SynthAFax aPTT reagent can be used for the quantitative measurement of FIX activity in N9-GP-containing patient plasma when calibrated with normal pooled plasma or N9-GP.

PB 1991 | Haemophilia A and B in Costa Rica - An Update of the National Register

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Background: Data of national register for haemophilia A(HA) and B(HB) patients in Costa Rica (CR), is of great importance for helping in planning the necessary amounts of treatment concentrates and improvement of treatment protocols and quality of patients' life.

Aims: Here we aim to present an update of epidemiological, genetic and clinical data for HA and HB patients in CR based on our national register.

Methods: The data were collected in 5 years period and recorded in a National register. The register included results for severity of the disease, type of mutation, inhibitor status and carrier status of females.

Results: 208 patients with HA and HB in CR are enrolled in the register. 178 are with HA and 30 with HB. From all HA patients 135 have the severe form, 18 moderate and 25 mild. The number of HA with mild form are underestimated most likely due to the rare bleedings of these patients and insufficient diagnosis. Genetic analyses were performed on 45 severe HA patients. 21(47%) patients were diagnosed with intron 22 inversion, 2 (4%) with missense, 7(15%) with nonsense, 7 (15%) with small del/ins and 7 (15%) with splice-site and 1 (1%) large deletion. The family genetic analyses reveal 37 carriers of HA. Most mutations in HB patients were missense. Only in 1 patient, a large deletion was identified. In 20 patients (44%), all with null-mutations, inhibitors were detected. Most patients are treated on demand, but in the last 2 years, the number of patients on prophylaxes slowly increased.

Conclusions: The mutation profile of HA and HB patients in CR do not differ from the general mutation profile for the disease. The correct diagnostic of carrier status of the females in hemophilia families is of extreme importance for our country, having in mind that some of the patients live in remoted regions of the country with a diminished access to medical care.

PB 1992 | Understanding Hemophilia A and B Factor Replacement Administration Patterns: A Feasibility Study

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Background: For both Hemophilia A and Hemophilia B, extended half-life factor replacement therapies (EHLs) require less frequent dosing; however, little is known of real-world administration patterns and cost for EHLs as compared with standard half-life therapies (SHLs). Large US commercial claims databases contain robust longitudinal transaction data, but sparse patient-level clinical data elements. Medical records contain the detailed clinical data, but lack complete medical resource use data and costs over time. Meanwhile, patients are the principal source of information regarding their behaviour, perception of outcomes, motivations and preferences to act, though this may be limited by recall.

Aims: To conduct a feasibility assessment to determine if a dataset of sufficient size and breadth could be constructed to compare the real-world administration patterns and resource utilization of SHLs vs EHLs for hemophilia by combining data from economic claims, medical charts and patient interviews.

Methods: After Central IRB approval, both physicians and patients/caregivers consented to participate in qualitative interviews to assess data availability. Physicians reviewed select patient charts for completeness. Patients were interviewed to review their inputs.

Results: A total of 23 patient/caregiver (15 Hemophilia A and 8 Hemophilia B) and 6 physician interviews were completed. 9 patients (40%) were able to obtain 12 months of claims details; all included pharmacy costs for factor replacement but some lacked specificity on diagnosis, or procedure codes for medical resources. All patients could complete the patient questionnaires. Physicians were able to locate and provide the relevant retrospective patient medical chart data.

Conclusions: This study demonstrates the feasibility of constructing a merged dataset of sufficient size for a robust analysis, which includes information from three distinct sources.

PB 1993 | Prevalence and Possible Risk Factors of Hyperhomocysteinemia in Patients with Severe Hemophilia from North-Western Russia

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Background: Severe hemophilia (SH) is often complicated by joint(s) destruction caused by chronic arthropathy due to recurrent hemorrhagic events and activation of such biological mechanisms as oxidative stress and inflammation. The frequency of increased homocysteine (HCy) plasma level, or hyperhomocysteinemia (HHCy), in patients with SH from North-Western Russia (NWR), it's possible reasons and role in development of arthropathy are not well known.

Aims: To assess the prevalence of HHCy and its possible risk factors in patients with SH from NWR.

Methods: We studied 22 men with severe hemophilia A or B (19 and 3 patients, respectively). Osteoarthritis of large joint(s) was detected in each patient, with the rate of recurrent hemorrhagic events in joint(s) from 6 to 13 per year. Standard ELISA assays to measure HCy plasma level and IgG to hepatitis C virus (HCV) were used. Polymorphism C677T in the methylenetetrahydrofolate reductase gene (MTHFR) was studied by PCR and subsequent restriction analysis. Fisher's exact test was used to assess statistical differences between the groups by calculating odds ratios (OR) with their 95% confidence intervals (CI) and p-value.

Results: Hyperhomocysteinemia (HCy level >13.4 µmol/l) was observed in 8 (36.4%) patients. This rate was more than 4-fold increased when compared to frequency of HHCy (8.8%) in healthy persons from NWR (OR=5.9, 95% CI: 2.2-15.5, p=0.0009). Seropositivity for HCV was detected in 75.0% hemophiliacs with HHCy compared to 57.1% in patients with normal HCy level (OR=2.3, 95% CI: 0.3-15.3, p=0.65). MTHFR 677T allele was present in genotype of 6 (75.0%) patients with HHCy and in 42.9% of other patients (OR=4.0, 95% CI: 0.6-27.3, p=0.2).

Conclusions: We have found high frequency of HHCy in patients with SH. Both genetic (MTHFR polymorphism) and acquired (HCV infection) factors could participate in development of HHCy in this group. Further studies are needed to confirm our data and identify the role of HHCy in development of arthropathy in SH patients.

PB 1994 | A Thrombomodulin Gene Variant Leading to Hyperthrombomodulinaemia and an Inherited Bleeding Disorder

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Background: Thrombomodulin (TM), expressed on endothelial cells interacts with thrombin driving activation of protein C and thrombin activatable fibrinolysis inhibitor (TAFI). TM variants are associated with thrombophilia, however familial cases with an autosomal dominant bleeding disorder due to a premature stop codon in *THBD* (p.Cys537stop) have been described.

Aims: We investigate a family with a novel *THBD* variant (*THBD: c.1487delC*, p.Pro496Argfs*10) identified in 6 family members with a bleeding phenotype.

Methods: The index case was originally diagnosed with an ‚unspecified bleeding disorder' and abnormal prothrombin consumption index - the molecular aetiology clarified by targeted high throughput sequencing (HTS) with the ThromboGenomics panel v2.8. Plasma TM levels were determined by ELISA and thrombin potential by calibrated automated thrombography. Lysis rates of plasma clots by tissue plasminogen activator were determined by turbidity assays and Hemacore ± carboxypeptidase inhibitor (CPI).

Results: Affected adults have a mild bleeding phenotype (ISTH BAT Scores 4-9) - traumatic haematomas, dental extraction and post-partum bleeds. Coagulation factors and platelet function were normal. Plasma TM levels were grossly elevated (median 556 ng/ml, range 364-698 ng/ml; normal 2.9-7.6 ng/ml) suggesting excess shedding of cellular TM leading to hyperthrombomodulinaemia. Endogenous thrombin potential was marginally reduced. Lysis of plasma clots was delayed compared to control plasma but was dramatically enhanced by inclusion of CPI, indicating that the change in lysis could be attributed to increased TAFIa.

Conclusions: This family represents only the second *THBD* variant to cause a modest bleeding phenotype which likely reflects a partial balancing of reduced thrombin procoagulant activity and increased protein C activation by the attenuated fibrinolytic activity. This finding underscores the utility of targeted HTS in diagnosis of bleeding disorders, permitting family screening and preemptive management strategies.

PB 1995 | Importance of the ADP-dependent Amplification Pathway in Platelet Activation is Influenced by CalDAG-GEFI Expression Level

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Background: Homozygous *RASGRP2* variants are associated with severe bleedings. We recently reported two new pedigrees with mutations in *RASGRP2* (p.N67Lfs*24 and p.E260*/C296R) resulting in the complete loss of the encoded protein, CalDAG-GEFI.

Aims: Characterize the platelet function defects in patients carrying homozygous and heterozygous *RASGRP2* variants.

Methods: Flow cytometry, platelet aggregation, adhesion under static and flow conditions and spreading; Rap1 GTPase activation assay: mice with variable CalDAG-GEFI expression.

Results: The mutations blunt CalDAG-GEFI protein expression in transfected GripTite™ 293 MSR cells and patient platelets. Activation of Rap1 and $\alpha_{IIb}\beta_3$ integrin was markedly impaired in patient platelets stimulated with low and intermediate doses of ADP and TRAP-6. In homozygous carriers, maximal aggregation in response to ADP is markedly reduced and slower aggregation velocities are observed upon stimulation with any doses of agonists. Platelets from heterozygous patients or mice expressing low levels of CalDAG-GEFI also showed reduced aggregation to low doses agonists, especially when activated in the presence of a P2Y12 inhibitor. Interestingly, platelets from homozygous and heterozygous patients were unable to form lamellipodia and spread upon stimulation with high dose TRAP-6, while the aggregation response was normal under these conditions.

Conclusions: Partial CalDAG-GEFI deficiency impacts both platelet aggregation and spreading. Our results indicate that the importance of the ADP-dependent amplification pathway is related to CalDAG-GEFI protein expression level.

PB 1996 | Association of Homozygous PAI-1 Deficiency with Cardiac Fibrosis in Humans

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Background: Complete PAI-1 deficiency is a very rare autosomal recessive bleeding disorder. We have followed for 24 years a family in the old order Amish community with a rare loss-of-function mutation in SERPINE1 encoding PAI-1. Apart from hemostatic changes, murine models of PAI-1 deficiency develop age-dependent cardiac fibrosis.

Aims: To evaluate the cardiovascular status of homozygous PAI-1 deficient patients.

Methods: Study participants included 9 homozygous, 3 heterozygotes and 6 unaffected individuals who underwent cardiac magnetic resonance imaging (MRI). A 32-year-old male with complete PAI-1 deficiency also underwent transthoracic echocardiogram (TEE) at baseline and at follow up.

Results: Six of the 9 homozygotes had cardiac fibrosis ranging from 1-19% (Table[SG1] 1) in contrast to none of the controls or heterozygotes. Only one homozygote (32 year male followed since 12 years of age) had a low ejection fraction (EF). The patient has a moderate bleeding phenotype with trauma-related hematomas, testicular hematoma post hernia repair and post-injury hemarthroses, treated intermittently with oral antifibrinolytics. TEE revealed moderate left ventricular enlargement with global hypokinesis, EF 35%; cardiac MRI revealed significant cardiac fibrosis (19%) in several areas (Figure[SG2] 1). The patient was started on medical therapy-beta- blocker, ACE inhibitor; latter was discontinued after 5 months by patient. Repeat TEE 1 year post-diagnosis revealed stable EF (35%).

TABLE 1 Age, sex and degree of cardiac fibrosis in 9 homozygous PAI-1 deficient patients

Age in yrs at present	Age at diagnosis	Sex	Cardiac fibrosis %
15	2 months	male	13
20	5 months	male	8
22	2 years	female	8
24	10 years	female	1
25	4 years	female	0
31	10 years	female	0
32	12 years	male	19
34	13 years	female	14
35	11 years	female	0

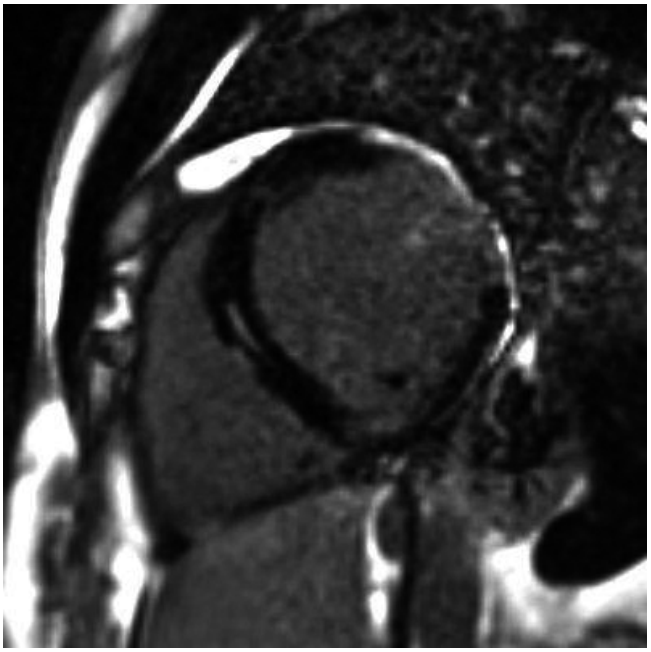

FIGURE 1 Cardiac fibrosis in a homozygous PAI-1 deficient male

Conclusions: Complete PAI-1 deficiency is associated with cardiac fibrosis in humans. We describe a case of fibrotic dilated cardiomyopathy in a patient with complete PAI-1 deficiency, evidence of cardiac abnormalities in the remaining 5 patients evaluated, emphasizing the need for diligent cardiac evaluation in this rare bleeding disorder.

PB 1997 | Molecular, Diagnostic and Clinical Features of 216 Patients from 46 Unrelated Families with FXI Deficiency Caused by Different Recurrent and Sporadic Mutations. Data from A 60000 Inhabitants Spanish Town

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Background: FXI is a key procoagulant protease whose deficiency, rare in Caucasians, has minor relevance in bleeding but protects against thrombosis according to animal models.

Aims: To characterize molecular and clinically the cases with FXI deficiency identified in a 60000 inhabitant Spanish town.

Methods: During 20 years, 32,4764 aPTTs from 51,366 patients were analyzed. 1,700 samples with prolonged aPTT (>1.3) were identified. Anticoagulated patients, with a not confirmed prolonged aPTT or with lupus anticoagulant were ruled out. Levels of coagulation factors were quantified by coagulometric assays.

Genetic analysis of *F11* (whole gene sequencing, MLPA and genotyping), biochemical and functional characterization of plasma FXI were performed in 311 members of 46 families and in 150 healthy subjects from the same town.

Results: We identified 46 unrelated cases with FXI deficiency carrying 4 recurrent mutations: p.Cys56Arg, which was present in 2% of the general population, p.Cys416Tyr, c.1693G>A, and p.Pro538Leu. 8 additional mutations (3 new and the first world-wide duplication causing FXI deficiency, Fig1) were also found.

Family studies revealed a total of 216 subjects with FXI deficiency.

aPTT was not prolonged in 31.5% of cases with FXI deficiency.

Bleeding was rare and mild. Interestingly, 9 patients were correctly anticoagulated with acenoucoumarol during 382 months, reporting no bleeding events.

Moreover, 6 cases developed acute myocardial infarction and 8 ischemic cerebrovascular events. Only 2 patients, both with local risk factors, developed venous thrombosis, even though 19 patients also carried prothrombotic SNPs, FV Leiden or prothrombin G20210A.

Conclusions: Our study supports that FXI deficiency, caused by recurrent or odd mutations, (i) might be underestimated in Caucasians; (ii) could protect against venous thrombosis, even if FXI deficiency is moderate; and (iii) causes minor risk of bleeding, also in patients under anticoagulant therapy.

Funding: PI15/00079; CB15/00055 (ISCIII); 19873/GERM/15.

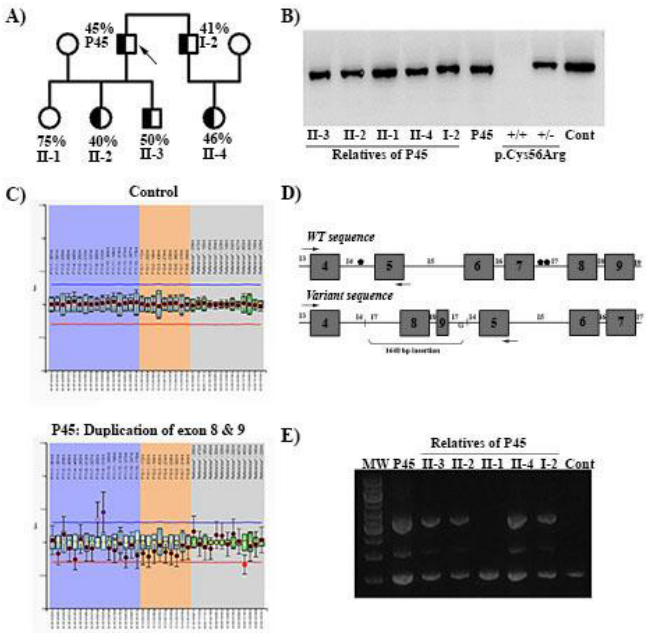

FIGURE 1 Identification of the insertion in P45. A)Family tree B)Plasma FXI in P45 family C)MLPA of F11 and F10 D)Sequence scheme E)PCR of the insertion

PB 1998 | The International Society on Thrombosis and Haemostasis - Bleeding Assessment Tool (ISTH-BAT) and the Risk of Future Bleeding

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Background: The International Society on Thrombosis and Haemostasis - Bleeding Assessment Tool (ISTH-BAT) is a validated diagnostic tool that is used in clinical practice in subjects with suspected inherited bleeding disorders.

Aims: To evaluate whether the ISTH-BAT, recorded in patients at first work-up in a tertiary care center, can predict their risk of subsequent bleeding events.

Methods: Observational cohort study that included all consecutive patients referred to Angelo Bianchi Bonomi Hemophilia and Thrombosis Center between 2011 and 2015 because of a suspected bleeding disorder of any sex, age and origin, and able to understand and speak Italian. Only those with ISTH-BAT≥3 were included. The incidence rates (IR) of major (MB) and clinically relevant non major bleeding (CRNMB) events were calculated as the number of events over accrued person-years (py). Main analysis was Cox regression analysis, assessing ISTH-BAT≤ 5 vs >5, and various co-variates, such as sex, age and presence or absence of a final diagnosis.

Results: We recruited 136 patients with a median ISTH-BAT of 4 (range 3-18). Eleven patients (8.1%) had a bleeding event during the follow-up period (one MB, 10 CRNMB). The overall incidence rate of bleeding events was 3.7 per 100 py (95% CI: 1.8-6.6). No difference was observed between patients with a ISTH-BAT ≤5 or >5 (HR 1.2, 95%CI: 0.3-4.6). Similar results were found when ISTH-BAT was considered as a continuous variable (HR 1.1, 95%CI: 0.9-1.4). The incidence rate of subsequent bleeding events was higher in patients with a diagnosis of a hemostatic defect than in those without (IR 7.5 vs 1.9 per 100py, HR 3.0, 95%CI: 0.8-11.8).

Conclusions: The ISTH-BAT does not predict future bleeding events.

PB 1999 | Efficacy and Safety of a New Fibrinogen Concentrate for the Treatment of Bleeding Episodes and Prevention of Excessive Bleeding during Surgery in Children with Congenital Fibrinogen Deficiency

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Background: A triply secured fibrinogen concentrate (LFB FGTW) was demonstrated to be efficient and well tolerated in adults with afibrinogenaemia. Limited data are available regarding treatment in children.

Aims: To investigate the efficacy and safety of replacement therapy in children with fibrinogen deficiency.

Methods: This was an international, open-label study, approved by Ethics Committees. All subject's parents signed an informed consent. Recovery-guided dosing was performed at study entry to obtain optimal plasma fibrinogen concentrations. Target levels were 1.2 g/L for major and 1.0 g/L for minor bleedings/surgeries, as for adults.

Results: Sixteen subjects ranging from 1 to 12 years (8 children ≤6 years) were enrolled. Recovery as measured in 12 subjects ranged from 14.0 to 30.7 g/L per g/kg (geometric mean: 19.1). Eleven children were treated on-demand with a median infusion dose of 0.090 g/kg [range 0.028-0.132] for 17 major and 0.070 g/kg [range 0.039-0.088] for 15 minor bleeding episodes. Physicians' global assessment of response to FGTW treatment (i.e. primary efficacy endpoint) was excellent or good for 97% of episodes. Ten children underwent 11 surgical procedures: 6 circumcisions including 1 orchidopexy were in ≤6 years group (median preoperative dose 0.092 g/kg) and other procedures, all dental extractions were in >6 years group (0.056 g/kg). Haemostasis was rated as excellent in all procedures. Most of the surgical or bleeding events (91%, 39/43) were managed with a single dose of FGTW. In response to the first FGTW administration, the median fibrinogen level was 1.37 g/L for the control of bleeding and 0.97 g/L before surgery. No serious adverse reactions were observed over a large survey period (> 1 year in 12 subjects).

Conclusions: These results provide clinical evidence for the excellent efficacy and safety of FGTW when used in paediatric subjects ≤12 years.

PB 2000 | In Vitro Study of a New Plasma-derived Factor V (FV) Concentrate: Dose-response Evaluation in Plasma from Patients with Congenital FV Deficiency

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Background: Congenital FV deficiency is a rare autosomal recessive bleeding disorder. Until now fresh frozen plasma has been considered the standard treatment, associated with possible hypervolaemia and other side effects. Recently, a new plasma-derived FV concentrate has been developed and is under evaluation by *in vitro* studies.

Aims: To assess the ability of the drug to restore the hemostatic levels of FV and the normal clotting times and generate safe amounts of thrombin in plasma from patients with congenital FV deficiency.

Methods: PT (prothrombin time), aPTT (activated partial thromboplastin time), FV activity and antigen levels and thrombin generation (TG) (triggered with 5 pM tissue factor) were measured in FV-deficient individuals (n = 10) pre and post *in vitro* spiking of plasma samples with

TABLE 1 Effect of FV concentrate on PT, aPTT, FV activity (FV:C) and FV antigen (FV:Ag) levels in plasma from patients with FV deficiency

Drug concentration (IU/mL)	PT ratio reference range (0.84-1.22)	aPTT ratio reference range (0.86-1.20)	FV:C IU/dL reference range (70-134)	FV:Ag IU/dL reference range (70-130)
Baseline	3.25 [2.67-3.49]	3.28 [2.54-4.11]	0.55 [0.43-0.95]	2.00 [0.92-2.15]
0.03	1.88 [1.81-1.95]	1.73 [1.65-1.92]	3.70 [3.58-4.00]	3.9 [3.49-5.09]
0.10	1.52 [1.48-1.53]	1.35 [1.31-1.51]	11.00 [10.3-11.43]	10.09 [8.33-11.36]
0.25	1.23 [1.21-1.24]	1.16 [1.12-1.28]	26.55 [25.88-27.28]	25.46 [24.60-26.93]
0.50	1.14 [1.13-1.16]	1.04 [0.99-1.13]	46.40 [45.85-47.05]	51.75 [50.20-53.08]
1.00	1.05 [1.04-1.07]	0.95 [0.93-1.04]	88.10 [84.63-90.10]	85.86 [79.85-86.52]

TABLE 2 Effect of FV concentrate on thrombin generation parameters in plasma from patients with FV deficiency

Drug concentration (IU/mL)	Lag time (min)	ttpeak (min)	Peak height (nM)	ETP (nM*min)
Baseline	9.00 [6.58-17.33]	15.67 [9.92-35.25]	51.28 [20.55-153.60]	479.30 [0.00-671.60]
0.03	4.00 [3.33-4.33]	5.33 [4.92-6.08]	238.80 [212.30-246.60]	749.00 [652.30-846.80]
0.05	3.17 [2.67-3.75]	4.67 [4.25-5.17]	249.80 [223.00-264.70]	753.40 [685.0-844.3]
0.10	2.50 [2.25-2.67]	4.00 [3.67-4.33]	234.20 [214.10-256.30]	780.70 [732.50-875.00]
0.25	1.67 [1.67-2.17]	3.3 3 [3.00-3.50]	231.50 [197.40-253.80]	742.80 [613.90-770.90]
0.50	1.67 [1.33-1.67]	3.00 [2.92-3.08]	213.20 [178.40-239.10]	678.30 [567.50-704.40]
1.00	1.33 [1.25-1.42]	3.00 [2.92-3.08]	201.70 [172.70-226.50]	701.00 [576.00-751.00]
Control group	1.67 [1.60-1.83]	3.34 [3.20-3.61]	225.80 [211.20-265.00]	748.30 [681.90-912.50]

increasing doses of the drug (0.03-1 IU/mL final concentration). TG was also assessed in a control group (n = 50). Data were evaluated by Wilcoxon test or Kruskal-Wallis test. P value < 0.05 was considered significant.

Results: Tables 1 and 2 report, respectively, coagulation and TG parameters measured with increasing drug concentration. Data are expressed as median and IQR. Normal values of PT, aPTT, lag time and ttpeak have been achieved by 0.25 IU/mL of FV concentrate. FV activity and antigen levels fell within the normal range following the addition of 1 IU/mL of FV concentrate to the plasma samples. A very low dose of FV concentrate (0.03 IU/mL) was enough to normalize peak height and ETP (endogenous thrombin potential).

Conclusions: Most of the parameters analyzed take on normal values using a drug concentration of 0.25 IU/mL. Therefore, the new FV concentrate is able to correct *in vitro* the coagulopathy associated with FV deficiency. Our study demonstrates that the new FV concentrate could potentially offer a major advance for the treatment of patients with FV deficiency.

PB 2001 | Multi-platform Characterization and Application of a Bleeding Assessment Tool in a Cohort of Patients with Factor XIII Deficiency

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Background: Factor XIII (FXIII) is involved in the terminal phase of the clotting cascade resulting in the formation of a stable hemostatic plug. Urea clot solubility test is the standard screening test and confirmation is by quantitative immunoassay for antigen and chromogenic tests for activity.

Aims:

- To characterize patients with inherited FXIII deficiency using all the above diagnostic methods.
- To determine if the ISTH/ SSC Bleeding Assessment Tool (BAT) can be used as a reliable screening tool in these patients.

Methods: The records of 44 patients with FXIII deficiency were reviewed from January 2011 to July 2015. Rotational thromboelastometry (ROTEM) (TEM, Munich, Germany), FXIII antigen assay (LIA, Milan, Italy), urea clot lysis (in-house) and FXIII activity (Berichrom, Dade Behring, Germany) were performed for screening and confirmation. Bleeding scores (ISTH BAT) were obtained from clinical records and were verified by patients.

Results: FXIII deficiency accounted for 1.36% (44/3219) of the total number of patients referred to our laboratory. The frequency of bleeding manifestations is shown (Table 1). The median FXIII antigen level was 0.8 U/dL (IQR: 0 - 2.1 U/dL) and the median FXIII activity level was 6.15% (IQR: 0-8.75%). Since FXIII activity had a lower limit of detection of 10%, it did not correlate with FXIII antigen, BAT and ROTEM. FXIII antigen correlated significantly with lysis on ROTEM (Likelihood ratio: 16.11, Pearson's chi square: 15.04) (Table 2). FXIII antigen with bleeding score (BS significant when ≥ 3 for children, ≥ 4 for males, ≥ 6 for females) also showed a positive correlation. 10/37 patients had maximum lysis of ≤15% even though their bleeding score was ≥ 4 (Sensitivity 72.2%). We found that a cut off of 5% for maximum lysis increased the sensitivity for detecting FXIII deficiency to 94.4%.

Conclusions: FXIII antigen level correlated with BAT and lysis on ROTEM. A cut-off of 5% for maximum lysis (on ROTEM) increased the sensitivity for detecting FXIII deficiency to more than 90%.

TABLE 1 Distribution of bleeding manifestations

SYMPTOMS	NUMBER	PERCENTAGE
Umbilical stump bleed	36/44	82%
Easy bruisability	25/44	57%
Prolonged bleeding following trivial trauma	12/44	27%
Subcutaneous hematomas	6/44	14%
Intracranial bleed	6/44	14%

TABLE 2 Correlation of FXIII antigen with lysis on ROTEM (FXIII antigen divided into 3 groups <= 1,1-5 and >5)

FXIII antigen (U/dl)	Lysis on ROTEM ≤ 15%	Lysis on ROTEM > 15%	Total
≤1	4	12	16
1-5	0	14	14
> 5	3	0	3

PB 2002 | New Bleeding Disorder with Enhanced Fibrinolysis due to Lysinuric Protein Intolerance and Renal Failure

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Background: Lysinuric protein intolerance (LPI), is a rare autosomal recessive transport disorder of the cationic amino acids lysine, arginine, and ornithine affecting intestine, lungs and renal tubules. LPI patients have a poorly characterized bleeding tendency, albeit some reports on low platelet counts and fibrinogen, while also enhanced thrombin and fibrin formation have been reported.

Aims: Our aim was to further depict hemostasis of these patients.

Methods: 15 adult LPI patients (8 female) were enrolled. Bleeding tendency was evaluated with the ISTH/SSC-BAT questionnaire. Routine metabolic and bleeding assays, PFA-100^{TM}, rotational thromboelastometry (ROTEM) and Calibrated Automated Thrombogram (CAT) were performed, data are reported mainly as median and range.

Results: In ISTH/SSC-BAT median bleeding score was 4 (-1-12). 5 patients had mild anemia (123 g/L; 106-147) and 6 presented with mild thrombocytopenia (median 154 E9/L; 95-309). PT and APTT were normal. In 11 patients PFA-100^{TM} adenosine-5-diphophosphate and epinephrine closure times were both prolonged. Functional fibrinogen was 2.5 g/L (1.0-4.3), in 4 patients at < 2.0 g/L. FXIII:C was low, 58 IU/dL (27-141). D-dimer was extremely elevated in all patients, 32 mg/L (13-109). 5 patients carried at least 4 abnormal ROTEM parameters and endogenous thrombin generation (ETP) was lower than in healthy individuals ($p< 0.01$). Interestingly, creatinine correlated negatively with fibrinogen and positively with D-dimer. D-dimer showed strong negative correlation also with base excess and bicarbonate.

Conclusions: LPI is associated with a variable bleeding tendency, characterized by abnormalities in platelet function, thrombin generation and fibrinolysis. Enhanced fibrinolysis associated with metabolic and renal disorder.

PB 2003 | Mutation Profile of Factor X Deficiency

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Background: FX deficiency is one of the rarest coagulation disorders with a great heterogeneity and complexity of the *F10* defects.

Aims: We aimed to evaluate the mutation profile of patients with FX deficiency.

Methods: The coding regions and intron/ exon boundaries of the *F10* gene have been sequenced in all patients. MLPA was used for detection of large deletions/duplications.

Results: The study includes 208 patients with FX deficiency sent for genetic investigation. In 74% (153) of cases disease causative mutation was detected. Most identified mutations were missense (85%), localized to the Gla (exon 2) and the catalytic domains (exons 7 and 8) of FX protein. The mutation Glu142Lys was identified to be with very high recurrence (10%). All other types of defects were distributed as following: nonsense 3%; small del/ins 4%, splice-site 2%; large deletion 6%. In 23 cases the genetic alterations were either compound or homozygous, leading in most cases to severe FX deficiency. In 10 cases combined FVII and FX deficiency was diagnosed, where in 4 cases a large deletion of 13q34 was the cause for the deficiency. The mean FX:C in heterozygous cases was 48%, while in compound or homozygous cases mean FX:C dropped to 4% corresponding to severe type of deficiency. Interestingly, the mutation Glu142Lys, although homozygous, showed moderate reduction in FX:C up to 45%.This genetic alteration, together with other *F10* mutations in compound heterozygous state, sowed FX:C reduction of 20 to 9%, indicating that marked effect of the other genetic defect and a minor effect of Glu142Lys substitution on FX:C.

Conclusions: The molecular genetic profile of FX deficiency is very heterogenic. A good correlation between the clinical phenotype, laboratory findings and genotype would bring more light in the mechanisms of the FX deficiency and would help in better evaluation of the clinical symptoms.

PB 2004 | Retrospective Evaluation of Phenotype and Management of Dysfibrinogenemia and Hypodysfibrinogenemia in a Cohort of Italian Patients

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Background: Dysfibrinogenemia (DF) and Hypodysfibrinogenemia (HDF) patients (pts) may experience hemorrhages or thromboses, and the clinical management can be difficult.

Aims: Aim of this study is to obtain information on DF/HDF clinical phenotype and management.

Methods: This is a spontaneous, retrospective, multicenter national study. Data are collected from clinical records.

Results: 41 pts have been enrolled in 3 centers: 35 DF (85%), 6 HDF (15%); 18M, 23F. Median follow up: 7.4 months (1-203). Median age at diagnosis: 36 years (range 3-81). Median fibrinogen activity/antigen level: 53 mg/dL (0-156) and 250 mg/dL (66-380), respectively. 14 pts reported bleeding episodes: epistaxis, hematuria (presence of kidney stones), hematomas, ecchimoses, menometrorrhagia, and gastro-intestinal (presence of esophageal varices), but no specific therapy was required. A portal venous thrombosis occurred in 1 DF patient who underwent splenectomy without replacement therapy; he was treated with warfarin without anti-hemorrhagic prophylaxis. 41 minor/major surgeries were performed in 23 pts. In 10/41 (24%) cases, prophylaxis was administered [Fresh Frozen Plasma in 3, Fibrinogen Concentrate (FC) in 1, Tranexamic Acid in 6]; in 5/41 (12%) cases, Low Molecular Weight Heparin (LMWH) was administered; no hemorrhage occurred. 13 pregnancies occurred in 9 women. In 1 case, LMWH prophylaxis was undertaken during pregnancy, and in 1 other during puerperium. In 2 cases, FC was administered at time of vaginal delivery (VD). 9 VD and 4 cesarean sections were performed without complications.

Conclusions: Pts from this case series experienced few hemorrhagic/thrombotic events. The majority was asymptomatic, and the most severe events were related to associated disorders. Nonetheless this study has the potential to collect data from a numerous population of pts who live in the same country, and therefore to provide useful information to better characterize and manage these rare diseases.

PB 2005 | Use of a Population Pharmacokinetic Model to Determine Pharmacokinetic Parameters of a New Fibrinogen Concentrate in Pediatric Afibrinogenemic Subjects ≤12-year Old

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Background: Pharmacokinetics (PK) of a triply secured fibrinogen concentrate (LFB FGTW) was first investigated in afibrinogenemic subjects ≥40 kg. Limited data are available in children.

Aims: To develop a population PK (PPK) model with plasma fibrinogen activity (Fg:C) data from afibrinogenemic subjects of all age and weight classes to describe FGTW PK in children ≤12 years and < 40kg and to compare it to other age groups.

Methods: Blood PK samples from 31 afibrinogenemic subjects (1-48 years) were collected over time to determine the Fg:C using a validated Clauss assay. Subjects received a single dose of 0.06 g/kg FGTW in one of 3 clinical trials conducted first in adults, then in adults/adolescents ≥40 kg and finally in children. All or parents thereof signed an informed consent. A PPK model was implemented with NONMEM® using the first order conditional estimation method with interaction.

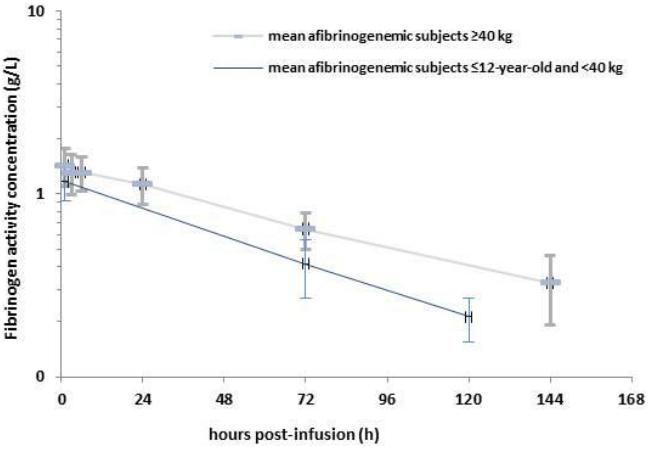

FIGURE 1 Fg:C levels over time in both groups of afibrinogenemic subjects

Results: There was a slightly faster elimination of FGTW in children ≤12 years and < 40 kg (n=12) compared to the other population (n=19) (Fig 1).

A one compartment model with allometric scaling was found to best describe the kinetics of FGTW; additional covariates did not improve the model. Conditional estimates of clearance and distribution volumes adjusted to bodyweight were found to be greater by 64% and 21%, respectively, in children compared to the rest of the population. This led to a shorter elimination half-life estimated at about 2 days in children. Incremental recovery (IR) determined at 1h post-infusion was found to be lower in children with a geometric mean at 19.1 compared to 23.3 (g/L)/(g/kg) in adults and adolescents.

Conclusions: IR and half-life were observed to be lower in the pediatric afibrinogenemic population ≤12 years and < 40 kg. Thus, an approximate 25% higher dose of FGTW would be needed in this pediatric population. An adapted FGTW dosing formula for this age group should be considered to reach the target level of fibrinogen by using 1/IR of 0.053 *vs* the 1/IR of 0.043 applicable to adults/adolescents.

PB 2006 | Safety and Efficacy of Recombinant Factor XIII (rFXIII-A2) in Patients with Congenital FXIII-A Subunit Deficiency Undergoing Minor Surgical Procedures: Results from the Mentor™2 Trial

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Background: Congenital FXIII-A subunit deficiency carries a high risk of intraoperative bleeding in the absence of FXIII replacement. Data on surgical haemostatic coverage using FXIII replacement is scarce. rFXIII-A_2 is the only recombinant product available for use in congenital FXIII deficiency. The safety and efficacy of rFXIII-A_2 when used for prophylaxis and treatment of bleeds was shown to be favourable in the mentor™ trial programme: patients receiving 35 IU/kg rFXIII-A_2 every 4 weeks achieved mean FXIII trough levels between 17 and 19%. Elective surgical procedures were included in mentor™2.

Aims: Report minor surgeries data in patients receiving rFXIII-A_2 prophylaxis during mentor™2.

Methods: mentor™2 was a multinational, open-label, single-arm extension of the pivotal mentor™1 trial. Eligible patients (aged ≥6 years) received 35 IU/kg rFXIII-A_2 every 28±2 days for ≥52 weeks. Elective surgery was permitted during the treatment period; if any procedure required additional FXIII substitution, plasma-derived FXIII was to be given according to local practice.

Results: mentor™2 enrolled 60 patients (median [range] age at enrolment: 26.0 [7.0-77.0] years). Twelve minor procedures (seven dental, five ‚other') were performed in nine patients (median [range] age at time of surgery: 31 [19-59] years; Table). Of these procedures, eight were performed within 7 days of the patient's last scheduled rFXIII-A_2 dose and four were performed 10-21 days after the last dose. No additional FXIII substitution was required for any procedure. Concomitant antifibrinolytics were used in four procedures (tooth extraction [n=3] and colonoscopy [n=1]). No adverse events assessed as possibly/probably related to rFXIII-A_2 treatment were observed.

Conclusions: These data suggests prophylaxis with 35 IU/kg rFXIII-A_2 every 4 weeks provides sufficient haemostatic coverage for the management of minor surgical procedures in patients with congenital FXIII deficiency without additional FXIII treatment.

TABLE 1

Table. Summary of surgical procedures

Type of procedure	No. of procedures	No. of patients	Days since last rFXIII-A_2 dose	No. of procedures requiring antifibrinolytics
Dental				
Tooth extraction + tooth root extraction	1	1	0	0
Tooth (including wisdom tooth) extraction	6	4	0–17	3
Other				
Anterior subcutaneous transposition ulnar nerve carpal tunnel syndrome repair	1	1	1	0
Removal of internal polyp from right ear and partial removal of internal polyp from left ear	1	1	21	0
Suture of left hand after cut	1	1	10	0
Colonoscopy	1	1	1	1
Circumcision revision	1	1	1	0

PB 2007 | Investigation of Medical Indications for PNH Screening Experiment by Flow Cytometry

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder leading to a partial or absolute deficiency of all glycophosphatidyl-inositol (GPI)-linked proteins. The classical approach to diagnosis of PNH by cytometry involves the loss of at least two GPI-linked antigens on RBCs and neutrophils. But there is not a uniform consensus about the medical indications for PNH clone testing by FCM.

Aims: In order to understand the clinical significance of the PNH screening.

Methods: 4694 individuals were submitted for diagnostic screening of PNH.CD59 was used for RBC analysis based upon physical parameters. We combined FLAER with CD45, CD24, allowing the simultaneous analysis of FLAER and the GPI-linked CD24 on neutrophil and monocyte lineages by CD45 vs side scatter.

Results: PNH clone cells were found in 309/4694 (6.6%) cases. Most commonly individuals were screened because of aplastic anemia (1010/4694,21.5%) and MDS(970/4694,20.7%), followed by hemoglobinuria (896/4694,19.1%), pancytopenia(656/4694,14.0%), hemolytic anemia (448/4694,9.5%), bone marrow failure (233/4694,5.0%), atypical venous thrombosis (76/4694,1.6%), other (405/4694,8.6%). Essentially all patients with classic PNH (108/309,35.0%)report gross hemoglobinuria at some point during the course of their illness. This symptom were absent in patients with PNH-subclincal (190/309,61.5%) because the clone size is often relatively small. PNH clone cell often were found in PNH combined with another specified bone marrow failure disorder, including AA (120/309), MDS(85/309), pancytopenia (4/309).

Conclusions: The FCM screening of GPI-deficient cells shows that the rate of positive results is higher when cases were tested because of hemoglobinuria, anemia,thrombosis and pancytopenia, and much higher in cases previously diagnosed of aplastic anemia or MDS, and therefore screening for PNH in patients with aplastic anemia, or MDS even in the absence of clinical evidence of hemolysis, is recommended at diagnosis and follow-up.

PB 2008 | Clinical and Molecular Data in 15 Italian Subjects with Congenital FXI Deficiency

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Background: Congenital Factor XI (FXI) deficiency is responsible for a bleeding disorder with a high variability in clinical phenotype. To date, many allele variants have been shown to be responsible for the Factor XI deficiency. However, a clear genotype-phenotype relationship is difficult to establish.

Aims: The objective of the study was to describe individuals with Factor XI deficiency, in order to provide novel clinical and molecular insights into such bleeding disorders.

Methods: Fifteen unrelated Italian index cases with congenital FXI deficiency and their relatives were investigated. After the identification of deficiency, we obtained DNA from each subject and performed a direct sequencing with standard procedures.

Results: We identified 5 and 10 individuals with severe and moderate deficiency of Factor XI activity, respectively. Most families (8/15) carried mutations in the Apple 2 domain and 13% of Glu117Stop (type II mutation) was observed. The Glu117Stop (type II) mutation showed a similar allele frequency (13.3%) in respect to other Italian series (18%). 4 novel compound heterozigosity were identified. Bleeding symptoms were present in only 2 severely deficient subjects. They carried the combinations Phe283Leu/Trp501stop and Glu297Lys/Trp501stop, respectively. Bleeding episodes occurred also in the presence of a moderate deficiency in 2 subjects heterozygous for Thr132Met and Gly400Val, respectively. The previously described Phe283Leu mutation (so-called type III) in the presence of a null allele was associated with Factor XI low levels and a bleeding phenotype.

Conclusions: We confirm an unclear prediction of phenotype from mutational data. Further characterization of FXI mutant alleles may allow for a more comprehensive understanding of bio chemical effects and predict for bleeding phenotype better than the unique FXI levels measurement.

PB 2009 | Are Heterozygotes of Factor XIII Deficiency Prone to Bleeding? A New Finding in Area of Rare Bleeding Disorders

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Background: Factor XIII (FXIII) deficiency is a rare disorder with high bleeding tendency. Factor activity is 30-70% in individuals with heterozygous FXIII deficiency (FXIIID) and they do not usually experience spontaneous hemorrhage.

Aims: . Since southeast Iran has the largest population with FXIIID, we design this study to assessed bleeding tendency among heterozygote of FXIIID.

Methods: This study was carried out on 53 heterozygote of FXIIID as well as same number of normal population. Initially all individuals were assessed for Trp187Arg FXIII mutation and FXIII activity. Both groups were interviewed by expert staff to record their demographic data and any abnormal clinical presentations in the past. In prospective arm in regular intervals of one-month for a period of three months, bleeding episodes of both groups were recorded.

Results: The mean of FXIII activity of the patient group was 57% and all the patients were heterozygote for Trp187Arg mutation. In control group, the mean of FXIII activity was 105% and all of them were non-mutant. Three (5.7%) individuals experienced umbilical cord bleeding and 16 (32%) women experienced at least on spontaneous abortion while in control group only 1 (2%) of women experienced this episode. Mean bleeding score among cases group for epistaxis, bleeding from minor wounds, bleeding from oral cavity as well as post dental extraction bleeding were higher than control group.

Conclusions: Our findings indicated that heterozygote of FXIIID may complicate by substantial bleeding events, which may threat the life and lead to the requirement of prophylaxis administration.

PB 2010 | FVII Deficiency: Characterization of Three Different Mutations in the FVII Catalytic Domain

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Background: Inherited coagulation factor (F) VII deficiency is the most common among the rare bleeding disorders. Although the mutational pattern of FVII deficiency is well characterized, the intracellular processing mechanisms of mutant FVII molecules have only been investigated for a few mutations.

Aims: To unveil the cellular and molecular mechanisms affecting biosynthesis of FVII in three different mutations in the FVII catalytic domain: the G420V missense change, the deletion p.I289del and the frame-shifted and elongated variant p.A354Vg-p.P464Hfs, all associated with severe bleeding.

Methods: The wild type (wt) and mutant FVII were transiently expressed in CHO-K1 cells. FVII antigen was measured by ELISA and FVII activity by a fluorogenic assay. Synthesis and stability of the FVII mutants were evaluated by pulse-chase stable isotope labelling followed by mass spectrometry based quantitative proteomics (pc-SILAC). Degradation studies were performed using proteasome and lysosome inhibitors. Intracellular localization was assessed by confocal immunofluorescence microscopy.

Results: Secretion of the FVII mutants was severely decreased compared to FVIIwt. No activity of the FVII mutants was detected whereas the activity of FVIIwt was 0.09 IU/ml. The synthesis rate of the FVII mutants was slower and significantly reduced, being between 8 and

20% of the FVIIwt at 6h of the chase. The FVII variants were localized in the endoplasmic reticulum (ER) and none of them were detected in the Golgi apparatus. Degradation by lysosomes or proteasomes was not detectable for the three mutants.

Conclusions: Due to the decreased secretion of the mutants, it is likely to assume that they are not folded correctly in the ER and remain there. Consequently, the mutants are unable to follow the normal transport to the Golgi apparatus.

PB 2011 | Correction of Coagulopathy Related to Severe Factor V Defects with a New Plasma-derived FV Concentrate: An In vitro Study with ROTEM

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Background: Fresh frozen plasma is the main therapeutic option in severe FV deficiency because no FV-specific concentrate is still available. However, plasma transfusion can lead to adverse reactions or events. A new plasma-derived FV concentrate has been developed and is under evaluation for clinical use.

Aims: To assess the ability of the new FV concentrate to correct *in vitro* the coagulopathy in severe FV defects.

Methods: ROTEM analysis was performed in 9 patients with severe FV deficiency pre and post *in vitro* spiking of blood samples with increasing doses of the drug (0.01-1 IU/mL) and in 20 healthy volunteers who served as a control group. Two ROTEM assays, INTEM and EXTEM, were used and the following parameters were evaluated: clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), maximum velocity (MAXV) and area under the curve (AUC). Data were presented as median and IQR and analyzed using Kruskal-Wallis test to compare vs. "control" group. P value < 0.05 was considered significant.

Results: FV-deficient patients had a significantly prolonged CT compared to the normal group (p< 0.0001) both in INTEM and EXTEM. CT values were corrected by the addition of 0.25 IU/mL of FV concentrate both in INTEM and EXTEM. As regards CFT, MCF, MAXV and AUC, no statistically significant differences were detected between patients and controls in none of the assays performed (Table 1 and Table 2).

Conclusions: Our study, conducted on whole blood by ROTEM, reveals that CT is the main parameter impaired in severe FV defects. Replacement with 0.25 IU/mL of the new FV concentrate was able to *in vitro* normalize this parameter. ROTEM could be a useful tool to monitor the replacement therapy by FV concentrate in FV-deficient patients.

TABLE 1 Effect of FV concentrate on ROTEM/INTEM parameters in FV-deficient patients and a control group

	FV (IU/mL)						
INTEM	Baseline	0.01	0.10	0.25	0.50	1.00	Control group
CT (s) reference range 100-240	572 [43-718]	403 [345-425]	256 [228-259]	208 [192-223]	191 [183-205]	173 [163-188]	177 [174-183]
CFT (s) reference range 30-110	80 [61-84]	65 [51-79]	54 [49-72]	63 [50-72]	58 [47-70]	57 [46-62]	67 [54-73]
MCF (mm) reference range 50-72	67 [60-70]	67 [59-70]	66 [59-70]	67 [59-69]	67 [59-70]	67 [63-70]	63 [61-70]
MAXV (mm*min-1)	17 [16-23]	21 [16-27]	23 [20-26]	22 [17-25]	21 [17-28]	21 [20-27]	19 [18-28]
AUC (mm*100)	6666 [6084-6963]	6698 [5908-6949]	6546 [5848-6969]	6635 [5817-6889]	6618 [5940-6996]	6623 [6212-6930]	6310 [6111-6927]

TABLE 2 Effect of FV concentrate on ROTEM/EXTEM parameters in FV-deficient patients and a control group

	FV (IU/mL)						
EXTEM	Baseline	0.01	0.10	0.25	0.50	1.00	Control group
CT (s) reference range 38-79	259 [234-502]	146 [111-168]	81 [74-90]	73 [70-77]	62 [59-68]	53 [51-57]	60 [45-64]
CFT (s) reference range 34-159	117 [83-391]	64 [48-69]	54 [48-66]	68 [54-78]	72 [54-81]	63 [51-74]	77 [50-84]
MCF (mm) reference range 50-72	72 [62-76]	72 [67-75]	69 [63-72]	67 [62-72]	67 [62-72]	68 [63-72]	67 [63-72]
MAXV (mm*min-1)	13 [8-19]	22 [19-29]	23 [19-26]	18 [16-24]	17 [16-23]	19 [17-24]	17 [16-24]
AUC (mm*100)	7217 [6133-7602]	7186 [6694-7421]	6830 [6280-7111]	6705 [6186-7163]	6606 [6185-7148]	6713 [6290-7037]	6234 [5975-6646]

PB 2012 | Molecular Analysis and Clinical Description of an Italian Cohort of Patients with FXI Deficiency

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Background: Factor XI deficiency is an autosomal recessive bleeding disorder (prevalence 1:1,000,000) caused by mutations in F11 gene (15 exons, 4q35.2). The absence of correlation between genotype, FXI activity and bleedings is described. Symptoms are really heterogeneous and infrequently spontaneous.

Aims: To evaluate relationships between genotype and bleeding manifestations in patients with FXI deficiency.

Methods: Thirty-five patients from Parma Haemophilia Center with FXI:C activity< 0.6 IU/mL were identified by pre-surgical/routine laboratory screening or bleeding history and classified according to clinical phenotype, FXI activity and genotype. The molecular screening of F11 gene was performed with HRM and direct sequencing.

Results: Median age at diagnosis was 25(1-71), median FXI:C 0.37 IU/mL (0.02-0.56). We identified 17 different mutations (missense or truncating), 5 not reported in FXI mutation database.

Four patients were compound heterozygous for pathological mutations: two (FXI 0.002 and 0.019 U/mL) had truncating mutations and were symptomatic (only one with spontaneous mucosal bleedings), the others (FXI:C 0.37 and 0.56 IU/mL) had truncating/missense mutations, without symptoms.

Thirty patients (0.20< FXI:C< 0.6 IU/mL) were heterozygous for pathological mutation, 51% was asymptomatic. Two patients, both with FXI 0.3IU/mL, presented severe bleedings: a female with spontaneous hemarthrosis, post partum and post-surgery bleeding and a boy with intracranial hemorrhage related to cerebral cancer. All the other patients had post-challenge or spontaneous mild bleedings, mainly mucosal.

Conclusions: In our cohort, truncating mutations in compound heterozygosis were associated with severe deficiency of FXI while the association with missense mutations or an heterozygous genotype did not. The bleeding tendency was not related to FXI activity, confirming results of the previous studies and leading to the hypothesis of other predictors of bleeding risk.

PB 2013 | Bernard-soulier Syndrome in Pakistan: Biochemical and Molecular Analysis Leading to Identification of a Novel Mutation

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Background: Bernard-Soulier syndrome (BSS) is a rare, autosomal recessive bleeding disorder with abnormalities of the platelet GPIb-IX-V complex. Typical are mucocutaneous bleedings, thrombocytopenia, presence of giant platelets and impaired ristocetin- induced agglutination. Three disease-related genes (*GP1BA*, *GP1BB*, *GP9*) harbor mutations of all types. Here, we investigated a Pakistani cohort of 6 juvenile patients out of 5 unrelated families presenting mild to severe bleeding symptoms and 10 family members using biochemical and molecular genetic analyses.

Aims: Aim of the study was to define the phenotype and reveal its underlying pathogenic mutation.

Methods: Blood count, smear and platelet characterization using aggregometry and flow cytometry was done for all 6 patients and partial for family members. Sequencing of coding- and splice site regions of all genes was done for index patients. Variants were analyzed by ALAMUT®. Occurrence in variant databases (dbSNP, EVS, ExAC), ClinVar, the latest BSS international consortium report and *in silico* pathogenic prediction (SIFT, MutTaster, PolyPhen2) were investigated. Genotyping was performed for all family members.

Results: Patients showed macrothrombocytopenia, impaired ristocetin-induced agglutination and severely reduced expression of GPIb/IX. We identified two nonsense mutations in *GP1BA* (NM_000173.6): one of them *novel* (c.21delG,p.Leu8Serfs*22), one reported (rs774388410). In *GP9* (NM_000174.4) we discovered the same missense mutation (c.70T>C,p.Cys24Arg, rs28933378) in 3 index patients from unrelated families. This mutation is already published to be found in BSS patients from India (13 to date) and Pakistan (4 to date).

Conclusions: We report a *novel* nonsense *GP1BA* mutation altering the signal peptide of the alpha chain of GP1b. Our finding of the *GP9* mutation in 3 unrelated families supports the conclusion that this mutation is a founder mutation for South Asians.

PB 2014 | Genotypic Abnormalities in Phenotypic Confirmed Patients with Rare Bleeding Disorders

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Background: Rare Bleeding Disorders (RBD) are characterized by increased susceptibility to develop hemorrhages because of genetic abnormalities in multiple components of the hemostatic balance. At the moment, at least 135 genes/loci are known as genetic causes for hemostatic disorders.

Aims: To screen for genetic abnormalities to confirm the patients' phenotype.

Methods: Targeted gene analyses were performed on 148 patients with RBD, by classical Sanger sequencing or by ion semiconductor next generation sequencing technology (IonTorrent PGM™). All coding regions including intron-exon boundaries were sequenced. Two

different classes of hemostatic genes were analysed: procoagulant genes (*F2*, *F12*, *F11*, *F7*, *F10*, *F13A1*, *F13B*, *FGA*, *FGB*, *FGG*) and platelet specific genes (*GFI1B*, *GP1BA*, *ITGB3*, *MYH9*, *NBEAL2*). Targeted single gene approach was based on patients' laboratory phenotype in combination with clinical symptoms.

Results: Of the 148 patients tested 38 carried (likely) pathogenic mutations and 8 carried variants of unknown pathogenicity (VUS) in procoagulant genes. For the more heterogeneous platelet abnormalities only 3 out of 38 patients had (likely) pathogenic mutations, 1 patient with a VUS.

Conclusions: The patients' phenotype was an important parameter for targeted single gene approach to detect genotypic abnormalities in RBD patients. The overall yield to detect pathogenic mutations was 27% in procoagulant abnormalies. The targeted single gene approach was resolved in only 11% in cases with primary hemostasis abnormalities. To increase the yield we recently implemented whole exome sequencing (WES) as a diagnostic test. WES allows analysis of a large panel of genes in one single test. The hemostatic gene panel we designed contains currently 135 genes proven to be involved in hemostatic disease including genes involved in primary hemostasis (platelet and vessel wall) and secondary hemostasis (coagulation and fibrinolysis). If the diagnostic panel reveals a negative result exome wide analysis will be performed.

PB 2015 | Evaluation of the Use of Bleeding Assessment Tools in FXI Deficiency

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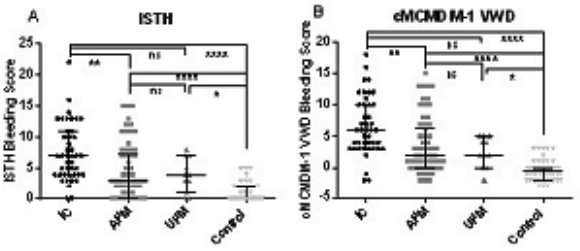
Background: The bleeding history is paramount to assessment of FXI deficiency; however variability exists as to what constitutes a significant bleeding history.

Aims: To assess utility of ISTH and condensed MCMDM-1 VWD bleeding assessment tools as descriptive, diagnostic and predictive measures in FXI deficiency and determine the impact of diagnostic setting on bleeding scores (BS).

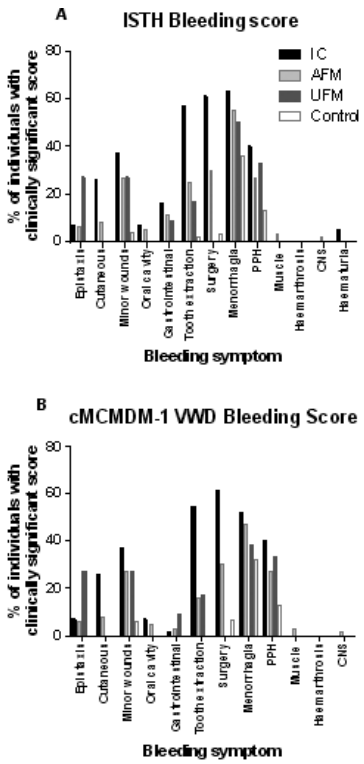
Methods: BS were determined in 116 adults from 69 FXI-deficient families and 50 healthy individuals. Plasma FXI:C levels and thrombin generation assays (TGA) were performed along with F11 mutation analysis. Ethical committee approval and participant consent were obtained.

Results: Statistically higher BS were demonstrated in index cases (IC) (n = 43) compared to affected family members (AFM) (n =62) (diagnosed through family screening); patterns of bleeding were similar but a higher percentage of IC reported symptoms. Unexpectedly, no difference in BS was seen between AFM and unaffected family members (UFM) (n =11) and UFM reported more bleeding symptoms and had statistically higher BS than normal individuals (n=50) (Fig.1 and Fig. 2).No correlation was seen between FXI:C levels and either BS (P>0.5), or between heterozygous mutation type and BS. Both BS correlated with ETP in IC (P< 0.003) but only with ETP in AFM who had undergone procedures in areas of high fibrinolytic activity (P< 0.0093). Neither BS had diagnostic utility in family screening (ROC AUC< 0.5212, P>0.82) and mucocutaneous scores derived from each BS held no predictive value for past bleeding (ROC AUC< 0.647, P>0.14).



Lines and bars represent median and interquartile range. * P < 0.05, ** P< 0.01, *** P < 0.001, **** P < 0.0001, ns, not significant. Tested with ANOVA Kruskal Wallis test with Dunn's multiple comparisons test.

FIGURE 1 Comparison of ISTH (A) and cMCMDM-1 VWD (B) bleeding scores in 4 different study groups



A clinically significant score was defined as = 1 for menorrhagia, post partum haemorrhage (PPH) ,muscle bleeds and haemarthrosis, =3 for central nervous system (CNS) symptoms and =2 for all other symptoms. Frequencies for tooth extraction, surgery, childbirth/PPH only include data from individuals who underwent these procedures. Frequencies for menorrhagia only included data from females.

FIGURE 2 Frequency of clinically significant bleeding symptoms in 4 study groups

Conclusions: Importantly we show that diagnostic setting influences BS in FXI deficiency and that UFM report more bleeding than expected. BS can be used as descriptive but not diagnostic tools. Correlation of ETP with BS raises the possibility that BS may determine bleeding phenotype in IC and a select group of AFM who have been previously challenged by procedures high risk for bleeding in FXI deficiency. Prospective studies are required to confirm this.

PB 2016 | Clinical Presentation and Management of Glanzmann's Thrombasthenia: A Case Study

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Background: Glanzmann′s thrombasthenia is a very rare, characterized by a life-long mucocutaneous bleeding tendency, absent or severely reduced platelet aggregation . Mucocuteanous bleeding, gingival hemorrhages, menorrhagia, gastrointestinal bleeding, hematuria can be seen in patients with Glanzmann′s thrombasthenia.

Aims: In this study, we aim to report the patients with Glanzman's thromboastenias bleeding sites and treatment modalities of 25 patients.

Methods: From 1995 to 2016, we retrospectively reviewed medical records of 25 patients with Glanzmann′s thrombasthenia and discussed with the literature.

Results: Twenty five patients with Glanzmann′s thrombasthenia, age range from 1 to 17 years were included in this study. The ages of onset were between 3 days and 7 years of age. Mucocutaneous bleeding was the most common clinical presentation (%92). Intracranial haemorrhage (ICH) was seen in one patient after trauma. Menorrhagia was seen in 3 girls who were in the age of menstruation. Three patients referred to our centre with gastrointestinal bleeding and one patient with hyphema. Three patients are diagnosed after circumcision. Twenty five of 4 patients have surgical operations including circumcision and teeth extraction. Thrombocyte suspensions, local therapies, antifibrinolytic agents and recombinant FVIIa were used for bleeding control before and during surgery patients performed surgeries. Oral contraceptives were given to the patients with menorrhagia.All bleeding episodes of these patients were successfully treated and there is no other complication or death.

Conclusions: Glanzmann′s thrombasthenia is a rare hematologic disorder. The clinical presentation with mucocutaneous bleeding can range from mild to severe to life threatening. Affected patients may manifest with severe haemorrhagic symptomps early in life. In this case series with Glanzman thromboastenias we report bleeding characterizations and management of different site of haemorrhage in 25 patients.

PB 2017 | Prevalence of Menorrhagia in Women Diagnosed with Congenital Bleeding Disorders

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Background: Menorrhagia is defined as abnormally heavy or prolonged bleeding during menstrual cycle andblood loss exceeding 80 ml per cycle. It is the most common symptom in women of reproductive age with congenital bleeding disorders. However, there is paucity of data on the incidence, diagnosis and treatment of bleeding in women with these disorders in developing countries.

Aims: Here we report the frequency, clinical picture and types of congenital bleeding disorders among women with menorrhagia.

Methods: An observational study was carried out amongst females diagnosed with congenital bleeding disorders. Frequencies of bleeding disorders with respect to menorrhagia were calculated and chi square test was applied to observe the association of prevalence of menorrhagia amongst all bleeding disorders.

Results: A total of 116 females with congenital bleeding disorders, including factor deficiencies (FI, FV, FVII, FXI, FXIII), platelet function disorders (Glanzmann's Thrombasthenia (GT), Bernard Soulier Syndrome (BSS), and Storage pool disorder (SPD) and Von Willebrand disease (VWD)were studied. The mean age was 12.69±10.13 years. Of the entire cohort, 65(56%) females were found to be in the reproductive age and out of them 41(63%) had menorrhagia with mean age of 22.2±7.4 years. Menorrhagia was more commonly observed in BSS, FI, FV, FXIII and SPD patients followed by VWD, FVII and GT.Pictorial blood assessment chart (PBAC) >100 were observed in all subjects. We intended to observe the association of frequency of menorrhagia amongst all bleeding disorders and it was found that menorrhagia was evenly prevalent in all bleeding disorders.

Conclusions: These results demonstrate that menorrhagia is prevalent among all congenital bleeding disorders. Therefore, women,especially in the reproductive age with menorrhagia, should be further evaluated for hemostatic disorders.

PB 2018 | Thrombotic Complications in a Patient with Congenital Afibrinogenemia

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Background: Congenital afibrinogenemia (CA) is a rare inherited bleeding disorder characterized by complete absence of plasma fibrinogen. Patients with CA present with bleedings but paradoxically thrombotic events have also been reported.

Aims: Present a case of a patient with CA and hepatitis C (HC) showing both bleeding and thrombotic events whose management remains difficult and challenging.

Methods: Female, 31 years old, diagnosed with CA, because of postnatal cephalhematoma. She was compound heterozygote for mutations in fibrinogen A gene.

Results: The patient presented recurrent mucosal hemorrhages, menorrhagia, hemoperitoneum and hemarthrosis and treated with plasma or fibrinogen concentrates (FC). She was on prophylaxis with FC since 2011. In 1996 she was diagnosed with HC virus infection and ten years later she successfully treated with antiviral drugs. At that time she developed hypertension, ischemic lesions of the toes and progressive renal failure and she received corticosteroids and rituximab for vasculitis associated with HC. A year later her renal function and hypertension deteriorated. Computed tomography angiography revealed stenosis of both renal and other intra abdominal arteries. The patient underwent an angioplasty of the right renal artery. During the following years she had two episodes of thrombosis (superior mesenteric artery and left internal jugular and subclavian vein). Her renal function gradually deteriorated and in 2015 she started on regular hemodialysis but she presented thrombosis on the left femoral vein, probably related to the dialysis catheter. Thrombotic events were treated with low molecular weight heparin. In 2016 she underwent renal transplantation from a living related donor (mother).

Conclusions: Although the combination of anticoagulation and replacement therapy is an effective treatment for the thrombotic events occurred to the patients with CA, the maintenance of the fragile balance between thrombosis and bleeding is quite difficult.

PB 2020 | Identification of Novel and Recurrent Mutations in F13B Gene Causing Mild FXIII Deficiency

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Background: Severe FXIII deficiency is a rare, autosomal recessive bleeding disorder affecting approximately one in 1-3 million people. On the other hand the heterozygous FXIII deficiency is expected to be more common occurrence affecting approximately one out of 1000 inhabitants.

Aims: Characterization of *F13B* gene mutations in mild FXIII deficiency.

Methods: We report the results of the screening of *F13B* gene mutations in a mild FXIII deficiency patient cohort (FXIII activity < 65% of normal) of German-Caucasian descent. Genomic DNA of these patients has been analyzed by direct sequencing of the *F13A1* and *F13B* genes between 2009 and 2015 using previously described protocols. All detected missense mutations were analyzed on mutation prediction servers and also by modeling them using protein modeling tools. The missense mutations were also cloned into expression vectors transfected heterologously into *HEK293t* cell lines and their expression products analyzed.

Results: Eight novel and one recurrent heterozygous *F13B* gene mutations were identified. The majority of patients (10/13) carried missense mutations, while the remaining three patients showed distinct genetic defects revealing one small deletion (c.1958delT), one small insertion (c.365insA) and one splice site (IVS5-1G>A) mutation in intron 5. Among six different missense mutations, five were novel (p.Phe42Val, p.Tyr163His, p.Cys213Trp, p.Val266Pro, p.Ala416Glu) all affecting highly conserved regions of the FXIII-B Subunit. One previously described missense mutation (pCys336Phe) was found in 5 out of 13 analyzed patients. *In vitro* and *in silico* analysis shed light on the causality of the missense mutations.

Conclusions: Eight novel potentially causative mutations underlying mild FXIII deficiency have been identified within *F13B* gene. The p.Cys336Phe mutation seems to be a common mutation causing mild FXIII deficiency in the German-Caucasian population.

PB 2021 | Bleeding Score as a Diagnostic Tool in Patients of Autosomal Recessive Inherited Bleeding Disorders

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Background: To establish the quantitative method for the assessment of bleeding manifestation is a big challenge for the clinicians.Bleeding pattern vary significantly between disorders and patients, even with the same disorder.The incidence of autosomal recessive bleeding disorders (ARBD) is rare as compared to other bleeding disorders. These are prevalent in those geographical locations where consanguinity is common and occur frequently in homozygosity.

Aims: To establish the utility of bleeding scores(BS) as a diagnostic tool for the patients
with ARBD in different regions of Pakistan.

Methods: Patient's data of this cross-sectional study was collected from different hematology centers in Pakistan using Tosetto *et al* bleeding score.Diagnosed patients with ARBD were recruited after obtaining written consent and approval from ethical committee during last four years. Data was recorded in a structured questionnaire.

Results: Out of 211 patients with ARBD; 95 (33.8%) had VWD type 3 with a BS of 13.5,GT in 27 (9.6%) with a BS of 11,BSS in 7 patients (2.5%) with BS of 11, deficiencies of fibrinogen in 34 patients (12%) and BS of 12.5, FXIII in 13 (4.6%) and BS of 11.5, FVII in 12 (4.3%) and BS of 12, FV 9 (3.2%) BS of 11,8(2.8%) vitamin K-dependent clotting factor and BS of 12, FX in 2 (0.7%) BS of 12, FII in 2 (0.7%) with a score of 10, FXI deficiency and combined FV &VIII in 1 (0.4%) each and a BS of 10 and 11.5 were found respectively.

Table 1.1: Frequency and Severity of Bleeding.

ARBDs	Gum bleeding %	Hem arthr osis %	Hemat oma %	Epistaxis %	Menorrh agia ~ %	Umbilical cord bleed%	Bleeding after trauma%	Bruise s %	ICH %	Bleeding Grades*	Bleeding score¶
VWD type 3 disorder.	53.2	7.6	16.15	32.3	11.4	10.45	-	12.3	-	II	13.5
Fibrinogen	6.25	18.75	50	25	-	-	68.7	43.75	-	II	12.5
Factor XIII	84.6		46.15	69.2	46.1	84.6	38.4	69.2	7.69	II	11.5
Factor VII	75	66.7	58.3	91.6	46.1		91.6	75	16.6	III	12
Factor V	77.8	22.2	66.7	55.6	55.6	-	44.4	55.6	11.1	II	11
Vitamin K dependent clotting factors	87.5	-	62.5	25	37.5	62.5	87.5	62.5	-	II	12
Glanzmann Thrombasthenia	85.7	-	14.2	71.4	28.5	57.1	100	71.4	-	II	11
Factor II	100	50	100	50	50	-	-	50	-	II	10
Factor X	50	100	100	-	100	100	-		-	II	12
Combined Factor V and VIII	100	100	100	-	-	-	-	100	-	II	11.5
Factor XI	100	-	100	100	-	-	100	100	-	II	10
Bernard Soulier syndrome	100	-	100	100	-	-	100	100	-	II	11

*Calculated on the basis of WHO bleeding grades. ¶based on Tosetto et al bleeding score calculation scale. ICH: intracranial hemorrhage.
~ Reported in adult female patients.

FIGURE 1 Frequency and Severity of Bleeding

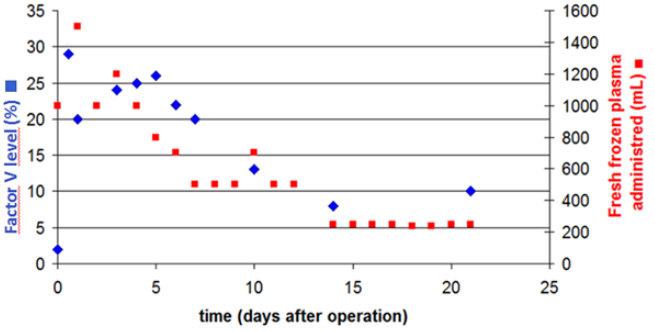

FIGURE 1 Factor V level and substitution with fresh frozen plasma in the peri- and postoperative period

Conclusions: At the end of this study it was found that bleeding score with an average of 11.5 is positively associated with an ARBD. This prediction is useful in diagnosing a patient with these disorders and help to make correct treatment decisions.

Keywords: Autosomal recessive, coagulation factors, BAT, bleeding scores.

PB 2023 | Acute Coronary Syndrome in a Patient with Factor V Deficiency: A Case Report

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Background: Occasional thrombotic phenomena are seen even in rare congenital bleeding disorders. Due to rarity of factor (F) V deficiency there are no guidelines in the available literature on how to manage such patients.

Aims: A patient with FV deficiency and acute coronary artery thrombosis is presented.

Methods: Standard coagulation tests were used, FV activity was determined by one-stage PT based assay.

Results: Case presentation: A 75-year old caucasian man with a congenital FV deficiency (FV level 2%), diabetes mellitus type 2, arterial hypertension and hyperlipidemia presented with unstable angina pectoris. His aPTT was 69,3 s, PT/INR was 0,35/2,09, antibodies against FV were negative. Prior to coronarography he received 1200 mL of fresh frozen plasma (FFP) (15 mL/kg), that raised his FV to 28%; he received standard antiaggregation therapy (acetylsalicylic acid, clopidogrel) and 5000 IU of standard heparin. Three-vessel coronary disease, including occlusion of LAD, and a 90% stenosis of right internal carotid artery was diagnosed. The patient was prepared for CABG and TEA ACI dex. We calculated the half- life of FV to be 19 hours. Prior to the operation he received 12 mL FFP/kg, raising FV level to 29%. FV level was kept above 20% the first 7 days after the surgery by administering FFP daily, along with acetylsalicylic acid 100 mg/d and a prophylactic dose of enoxaparin. Postoperative complications were serous pleural effusions and paroxisms of atrial fibrillation. From the second week after the operation till complete healing of the wounds FV level was maintained above 10%. No bleeding was noticed. Patient was released from the hospital 25 days after the surgery taking 100 mg acetylsalicylic acid per day.

Conclusions: Level of FV above 20% was sufficient to maintain proper haemostasis during invasive coronary diagnostics and a major cardiac surgery in a FV- deficient patient, receiving also standard antiaggregation therapy and a prophylactic dose of low-molecular weight heparin.

PB 2024 | Congenital Hypoprothrombinemia Presenting in the Neonatal Period: Report of 2 Cases in South of Iran

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Background: Prothrombin deficiency is the rarest inherited bleeding disorder. It usually presents with mucocutaneous bleeding, hemarthrosis and post surgery or post trauma hemorrhage. Rarely, the disease may manifest in the neonatal period with bleeding tendency.

Aims: We are going to introduce 2 babies with prothrombin deficiency who were diagnosed in the neonatal period with umbilical, gastrointestinal and intracranial hemorrhage.

Methods: The babies (a girl and a boy) were admitted simultaneously to a nursery ward in Shiraz, south of Iran. The girl was a preterm baby who was admitted due to GI bleeding and IVH grade II detected by transcranial ultrasonography. The boy was a term neonate who was admitted for umbilical stump bleeding and cephalhematoma following a difficult NVD (figure 1). Both families were living in a village near Shiraz and the parents were first cousin.

Results: The coagulation profile revealed a prolong PT and aPTT in both cases which were corrected with mixing study. Vitamin K administration didn´t correct their tests. Screening for common pathway factors showed prothrombin deficiency in both cases while other factors were normal (3.5 and 14 U/dL respectively). Their parents were heterozygote for prothrombin deficiency with factor II levels ranging from 61 to 76 U/dL. Fresh frozen plasma was infused twice weekly and they were discharged from hospital in good general health.

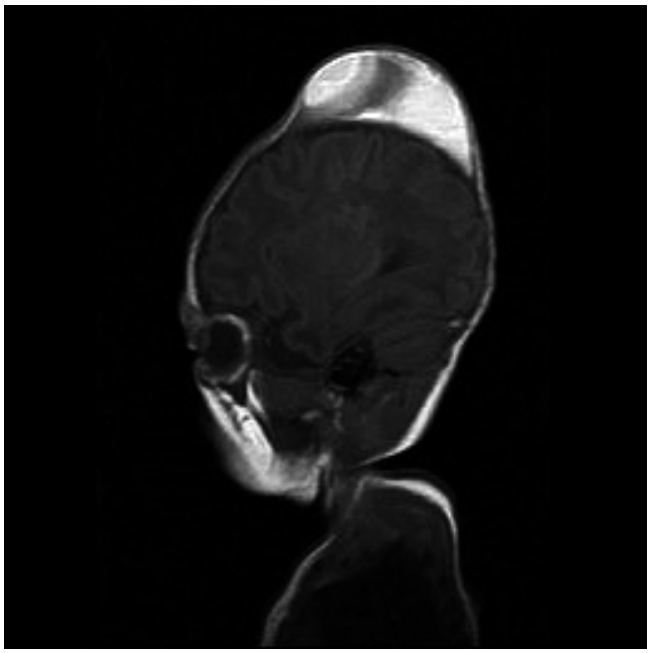

FIGURE 1 A large postero-lateral aspect cephalhematoma in the case with prothrombin deficiency

Conclusions: Prothrombin deficiency can present in the neonatal period with cephalhematoma as well as bleeding from CNS, GI and umbilical stump. As consanguineous marriage is common in some part of Iran, it is recommended to perform a mass screening program in this regions provided that an index case is detected.

PB 2025 | Rare Bleeding Disorders in the Netherlands (RBiN)

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Background: Rare Bleeding Disorders (deficiencies of fibrinogen, factor II, V, V&VIII, VII, X, XI, XIII, α2-antiplasmin or plasminogen activator inhibitor 1) have a diverse clinical presentation, varying bleeding scores, bleeding episodes, health-related quality of life and laboratory parameters.Therefore, correlations between genotype and phenotype are difficult to establish.

Aims: To describe epidemiology, bleeding tendency, laboratory parameters, quality of life and molecular genetic spectrum of all known patients in the Netherlands with rare bleeding disorders (RBD). In addition, the study aims to further explore the relationship between clinical and laboratory phenotype and genotype.

Methods: Cross-sectional multicentre observational study in all patients registered in Dutch Haemophilia Treatment Centers (HTC) with known RBD, aged 1-99 years. After informed consent patients are asked to fill out questionnaires on bleeding, socio-demographic characteristics, clinical characteristics (bleeding tendency, treatment, development of antibodies), medical history (hospital admissions, medication used), needle phobia, sports and physical activity, quality of life and functional limitations. ISTH-BAT and other bleeding assessment tools will be performed and validated for RBD. Blood and saliva samples will be collected for laboratory testing, including the Nijmegen Haemostasis Assay (NHA). Whole exome sequencing (WES) will be performed to unravel the patients genotype. Currently our WES strategy includes 128 bleeding related OMIM-proved genes, which will be further expanded when indicated.

Results: Inclusion of patients for the Rare Bleeding disorders in the Netherlands (RBiN) study will start in 2017. So far, 269 patients have been identified in all HTC in the Netherlands.

Conclusions: The RBiN study will provide new insight in the relationship between the clinical and laboratory phenotype and the genotype in rare bleeding disorders.

PB 2026 | Identification of Two Novel F5 Compound Heterozygous Mutations in Two Norwegian Patients with Severe Factor V Deficiency N

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Background: "Mary" was the first factor V (FV) deficiency patient discovered by Owren (1943). FV deficiency is a rare autosomal recessive bleeding disorder (1:1 mill). The bleeding symptoms range from mild to severe.

Aims: In two unrelated Norwegian FV deficient patients, and their parents, we characterized the F5 gene mutations and the effect on FV antigen (ag)/activity and thrombin generation (TG).

Methods: FV ag (ELISA)/activity and TG (CAT) were measured. Mutation analysis of the F5 gene was performed by Sanger sequencing of the exons and the exon/intron boundaries.

Results: Proband 1 (girl, 13 yrs) had severe bleeding tendency with mucosal bleedings since early childhood, easily bruising and heavy menstrual bleedings. FV ag/activity were < 1%. Lag time/peak (TG parameters) were not detected during 90 min. Two heterozygous missense mutations in the F5 gene were found; c.6293 C>T (Pro2098Leu) in exon 23, which is identical to Mary's mutation, and c.5990 C>G (Tyr1997Cys) in exon 21. The second mutation in proband 1 is novel, and an inhibitor against FV has evolved after treatment with

Octaplasma. NovoSeven is successfully used to limit her bleeds. Proband 2 (girl, 16 yrs) had moderate bleeding tendency with easily bruising and heavy menstrual bleedings. FV ag/activity were < 1% and lag time/peak evidently reduced. She also carried two compound heterozygous missense mutations in F5: c.6293 C>T (Pro2098Leu) in exon 23 (Mary's mutation) and c.5408 A>G (His1803Arg) in exon 16. For both probands, the healthy parents had FV ag of 60-98% and FV activity of 42-59%.

Conclusions: We identified that both FV deficiency patients had inherited compound heterozygous F5 mutations, with Mary's mutation in common. Both probands had Type I FV deficiency and evidently reduced TG. The different bleeding phenotypes in these two probands, and perhaps also the inhibitor evolvement, is likely to be explained by the c.5990 C>G (Tyr1997Cys) mutation, which was exclusive for proband 1.

PB 2027 | Retrospective Evaluation of Phenotype and Management of A-hypo-Fibrinogenemia in a Cohort of Italian Patients

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Background: A-Hypo-Fibrinogenemia (AF, HF) patients (pts) may experience hemorrhages or thromboses, and the clinical management can be difficult.

Aims: To obtain information on AF/HF clinical phenotype and management.

Methods: This is a spontaneous, retrospective, multicenter national study. Data are collected from clinical records.

Results: 2 AF and 12 HF pts have been enrolled (6M, 8F). Median follow up: 39 months (1-553). Median fibrinogen activity/antigen level: 78 mg/dL (0-150)/73 mg/dL (0-140). 5 pts had epistaxis, hematomas, ecchimoses, menometrorrhagia, intra-abdominal bleeding, gum bleeding, hemarthrosis. Fresh frozen plasma (FFP), Fibrinogen Concentrate (FC), cryoprecipitate, whole blood, tranexamic acid were administered in the majority of these events.

One ischemic stroke, 1 lower limb arterial and 1 cerebral sinus thrombosis, 1concomitant aortic and inferior vena cava thrombosis occurred: 3 events during FC therapy, 1 during puerperium. Heparin, LMWH, anti-platelet agents, fibrinolytic agents, warfarin were then administered.

One gastrectomy, 1 lower limb amputation, 5 gynecological, 1 otorhinolaryngological and 1 plastic surgery were performed in 2 AF and 3 HF pts: in AF pts, FC or FFP prophylaxis was administered while in HF pts was not. Bleeding was observed after 2 surgeries performed without prophylaxis.

8 pregnancies occurred in 3 HF women: 2 vaginal deliveries (VD) and 2 cesarean sections (CS) were performed; 4 spontaneous abortions occurred. In 3 cases, FC prophylaxis and in 4 LMWH was administered during pregnancy. One venous thrombosis, 2 hemorrhages, 1 DIC, complicated 4 pregnancies. FC was administered at delivery and LMWH during puerperium, for the 2CS. No complications at delivery occurred.

Conclusions: AF/severe HF (≤50mg/dL) pts may experience significant hemorrhagic/thrombotic events, which are difficult to manage. The need exists to collect more data, to provide useful information to better characterize and manage these rare diseases.

PB 2028 | Factor X Deficiency Followed by a Tertiary Pediatric Hematology Center

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Background: Congenital factor X (FX) deficiency is a rare bleeding disorder inherited as an autosomal recessive trait with an incidence of 1 : 500 000-1 000 000. It is more common in populations with high rate of consanguineous marriages. A total or partial deficiency of FX causes an impairment of clot formation, leading to a haemorrhagic disease, which manifests with bleeding symptoms of different severity,also unprovoked.

Aims: We analysed the clinical manifestations, laboratory phenotype in 19 patients from Turkey affected with severe FX deficiency.

Methods: The clinical findings, laboratory data, management and outcome in a group of Turkish children diagnosed with FX deficiency in Hematology Clinic of Kanuni Sultan Suleyman Education and Research Hospital were evaulated.

Results: The most frequent bleeding episodes in patients were epistaxis and easy bruising. As a life threatening event intracranial bleeding was reported in 4 of our 19 cases. All of **3** adolescent girls have severe menorrhagia with accompanying iron deficiency anemia. Four patients have intramuscular bleeding, 3 patients have often gingival bleeding, two patients hemarthrosis and two patients bleeding due to operation. Four patients with mild deficiency didn't have any bleeding.One patient with severe intracranial bleeding and recurrent hemarthrosis is under PCC prophilaxis. The adolescent girls with menorrhagia are using oral contraceptives and often PCC during menstruation.

Conclusions: In frequent intracranial attacks prophylaxis is still discussed. There is limited number of reports about PCC prophylaxis. We think that, prophylactic treatment used for hemophilia patients should be considered as an initial therapeutic option for patients with rare factor deficiencies and a severe clinical course, and for those with a factor deficiency that can lead to severe bleeding.

PB 2029 | Correlation of Hemorrhagic Complications with Novel Mutations in FGA And FGG: In Punjabi Congenital Afibrinogenemia Patients

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Background: Congenital afibrinogenemia (OMIM # 202400) inherited as an autosomal recessive trait,manifests with little or absent levels of fibrinogen in plasma. Phenotypic expression of factor I deficiency is still not unanimously established in research literature globally due to vast variability in symptoms which may ranges from minimal bleeding to catastrophic hemorrhage.

Aims: To assess and establish the association between phenotype expression and identified genotype as a reference for future cases.

Methods: This descriptive and cross sectional study was complied with the Declaration of Helsinki, conducted in Karachi and Lahore. Patients with fibrinogen deficiency (Tested by fibrinogen functional assay from Laboratoire Stago, Asnieres, France) were screened for mutations in fibrinogen gene by direct sequencing. Bleeding score also calculated by using Tosetto'sbleeding assessment tool (Tosetto et al).

Results: Total 18 patients were evaluated with mean age group10±2 yrs. All patients were with markedly prolonged PT/APTT and fibrinogen levels (< 0.1g/l). The complication other than common bleeding symptoms in FGA was an intracranial bleed with a history of bone cyst while patient with mutation in FGG showed hemorrhagic appendix and repeated episodes of minor intracranial bleeds.

Conclusions: A high bleeding score is correlated well in both cases. The clinical manifestations of congenital afibrinogenemia in our local population are more or less same as reported in global literature. Few exceptions do exists as we are now observing the slight frequent occurrence of rare complications in our set of patients.

PB 2030 | Treatment of a Rare Case of Congenital A/Hypo Dysfibrinogenemia with Fibrinogen Concentrates: Comparison between Fibrinogen Levels and ROTEM® Parameters

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Background: Congenital hypodysfibrinogenemia is a rare autosomal recessive disorder characterized by extremely low levels of plasma clottable fibrinogen (Fg:Act) and disproportionate immunoreactive Fg (Fg:Ag) concentrations. Fg replacement therapy is effective for treating bleeding episodes in congenital Fg disorders and Fg concentrate has become a standard-of-care in several centers and is recommended for severe bleeding in surgical and trauma patients.

Aims: We report a case of a/hypodysfibrinogenemia in a woman treated with 2 gr of Fg concentrate (Haemocomplettan P®, CSL Behring) before a bone marrow biopsy and the effectiveness of treatment was monitored by Fg:Act, Fg:Ag and ROTEM®

Methods: 60-year-old female with a life-long mild disorder characterized by heavy menorrhagia and prolonged bleeding after tooth extractions. Her mother and a cousin presented similar symptoms. At age of 56, she had a large hematoma in the upper third of the left leg after a sprained knee. In 2010, she was diagnosed with non-Hodgkin lymphoma and underwent chemotherapy, being declared free of disease in late 2014. Laboratory studies showed prolonged prothrombin time, aPTT, thrombin and reptilase time but normal coagulation factors and degradation fibrinogen/fibrin products. Fg:Act and Fg:Ag levels were 15 and 40 mg/dL, respectively (reference range: 200-400 mg/dL).

Results: Minimal bleeding was reported with negligible estimated blood loss. No further treatment was administered and no bleeding complications were reported despite the low levels of Fg (< 100 mg/dL). No changes were observed 60 min after administration of Fg concentrates by ROTEM®. EXTEM, FIBTEM or INTEM parameters were compared to basal values.

Conclusions: In this patient with a/hypodysfibrinogenemia, Fg concentrate was not effective. The results provided by ROTEM showed a correlation with the levels of Fg: Act and Fg: Ag.

PB 2031 | A New Autosomal Dominant Inherited FVIII Defect: Evidence of a Novel Genetic Determinant of Circulating FVIII Levels

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Background: Inherited coagulation factor VIII (FVIII) deficiency is associated with hemophilia A due to mutations in the X-linked FVIII gene; von Willebrand disease (VWD) types 1 and 3, with little or no von Willebrand factor (VWF), the major transporter of circulating FVIII; type 2N VWD with a defective capacity of VWF to bind FVIII; LMAN1 or MCDF alterations causing a combined FV-FVIII defect. We describe a family with a FVIII deficiency caused by none of the above.

Aims: To genetically characterize the disorder and identify new genetic determinants of circulating FVIII levels.

Methods: We investigated the patients' hemostatic and genetic profile using DNA and RNA analyses, and we performed a genome-wide association study (GWAS).

Results: Fourteen family members were studied, and 8 of them (both males and females, from three generations) carried the FVIII defect. The proband, a 51-year-old man with a lifelong mild bleeding history (bleeding score=8), had low FVIII levels and a low FVIII/VWF:Ag ratio, inherited as an autosomal dominant trait (Table 1). All other hemostatic parameters explored were normal. No FVIII or VWF gene mutations were found. FVIII survival and clearance (after DDAVP

TABLE 1 Main hemostatic findings in the proband and some of his relatives

Patients	PT %	PTT sec	FVIII U/dL	VWF:Ag U/dL	FVIII:C ratio	VWF:CB U/dL	VWF:CB ratio	VWF:FVIIIB U/dL	VWF:FVIIIB ratio
Proband	96.7	43.9	27.4	61.3	0.45	57.5	0.93	60.5	1.09
Brother	90.0	42.3	82.0	186.7	0.44	167.1	0.89	210.9	1.13
Sister	63.1	46.1	41.0	92.3	0.45	78.7	0.86	84.4	0.92
Father	94.0	37.3	76.0	77.4	0.99	59.9	0.75	72.6	0.88
Mother	104.0	38.2	85.0	142.3	0.61	161.8	1.16	121.4	0.97
Normal range	80-100	24-36	60-160	60-160	≥0.75	65-150	≥0.75	65-150	≥0.75

infusion) were normal, and no anti-FVIII antibodies emerged. Whole-family GWAS revealed four genomic regions segregating with the defect (chr5:77,218,488-79,351,544; chr7:78,193,129-79,791,947; chr15:40,377,092-45,123,614; and chrX:142,475,363-155,270,560) spanning 21.3Mb (< 1% of the whole genome). The proteins currently known to be involved in FVIII synthesis, release, survival and catabolism all mapped outside these regions, ruling out their involvement as potential disease-causing genes.

Conclusions: Our results suggest a new mechanism and/or gene(s) involved in determining FVIII levels, that seems to have a role in the biosynthetic/secretory phases of FVIII production, mapping within a restricted genomic portion with clearly defined boundaries.

PB 2032 | Rare Bleeding Disorders: Snapshot from a Tertiary Care Hospital of Pakistan

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Background: The rare bleeding disorders (RBD) include inherited deficiencies of coagulation factors other than factor VIII and IX. RBD are transmitted in an autosomal recessive fashion and represent 3-5% of all inherited disorders. Patients with RBD can present with mild to severe life-threatening bleeding. RBD often remain undiagnosed and may result in fatal outcome.

Aims: To determine the frequency of RBD in Pakistani population presented to a tertiary care hospital.

Methods: A retrospective three years analysis was done from January 2014 till December 2016 at Aga Khan University Hospital, Pakistan. Ethical exemption was sought from institutional ethical review committee. Data was extracted by using ISD 9 coding for clotting factor deficiency including factors I, II, V, VII, X, XI, XII, XIII and combined deficiencies of factor V & VIII. Frequencies were generated for quantitative variables.

Results: Out of 335 patients with clotting factor deficiency, 44 (13%) were diagnosed with RBD. The mean age at presentation was 21 years (1 month-75 years). Male to female ratio was 1:1. Of 44 patients, 16 (37%) were found to be factor VII deficient followed by factor XIII,13 (33%); factor V and Fibrinogen, 5 (11%) each; factor X, 3 (7%); factor XI, 1(2%) and combined deficiency of factor V and VIII, 1 (2%). The most common presenting symptom was gum bleeding (34%), epistaxis (23%), hematuria (18%), intracranial bleeding (9%), joint bleeding (4%), bruising (7%) while 5% were asymptomatic and diagnosed on pre-operative workup of prolonged coagulation profile.

Conclusions: Thirteen percent patients were diagnosed with RBD, factor VII deficiency (37 %) being the most common. The data on rare bleeding disorders would serve to constitute RBD database registry on national level that will help in genetic counseling, early diagnosis, validation of bleeding scores and evaluation of bleeding risk assessment; especially prior to surgical interventions and formation of consensual therapeutic measures.

PB 2033 | Severe Congenital Factor VII Deficiency in Northern Region of Turkey

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Background: Congenital factor VII deficiency is a rare genetic coagulation disorder with a prevalence of 1/500.000 population. However, it is possible more common in populations in which consanguineous marriage ratio is high. Thus, frequency will be different in each country. Patients with factor VII deficiency may have severe bleeding symptoms or be asymptomatic.

Aims: In this study, we present clinical presentation and demographic features of our patients of factor VII deficiency retrospectively.

Methods: We retrospectively reviewed medical records of 12 patients with FVII deficiency in Ondokuz Mayis University, Department of Pediatric Hematology between 2005 to 2014 and discussed with the literature.

Results: Twelve patients (7 male and 5 female) with FVII deficiency were included in the study. Ages at diagnose were ranged from six month to sixteen years. Median factor VII level was 5.6% and ranged from 2% to 17.4%. Mucocutaneous bleeding was the most common clinical presentation. Four cases undergo surgical operations, four circumcision and one thyroid biopsy. Activated factor VII were used for bleeding control. Activated factor VII dose was 30 mcg/kg/dose in 4 hour interval and total 6 doses in circumcision operations; and 30 mcg/kg/dose in 4 hour interval total 3 doses in thyroid biopsy. Bleeding control was good in operations.

Conclusions: Our cases in this study had relatively less bleeding episodes and no patient has a severe bleeding episode. We didn't see any

thromboembolic complications during and after therapy. No bleeding was seen after surgery. FVII deficiency is relatively frequent in Turkey. Most of them is mild and asymptomatic cases. Presented cases is severe cases but they have no severe bleeding history and a prophylaxis requirement.

PB 2034 | Evaluation of Rare Factor Deficiencies in Presurgical Plasma Samples

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Background: Rare factor deficiencies account for 3% to 5% of all inherited coagulation factor deficiencies. Most asymptomatic cases of rare factor deficiency are diagnosed based on PT and APTT.

Aims: We retrospectively investigated the results of rare factor levels in patients who had prolonged PT and/or APTT on preoperative screening.

Methods: Data were collected from 719 patients who, over a 1-year-period, had been referred to our hemostasis laboratory to determine the cause of prolonged PT and APTT in presurgical plasma samples. The samples were analyzed in an ACL TOP 700 coagulation analyzer. All patients had normal lupus anticoagulant tests. Factor deficiency samples were classified based on factor activity levels below 50% of the lower limit of our laboratory's range.

Results: Fifty-six patients (median age, 8 years; range, 1-21 years; 54% males) were identified as having rare factor levels. Among these cases, the respective frequencies of FVII deficiency, FXI deficiency, FXII deficiency, and FV deficiency were 76%, 14%, 6% and 4%. The level of FVII below 40% was observed in 23/56 (41%) patients. PT was 4s longer than the upper limit of our laboratory's normal range in 16 cases of FVII level below 30%, PT was prolonged by 2-4 s in 24 cases of FVII level 30% to 50%, and PT was prolonged by less than 2 s in three cases of FVII level below 15%. APTT was 4 s longer than the upper limit of our laboratory's normal range in all cases of FXI and FXII deficiency with factor level below 50%. Both PT and APTT were prolonged more than 4 s in two cases of FV level below 1%.

Conclusions: The frequency of rare factor deficiencies in our study is similar to previous reports. Measurement of rare clotting factor levels may not be necessary in samples with mildly (< 4 s) prolonged APTT, particularly in cases of presurgical screening.

PB 2035 | Spotting of Two Novel Mutations in FGG Gene: In Unrelated Patients of Congenital Afibrinogenemia in Pakistan

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Background: Congenital afibrinogenemia (OMIM # 202400) transmitted as autosomal recessive trait is considered as a rare inherited bleeding disorder with little or absent levels of fibrinogen in circulating blood. The swiftly increasing numbers of new cases of this disorder in Pakistan in strong association with consanguinity is now placing a big question mark on its rarity.

Aims: To screen FGG gene for possibly existing mutations after sequencing FGA and FGB genes.

Methods: This descriptive and cross sectional study was conducted in Karachi and Lahore, fully complied with the Declaration of Helsinki. Patients with fibrinogen deficiency (Tested by fibrinogen functional assay from Laboratoire Stago, Asnieres, France) were screened for mutations in fibrinogen gamma (FGG) genes by direct sequencing after sequencing of FGA and FGB.

Results: Out of 18patients, three were found to have novel mutations in FGG. These mutations are so far the first to be detected and reported in FGG in Pakistan. Out of three, two mutations are Nonsense and were identified in two siblings and one is a frameshift mutation.

Conclusions: Congenital afibrinogenemia is a rapid growing problem in countries such as Pakistan where consanguinity is frequently practiced. This study illustrates the fact that the incidence of mutations occurring in FGG is also growing fast in our populationand these are no longer rare mutations.

PB 2036 | Factor VII Deficiency: One Novel Mutation and Genotype-phenotype Correlation in Patients from Southern Italy

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Background: Inherited FVII deficiency is an autosomal recessive trait and constitutes the most frequently diagnosed rare coagulation disorder. Genotype and phenotype are poorly correlated.

Aims: To add insights into molecular and clinical aspects of FVII deficiency. Here, we present molecular and clinical findings of 7 apparently unrelated patients with FVII deficiency.

Methods: From 2013 to 2016, 7 (4 male, 3 female) subjects were referred to our Centre because of a prolonged PT identified during routine or pre-surgery examinations or to undergo investigations for a bleeding episode. Informed consent was provided by patients. Mutation characterization has been performed by using a series of *in silico* applications: PROMO, SIFT, and Polyphen-2. Structural changes in FVII protein were analysed by using the SPDB viewer tool.

Results: We identified 3 compound heterozygous/homozygous and 4 heterozygous individuals with FVII deficiency. Overall, molecular investigations revealed 8 mutations. To the best of our knowledge, 1 mutation identified is novel: the c.1199G>C (p.Cys400Ser) mutation

within the exon 9. Also, we identified the c.-54G>A polymorphism (rs367732974) in *F7* 5'-upstream region, that has been never reported in FVII deficiency, although in EXAC database, this polymorphism shows an allele frequency < 1%. *In silico* predictions identified differences in binding sites for transcription factors for the c.-54G>A variant. In a silico approach, by means of the SPDB viewer tool, we visualized the possible damaging effect of p.Cys400Ser on FVII active conformation, leading to a breaking of a disulphide bridge. Also, we identified the c.-30A>C, c.430+1G>A, p.Val214Gly, p.Ala304Val, p.Gly391Ser, p.Cys400Ser, c.805+1G>A mutations. We recorded a CNS bleeding. Bleeding tendency was heterogeneous and no genotype/phenotype relationship have been observed.

Conclusions: Our findings further suggest that independently of the mutation affecting FVII levels, environmental factors heavily influence the clinical phenotype.

PB 2037 | Successful Delivery in an Afibrinogenemia Patient after Three Abortions: A Case Report and Review of Literature

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Background: Afibrinogenemia is accompanied with miscarriage and prophylaxis is recommended as soon as pregnancy is confirmed.

Aims: We present an afibrinogenemia patient with a novel mutation having successful delivery after three abortions.

Methods: A 23 -year- old female who was diagnosed as an afibrinogenemia at the age of 5 years old. The genetic analysis showed a homozygote new nonsense mutation [g.4347G>T; Gly358Stop]). The patient was on prophylaxis on the third pregnancy starting at gestational age of 7 weeks but was unsuccessful.

Fibrinogen concentrate (Haemocomplettan P, CSL Behring) was given with dose of 60 mg kg^{-1} thorough her 4th pregnancy. The total dose or frequency (one to three times per week) was increased as pregnancy advanced based upon her weight and fibrinogen activity levels. Tranexamic acid intravenously (15 mg kg^{-1}) was started one hour prior to delivery followed by 1 mg/kg/hour continuous infusion for 48 hours. It was switched to oral throughout post-delivery day 7.

Results: NTProphylaxis was commenced at gestational age of 4 weeks for her 4th pregnancy. Our case had the keep target level of between 50-100 mg dl^{-1} during first and second and 100 mg dl^{-1} during third trimesters and at term (table 1). The patient had no bleeding event during pregnancy leading to successful normal vaginal delivery at gestational age of 40 weeks.

Conclusions: Maintaining fibrinogen trough level of 50-100 mg dl^{-1} during pregnancy with the aim of keeping level of 100 mg dl^{-1} as pregnancy ended seems to be safe. This case report underscores prophylaxis should be started early in pregnancy. Moreover, normal vaginal delivery is superior to cesarean section because of lesser risk of thrombosis if clinically implemented. Antifibrinolytic drugs might be an alternative option to hold fibrinogen concentrate and reduce the risk of thrombosis if not contraindicated. This strategy was successful in our case without any thrombosis or significant bleeding events.

PB 2038 | Are Screening Coagulation Tests Useful to Predict the Clinical Phenotype in Patients with A/Hypo/Dysfibrinogenemia? A Single Institution Experience

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Background: A/hypo/dysfibrinogenemia (a/hypo/dys) is a rare congenital disorder characterized by mutations in any of the three fibrinogen (Fg) genes (FGA, FGB, FGG) that encode the three polypeptide chains (Aα,Bβ,γ) of the fibrinogen molecule. Most carriers of this disorder are asymptomatic (A), while a few patients may present either thrombotic (T) or bleeding (B) disorders.

Aims: We intended to establish whether the use of standard coagulation assays allows predicting the clinical phenotype in 17 patients with a/hypo/dys and thus, improving the use of the therapeutic resource.

Methods: Patients were recruited at Sanatorio Allende, Córdoba, Argentina and 4, 2 and 11 of them respectively presented different mutations in *FGA*, *FGB* and *FGG* genes; the clinical phenotypes were: 9 A, 3 T and 5 B. Prothrombin time (PT); aPTT, thrombin and reptilase time (TT and RT) as well as functional and antigen Fg (Fg:f and Fg:Ag) were performed using standard procedures.

Results: TP and aPTT showed normal or slightly prolonged times while TT and RT were significantly prolonged with a mean of 65 sec (range: 17-200 sec, normal range: 11-14 sec) and 45 sec (range: 15-75 sec, normal range: 12-15 sec), respectively. Fg:f and Fg:Ag showed an average of 92 mg/dl (range: 9-170 mg/dl, normal range: 200-400 mg/dL) and 168 mg/dl (range: 31-301 mg/dl, normal range: 200-400 mg/dl), respectively.

Conclusions: Unfortunately, screening clotting assays do not allow to predict the clinical phenotype in a/hypoDysFg patients and are also unhelpful for improving early prophylaxis to protect them from thrombotic and/or hemorrhagic events.

PB 2039 | Congenital Afibrinogenemia Case Presenting with Surrenal Bleeding and Cervical Hematoma

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Background: Congenital afibrinogenemia is a rare autosomal recessive disorder. Clinical manifestations vary in severity.

Aims: Here we present a congenital afibrinogenemia case who presented with surrenal bleeding and cervical hematoma.

Methods: -

Results: A 50 day baby presented with a cervical mass. He was born full -term from second degree consanguineous parents. His history revealed severe umblical cord bleeding. Plasma fibrinogen level was undetectable. Cervical USG revealed a hematoma which caused torticollis. Abdominal USG showed a surrenal mass consistent with hematoma. Prophylactic treatment with plasma derived fibrinogen concentrate was started. Surrenal hematoma regressed and disappeared on follow-up. Cervical hematoma regressed however persisted under treatment. Physiotherapy was started under factor replacement.

Conclusions: Afibrinogenemia is usually associated with mild to moderate bleeding and patients may present with umblical cord, gum and nose bleeding. There is still no consensus on standart treatment approach. Some patients may have a more severe phenotype and experience intracranial bleeding or atypical presentation. Secondary prophylactic replacement therapy is recommended in selected cases however optimal fibrinogen level to prevent spontaneous bleeding is yet to be investigated.

PB 2040 | Severe Congenital Factor X Deficiency in the Northern Region of Turkey: Successful Prophylaxis with Activated Prothrombin Complex Concentrates

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Background: Congenital factor X (FX) deficiency is a rare autosomal recessive bleeding disorder that is estimated to occur in a frequency of 1:1 000 000. Bleeding symptoms in patients with FX deficiency range from a mild bruising tendency and intracranial haemorrhage.

Aims: In this study, we evaluated children with severe congenital FX deficiency in our Pediatric Hematology Department which is a tersier reference center in the northern region of Turkey.

Methods: Between last ten-year period, three patients have been diagnosed as severe congenital FX deficiency in our center. All of them had been admitted to hospital spontaneous life-threatening intracranial haemorrhage (I) in a few days old. INR and APTT were both markedly prolonged. All coagulation factors analysis demonstrated normal results, except for FX activity (FX:C)< 1%. The family histories revealed a consanguineous marriage in the parents. The children were treated and operated with fresh frozen plasma (FFP) and activated prothrombin complex concentrates (aPCC). But a few days later, spontaneous life-threatening ICH were recurred and treated and operated.

Results: In Turkey, because purified FX concentrate and prothrombin complex concentrate is not yet available, FFP are the main therapeutics used. For patients who suffer intra-cranial haemorrhage, FFP infusion results in severe squeal. FFP is no available in living site of patient, compliance will be poor and long-term use of FFP has other risk. For this reason, we seek an alternative treatment for prophylaxis. aPCC contain some amount of factor X. It is a commercial product and ready to use in Turkey as bypass agent. Our patient can be used this concentrate We obtain an approval for each patient from national health authority to use off-label aPCC prophylaxis two times a week, 50IU/kg/dose.

Conclusions: Our patients were used aPCC prophylaxis for 10, 4 and 2 years respectively. They did not experience any new bleeding under prophylaxis. We think that aPCC may be used in prophylaxis of FX deficiency.

PB 2041 | Pseudoxanthoma Elasticum-like Disease with Deficiency of Vitamin K-dependent Clotting Factors, Hypofibrinogenemia and Cutis Laxa Features: A Case Report

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Background: Pseudoxanthoma elasticum (PXE)-like syndrome is characterized by the association of PXE and cutis laxa (CL) features with a deficiency of vitamin K-dependent clotting factors. It was first described in 1971 and was identified as a distinct genetic entity in 2007 with analysis of the GGCX (-glutamyl carboxylase) gene, which is involved in congenital defciency in vitamin K-dependent clotting factors. Here we report a new case of this extremely rare syndrome.

Aims: A 30-year-old female patient was seen for the emergence for recurrent cardiac tamponade after pericardial and pleural puncture and pericardial drainage; clinical survey found signs of slight panhypopituitarism. The interrogation of patient revealed gastrointestinal bleeding in 2015.

Physical examination revealed yellowish papules and plaques and retinal angioid streaks. A skin biopsy revealed polymorphous and fragmented elastic fibers in the reticular dermis. These were mineralized, as was demonstrated by Von Kossa staining.

Methods: Laboratory tests showed insufficient thyroid-stimulating, low cortisol, a hypogonadism and low coagulation factors.

VitaminK-dependent clotting factor deficiency led us to a diagnosis of PXE-like syndrome. A molecular study of the GGCX gene showed compound heterozygosity.

Results: The GGCX gene is usually responsible for PXE-like syndrome. GGCX encodes a -

glutamyl carboxylase necessary for activation of gla-proteins. Gla-proteins are involved both in coagulation factors in the liver and in the prevention of ectopic mineralization of soft tissues. Uncarboxylated forms of gla-proteins in fibroblast would thus enable mineralization and fragmentation of elastic fibers.

Conclusions: Digestive manifestations are unusual; however, pseudoxanthomaelasticum should be considered in all cases of gastrointestinal bleeding for no apparent reason. Early diagnosis allows prevention and measures to control the risk factors and limit the progression of complications.

PB 2042 | Rare Inherited Coagulation Disorder (RICD) in Females - Data from a Developing Country

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Background: Clinical spectrum of RICD is less clearly defined in females. Pakistan has poor health resources and a high rate of consanguinity. This makes the likelihood of RICD relatively higher in our population. Due to the societal pressure to marry and reproduce at an early age, the risk of bleeding during childbirth is also increased.

Aims: To assess the clinical spectrum of RICD in females registered at a not-for-profit organization.

Methods: In this case series from 1st October till 31st December 2016, we analyzed retrospective data collected from medical record files. Tests performed included prothrombin time (PT), activated partial thromboplastin time, mixing studies, thrombin time, factor assay, clot solubility test in 5 M urea and fibrinogen level. Socio-demographic data, bleeding phenotype and treatment details were also recorded.

Results: Twenty three females with RICD were identified. Of these, 5 (22%) were below 12 years of age and 18 (78%) were in the reproductive age group (12 to 49 years). History of consanguineous marriage was present in 10 (44%). Median age of onset of the first episode of bleeding was at 2 years (range; 2 months to 12 years). Factor V deficiency (n=5, 22%) was the most frequent followed by factor X deficiency (n=4, 17%), hypofibrinogenemia (n=4, 17%), afibrinogenemia (n=4, 17%), factor VII (n=2, 9%) and factor XIII deficiency (n=2, 9%). Based on the results of mixing studies, factor X and factor V deficiency were suspected in n=2 (9%) females. Mucocutaneous bleeding (n=19, 83%) was the most common presentation. Menorrhagia (n=3, 13%), umbilical stump (n=7, 30%) and musculoskeletal (n=10, 44%) bleeding were also present. Miscarriage (n=1) and postpartum haemorrhage (n=1) occurred in females with afibrinogenemia and factor V respectively. All females were treated with fresh frozen plasma and cryoprecipitate.

Conclusions: The most common RICD in females was factor V deficiency. Clinical spectrum was different from that of western population. Treatment was mainly with blood components.

PB 2043 | Congenital Factor V Deficiency

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Background: Congenital FV deficiency is rare bleeding disorder with an estimated incidence of 1 in 1000000. There is a variable spectrum of bleeding manifestations ranging from epistaxis to life-threatening hemorrhages. Severity is defined as mild (> 5% of factor activity), moderate (1%-5%) and severe (< 1%).

Aims: To assess the clinical-laboratory features of FV deficiency in 2 patients.

Methods: A 25- year-old woman (patient A) and a 30-year- old man (patient B) were examined. Patient A suffers from menorrhagia. Bleeding episodes of patient B consist trauma-related bleeds, hemartroses, muscular hematomas, gastrointestinal bleeding. Both have a negative family history for bleeding disorder.

Were performed standard coagulation assay, as well as global hemostatic tests: activated partial thromboplastine time (APTT), prothrombin ratio (PR), international normalized ratio (INR), thrombin time (TT), concentration of fibrinogen, factors of prothrombin complex, thromboelastography (TEG) and rotational thromboelastometry (ROTEM).

Results:

Data of patient A: APTT 35s (29-36); PR 53,4%(70-120), INR 1,44(0,85-1,35); FV 34% (70-120). Parameters of TEG (R, K, α MA, LY 30) and ROTEM (EXTEM, INTEM: CT, CFT, α, A10, A20, MCF, ML) are normal.

Data of patient B: APTT 155s; PR 12%; INR 5,18; FV 0,4%. Parameters of TEG are changed: α 7,9° (22-58); R 75 min (9-27); MA 34mm (44-64).

Results of ROTEM are changed too. EXTEM: CT 697s (38-79); CFT 425s (34-159); α 33° (63-83), A10 28mm (43-65); A20 47mm (50-71). INTEM: CT 1490s (10-240); CFT 299s (30-110); α 43° (70-83), A10 33mm (44-66); A20 43mm (50-71).

Conclusions: Patient A has mild FV deficiency according to factor FV activity level and mild bleeding phenotype of the disease. Patient B has severe FV deficiency according to factor FV activity level and severe bleeding phenotype of the disease. In both cases, the FV activity level has correlation with the severity of bleedings. The activity FV reduction by half does not cause changes in the TEG and ROTEM.

PB 2044 | Von Willebrand Factor Deficiency Reduces Experimental Liver Fibrosis in Mice

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Background: Liver diseases are associated with complex changes in the hemostatic system and elevated levels of the platelet-adhesive protein Von Willebrand factor (VWF) are reported in patients with acute and chronic liver damage. Although elevated levels of VWF are associated with fibrosis in the general population, the role of VWF in acute and chronic liver injury has not been examined in depth in experimental settings.

Aims: We tested the hypothesis that VWF deficiency inhibits experimental liver injury and fibrosis.

Methods: Wild-type and VWF-deficient mice were challenged with a hepatotoxic dose of carbon tetrachloride (1 ml/kg; CCl_4) either once to induce acute liver injury, or twice weekly for six weeks to induce liver fibrosis.

Results: VWF deficiency did not significantly affect acute CCl_4-induced hepatocellular necrosis in mice. Chronic CCl_4 challenge, twice weekly for 6 weeks, significantly increased hepatic stellate cell activation and collagen deposition in livers of wild-type (WT) mice. Interestingly, hepatic induction of several profibrogenic and stellate cell activation genes was attenuated in VWF-deficient mice. Moreover, birefringent sirius red staining (indicating type I and III collagens) and type I collagen immunofluorescence indicated a reduction in hepatic collagen deposition in CCl_4-exposed VWF-deficient mice compared to CCl_4-exposed WT mice.

Conclusions: The results indicate that VWF deficiency attenuates chronic CCl_4-induced liver fibrosis without affecting acute hepatocellular necrosis induced by this classic hepatotoxicant. Moreover, the results constitute experimental evidence to suggest that elevated plasma VWF levels in patients with cirrhosis are mechanistically linked to the progression of liver fibrosis.

PB 2045 | Genotypical Classification of Patients with von Willebrand Disease

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Background: Von Willebrand disease (vWD) is a bleeding disorder caused by inherited defects in the concentration, structure or function of Von Willebrand Factor (vWF). vWD can be divided in three subclasses, but classification remains difficult.

Aims: We improved vWD classification by performing *VWF* direct sequencing for diagnostic purposes.

Methods: Targeted gene analysis was performed on 504 patients suspected to have vWD, either by classical Sanger sequencing or by ion semiconductor next generation sequencing technology. All coding regions including intron-exon boundaries were sequenced. Large deletions/duplications in the *VWF* gene were detected by MLPA. Recently whole exome sequencing (WES) was implemented to search for *VWF* and other genetic abnormalities.

Results: 504 patients were analysed. 311 patients had a *VWF* genetic abnormality. Mutations found were 11 nonsense, 299 missense and 9 splice-site mutations, 25 small deletions, 7 duplications and 15 large deletions detected by MLPA. 193 patients had no mutations in the *VWF* gene. Of all mutations detected 315 were reported previously and 51 were novel. The novel mutations found were 3 nonsense, 29 missense and 4 splice-site mutations, 3 duplications and 12 small deletions. 78 patients were carrying the D1472H polymorphism affecting the von Willebrand Factor Ristocetin cofactor activity. The first WES results showed combinations of *VWF* abnormalities with other autosomal recessive abnormalities in genes involved in other primary hemostasis disorders like Hermansky Pudlak Syndrome.

Conclusions: Our systematic targeted genotypic approach resulted in 62% of the cases in vWD confirmation and in 91% of the confirmed cases a vWD classification. Since the overall yield to detect (likely) pathogenic mutations was only approximately 60% with the conventional targeted gene analysis approach and bleeding scores do not always match, we recently implemented WES. That approach resulted in additional genetic abnormalities affecting primary hemostasis including vWD.

PB 2046 | Common VWF Sequence Variants Associated with Higher VWF and FVIII Are Less Frequent in Subjects Diagnosed with Type 1 VWD

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Background: Genetic variation in the *VWF* gene is associated with von Willebrand Factor (VWF) and factor VIII (FVIII) levels in healthy individuals.

Aims: We hypothesized that *VWF* DNA variants associated with higher VWF or FVIII could impact the diagnosis of type 1 von Willebrand disease (VWD).

Methods: We examined VWF antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo), VWF propeptide (VWFpp), and FVIII levels along with *VWF* gene sequencing in subjects enrolled in the Zimmerman Program for the Molecular and Clinical Biology of VWD.

Results: We found *VWF* c.2880G>A associated with higher VWF (means 149, 107) and FVIII levels in healthy controls ($p < 0.001$), as previously reported. *VWF* c.2880G>A was significantly more common in controls (36% European American, 61% African American) than in subjects diagnosed with type 1 VWD and VWF:Ag < 30 (20%, $p < 0.005$). Two other *VWF* variants in linkage disequilibrium, c.2365A>G and c.2385T>C, were associated with higher VWF and FVIII ($p < 0.001$). *VWF* c.2365A>G was also more common in healthy controls (49% European American, 88% African American) than type 1 VWD subjects (35%, $p < 0.001$). VWF:Ag, VWF:RCo, and FVIII were not statistically different in type 1 VWD subjects who had these *VWF* variants compared to type 1 VWD patients without them. There was no difference in ABO blood group, VWFpp levels (excluding subjects with known VWF clearance defects), or bleeding score using the ISTH bleeding assessment tool. When other pathogenic *VWF* sequence variants were excluded, similar results were obtained. *VWF* variant frequencies were similar to healthy controls for subjects with a historical diagnosis of type 1 VWD but current VWF:Ag >50 or with a diagnosis of type 2 VWD.

Conclusions: These data suggest that certain *VWF* sequence variants associated with elevated FVIII and VWF levels may be protective against a diagnosis of type 1 VWD. These findings were independent of other sequence variants in *VWF*, supporting the independent effect of c.2880G>A and c.2365A>G/c.2385T>C on VWF levels.

PB 2047 | Interactomics of the Weibel-palade Body Exocytotic Machinery

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Background: Endothelial cells store Von Willebrand factor (VWF) in elongated secretory organelles, so called Weibel-Palade bodies (WPBs). Upon vascular damage or stress WPBs undergo exocytosis, which is orchestrated by a complex network of proteins consisting of Rabs, Rab-effectors and the SNARE complex. Genome wide association studies (GWAS) for genetic determinants of VWF levels have identified a number of new regulators that are part of the SNARE complex (STXBP5 and syntaxin-2) arguing that exocytosis of WPBs is a significant determinant of VWF levels. We have previously identified STXBP1, syntaxin-2 and syntaxin-3 as downstream targets of the Rab27A-Slp4-a complex which promotes WPB exocytosis. Despite the overlap between mechanistic and GWAS studies, we currently do not know the exact composition of the WPB exocytotic machinery and how all these individual components together orchestrate WPB release.

Aims: We used an unbiased iterative interactomics approach to identify new components of the WPB exocytotic machinery to further elucidate the mechanisms that control VWF secretion.

Methods: Lentivirally expressed mEGFP-tagged fusion proteins were pulled down from endothelial cells using GFP nanobody conjugated magnetic beads. Specific interactors were identified using mass spectrometry.

Results: We determined the endothelial interactome of syntaxin-3 and identified a number of SNARE proteins (NSF, SNAP23, STXBP2 and STXBP5) as well as regulators of microvilli formation. Using pull-downs we show that the C-terminal VAMP-like domain (VLD) of STXBP5 is indispensable for the interaction with syntaxin-2, -3 and -4. Subsequent proteomic profiling of the STXBP5 VLD interactome identified a large number of additional SNARE proteins. STXBP5 N436S, a variant containing a nonsynonymous SNP that is linked to decreased VWF levels, still interacts with syntaxin-2, -3 and -4.

Conclusions: Our data provide mechanistic detail on how SNAREs proteins regulate release of VWF from endothelial cells.

PB 2048 | Structural Thermodynamics of a Clinically Elusive High-affinity von Willebrand Factor Binding to Platelet GPIbα

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Background: Mutation of the cysteines forming the disulfide loop of the platelet GPIbα adhesive A1 domain of von Willebrand factor causes quantitative VWF deficiencies in the blood and von Willebrand disease. Two patients with C1272W and C1458Y mutations presented with DDAVP-induced severe thrombocytopenia that quickly relapsed to normal platelet counts and deficient plasma VWF.

Aims: The focus of this study is to determine how loss of the VWF A1 domain disulfide bond induces gain-of-function and identify the structural determinants for high-affinity by comparing the structural and functional properties of normal disulfide intact A1 with a reduced and carboxyamidated A1 that blocks the formation of the disulfide.

Methods: Using surface plasmon resonance, analytical rheology, and hydrogen-deuterium exchange mass spectrometry (HXMS), we decipher thermodynamic mechanisms of A1-GPIbα mediated platelet adhesion and resolve dynamic secondary structure elements that regulate the binding pathway.

Results: Constrained by the disulfide, dynamic selection between weak and tight binding conformations of A1 by GPIbα takes precedence and drives normal platelet adhesion to VWF. Unrestrained, loss of the disulfide partially disorders A1 and preferentially diverts

binding through an induced fit pathway with high-affinity contacts between locally disordered structures in both A1 and GPIbα. HXMS identifies an asymmetry of structurally dynamic and ordered regions common to both variants indicating that A1 lacking the disulfide retains native-like structural properties in the partially disordered state. Firm platelet adhesion to VWF is complemented by the dynamic flexibility of GPIbα and its ability to efficiently sample disordered conformations of A1 for high affinity contacts.

Conclusions: With these methods in place, structure-resolved mechanistic phenotyping of VWD becomes a real possibility that when combined with genotyping enables the prediction of how VWD patients manifest the symptoms of disease.

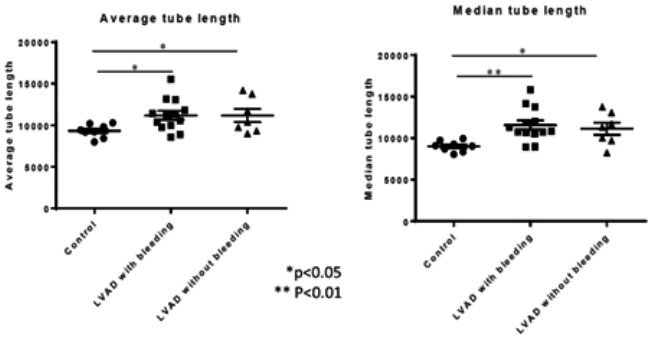

FIGURE 1 Plasma angiogenesis assay results in LVAD and control plasma

PB 2049 | Pro-angiogenic Signal in Plasma of Left Ventricular Assist Device Patients

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Background: All continuous flow left ventricular assist device (LVAD) patients have loss of high molecular weight multimers (HMWM) of von Willebrand factor (VWF),.20-40% develop gastrointestinal bleeding (GIB), and arteriovenous malformation (AVM) is the most common finding. AVMs are a known complication of congenital von Willebrand disease(CVWD), and CVWD plasma is pro-angiogenic in vitro.

Aims: We tested the hypothesis that LVAD plasma would emit an exaggerated pro-angiogenic signal.

Methods: We compared results of plasma angiogenesis assays (PAA), an in vitro assay that stimulates endothelial cells to proliferate and form new blood vessels in matrigel, and angiogenic cytokine/protein levels in 20 LVAD patients, compared to those of 8 controls.

Results: PAA experiments revealed increases in average tube length and median tube length in the LVAD plasma compared to controls, with a trend toward more tube formation in LVAD patients with a history of GIB (figure).

Loss of VWF HMWM was found in all LVAD patients, but in none of the controls, and LVAD patients also had far higher total VWF antigen levels compared to controls (188±72 U versus 89±20 U, p< 0.001). Compared to control plasma LVAD plasma showed significantly increased pro-angiogenic cytokines, endothelin 1, angiopoietin 2, and macrophage inhibitory protein 1a. VEGF2 levels did not show significant differences in the LVAD plasma when compared to the controls. Surprisingly, there were also significant elevations of anti-angiogenic proteins; tissue inhibitors of metalloproteinases 1 and 2, and endostatin (p< .01). These cytokine signals in LVAD plasma also tended to be the strongest in the LVAD patients with GIB.

Conclusions: As in CVWD, LVAD plasma increases in vitro angiogenesis. In LVAD patients, several pro-angiogenic cytokines are amplified, but not VEGF2, as are compensating anti-angiogenic factors, such as endostatin, suggesting a feedback loop in this pathologic condition.

PB 2050 | Characterisation of Q1541R, a Novel von Willebrand Factor Variant Causing Type 2A von Willebrand Disease - ADAMTS13 Cleavage of Type 2A Variants under Shear Stress

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Background: Type 2A Von Willebrand Disease (VWD) is characterised by loss of high molecule weight multimers caused by either defects in dimer or multimer formation, or enhanced susceptibility to ADAMTS13 proteolysis. Here we describe a novel VWF variant, Q1541R located in the A2 domain of VWF that results in a moderate to severe bleeding phenotype.

Aims: To characterise the effect of the Q1541R mutation on VWF function and assess cleavage under shear stress.

Methods: Expression of wild type (wt)VWF or VWF-Q1541R in HEK293 cells was assessed by VWF ELISA & pseudo Webiel-Palade body formation examined by confocal microscopy. Multimer formation, collagen binding, GPIb binding & static ADAMTS13 cleavage assays were performed using standard methods. Unfolding of the A2 domain was determined using optical tweezers. A shear based assay system was used to investigate the effect of the mutation on VWF function under shear stress.

Results: VWF-Q1541R was expressed at a marginally lower level than wtVWF, but formed normal storage vesicles in HEK293 cells & exhibited a full range of multimers with normal collagen & platelet binding function. Under static conditions VWF-Q1541R was more susceptible to ADAMTS13 proteolysis & required less force to unfold the A2 domain. Furthermore when HEK293T cells were co-transfected with ADAMTS13, the type 2A variants were proteolysised significantly faster. Under shear stress VWF-Q1541R mediated comparable VWF mediated platelet capture to collagen. Intriguingly,

when VWF-Q1541R was perfused over collagen with ADAMTS13, although a reduction in platelet capture was observed the reduction was similar to that seen with wild type VWF. Similar observations were also made with two common type 2A variants G1629E and E1638K.

Conclusions: The novel Q1541R variant results in VWD due to enhanced ADAMTS13 proteolysis. Interestingly, the increased ability of ADAMTS13 to proteolyse type 2A variants is lost when VWF is exposed to a collagen surface under shear stress.

PB 2052 | FVIIIa Mimicking Bispecific Antibody (Emicizumab) Improved Thrombus Formation of Type 2N von Willebrand Disease (VWD) under Both High and Low Shear Conditions

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Background: Type 2N VWD is characterized by low FVIII:C attributed to the defect in the ability of VWF to bind FVIII and the phenotype is similar to mild and moderate hemophilia A. emicizumab is a humanized bispecific antibody that promotes coagulation by bridging FIXa and FX, replacing the function of missing FVIIIa. Threfore, we hypothesized that emicizumab may benefical for the management of type 2N VWD.

Aims: We performed the *ex vivo* flow chamber experiments to analyze the effect of emicizumab in type 2N VWD.

Methods: Whole blood obtained from patients with type2N VWD (n=5) was perfused into the collagen-coated flow chamber with or without FVIII/VWF, FVIII, VWF and emicizumab under high($2500s^{-1}$) and low ($50s^{-1}$) shear conditions. Formed thrombus was fixed and immunostaining was performed to visualize platelets, VWF and FVIII. Thrombi were observed with confocal laser scanning microscopy. The obtained images were analyzed by Image Pro Premier 3D, and surface coverage (SC) and average thrombus height (HT) were calculated.

Results: Type 2N VWD demonstarated defective thrombus formation under both shear (high: SC 13%, TH 0.91μm, low: SC 3.8%, TH 0.47μm). Addition of FVIII/VWF (final concentration; *f.c.* 1U/mL) most improved thrombus formation. Addition of VWF (*f.c.* 1U/mL), or FVIII (*f.c.* 1U/mL) demonstrated partial improvement (high; SC 31, 28, 28%, TH 3.2, 2.4, 2.4 μm, low; SC 7.0, 3.7, 8.5%, TH 0.63, 0.42, 0.62 μm, respectively in order). Addition of emicizumab (100 μg/mL) improved thrombus formation under both high and low shear conditions (high; SC 27%, TH 2.8μm, Low; SC 8.4%, TH 0.42μm).

Conclusions: The reason why emicizumab improved thrombus formation especially under high shear can be explained by its rapid function since emicizumab is readily active and does not require the activation process. Therefore, emicizumab could be an alternative therapy for type 2N VWD.

PB 2053 | Perioperative Management of von Willebrand Patients with Desmopressin; Towards a Predictive Population PK Model

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder. In low to moderate risk procedures, patients in whom desmopressin (DDAVP) has been proven effective in a test, can be treated with DDAVP, aiming for FVIII/VWF target levels as defined in Dutch National Guidelines.

Aims: To evaluate perioperative management with DDAVP in VWD patients in relation to FVIII/VWF target levels, in order to improve prediction of effect by construction of a population pharmacokinetic (PK) model.

Methods: In this retrospective observational cohort study, VWD patients (historical VWF levels ≤0.30 $IUmL^{-1}$ or FVIII ≤0.40 $IUmL^{-1}$) treated in the Hemophilia Treatment Center of Erasmus University Medical Center- Sophia Children's Hospital undergoing a procedure between 2000-2016 were included. DDAVP dosing and achieved perioperative FVIII/VWF levels were compared to target levels.

Results: A total of 159 procedures in 79 patients were analyzed (Table 1). FVIII/VWF levels were available for 20 surgical procedures in 16 patients. During the first 36 hours, 86.4% of FVIII levels (Figure 1) and 63.3% of VWF:RCo levels were above target level (0.80 $IUmL^{-1}$). In 7 dental procedures, all FVIII/VWF levels were above target level (0.50 $IUmL^{-1}$) in this period. Bleeding complications occurred in only 3.8% and were unrelated to FVIII/VWF levels. Very high FVIII plasma levels (≥ 2.0 $IUmL^{-1}$) were reached in 59.1% of patients. DDAVP was administered 2-3 times in 9 procedures.

TABLE 1 Patient characteristics

	Total cohort	Patients with measured perioperative FVIII/VWF levels
Patient characteristics	N (%) or median [IQR]	N (%) or median [IQR]
Number of patients	79	22
Type 1 VWD	75 (95)	21 (95)
Total procedures	159	27
Dental procedures	71 (45)	7 (26)
Surgical procedures	88 (55)	20 (74)
Baseline VWF:Ag	0.32 [0.27-0.43]	0.31 [0.26-0.43]
Baseline VWF:RCo	0.25 [0.20-0.29]	0.25 [0.20-0.29]
Baseline FVIII	0.47 [0.35-0.57]	0.50 [0.44-0.58]

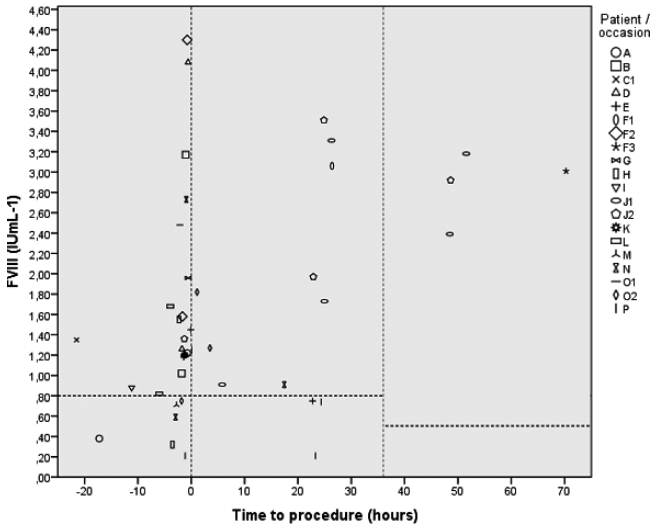

FIGURE 1 FVIII levels (IUmL-1) during the perioperative period in surgical procedures

Conclusions: DDAVP treatment is effective in this group, as patients achieve adequate FVIII/VWF plasma levels. Perioperative bleeding complications are rare, although some patients do not reach target levels. However, a large proportion of patients achieve very high FVIII plasma levels, with a potential risk of thromboembolic complications. In conclusion, better prediction of DDAVP response, both after first and consecutive dosing, may be realized by construction of population PK models, thus personalizing treatment in VWD patients.

PB 2054 | In-Frame Heterozygous Large Deletion in von Willebrand Factor (VWF) Gene in a Patient with Severe Bleeding Manifestations Might Accelerate VWF Clearance

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Background: We identified an in-frame heterozygous large deletion spanning exons 4 to 34 of von VWF gene in an index patient (IP) with type 3 von Willebrand disease (VWD). Curiously, the IP suffers frequently from bleeding episodes in spite of prophylaxis treatment.

Aims: This study aimed to determine the pathological mechanisms by which this single gene defect interferes with the VWF life cycle.

Methods: Blood outgrowth endothelial cells (BOECs) were isolated from blood of IP and healthy individuals. The wild-type or deleted VWF cDNA was transiently expressed in HEK293T cells. Assessment of antigen levels and multimer profile of the VWF in supernatant of the BOECs and HEK293T cells were performed. Subcellular location of the VWF in BOECs was evaluated by confocal microscopy scanning. VWF transcript analysis was done by using RNA extracted from the BOECs.

Results: RNA analysis assured biosynthesis of normal VWF transcription allele as well as an aberrant deleted transcript. Surprisingly, the mean of secreted VWF levels from the patient-derived BOECs was only slightly reduced compared with those of the healthy donors (63% vs. 72% respectively). Multimer analysis of the secreted VWF displayed loss of large and intermediate multimers along with shift in mobility of small multimers. Likewise, studies in HEK293T cells showed nearly normal secretion but a major defect in multimer structure. Confocal immunofluorescent analysis revealed a relatively strong VWF staining in IP-derived BOECs. However, the Weibel-Palade-bodies in IP-BOECs seemed smaller compared to those within normal BOECs.

Conclusions: Our results suggested that the deleted VWF protein (p.Asp75_Cys1948del) *is incorporated into VWF multimers and has a dominant-negative impact on multimer synthesis.* We speculate that the secreted chimeric VWF is cleared from plasma more rapidly than normal VWF.

PB 2055 | Acquired von Willebrand Disease in Patients with Extracorporal Membrane Oxygenation: The VWF:GPIbM/VWF:Ag Ratio is a Sensitive Diagnostic Tool

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Background: Extracorporeal membrane oxygenation (ECMO) is associated with bleeding that is not fully explained by the use of anticoagulant/antiplatelet drugs. The loss of high molecular weight multimers (HMWM) of von Willebrand factor (VWF) has been described in patients with extracorporal assist devices, leading to acquired von Willebrand disease (AVWD). Both frequency of AVWD in patients on ECMO and appropriate support strategies are still unsettled.

Aims: We examined the presence of AVWD in adult patients on ECMO with unexpected bleeding (n=13).

Methods: The diagnosis of AVWD was based on the ratio of activity (VWF:GPIbM) to VWF antigen and multimer analysis. Bleeding episodes and use of blood products were monitored.

Results: Mean patient age was 60 (range, 40-86). Only 3 patients were on veno-venous ECMO for respiratory failure, while 10 were on veno-arterial ECMO after cardiac surgery. ECMO was started within 2.5 days after surgery (range, 0-14). Bleeding symptoms commenced around day 10 (range, 1-47). VWF:Ag and VWF:GPIbM were elevated in most patients (358%; range, 194-600; and 205%, range 80-348). The VWF:GPIbM/VWF:Ag ratio was decreased in all patients (mean 0.59; range, 0.23-0.82). In the multimer analysis, loss of HMWMs was seen in all 13 patients. All patients had platelets >50 G/l, normal prothrombin time, and most had a prolonged aPTT (mean 49 sec; range

35-71) under Argatroban. Out of 13 patients, 11 underwent massive transfusion protocols including VWF concentrate (mean 14 TIE; range, 5-58 TIE). Bleeding was successfully controlled in all patients. However, 12 patients died in the due course.

Conclusions: AVWD is frequently present in bleeding patients with ECMO support. At a cut-off of 0.8, VWF:GPIbM/VWF:Ag ratio is a sensitive diagnostic tool for AVWD. No additional markers could be identified for patients with severe bleeding. Clinical trials assessing early diagnosis and intervention strategies for patients with AVWD on ECMO are urgently required.

PB 2056 | Different Modes of Association between Protein Disulfide Isomerase PDIA1 and VWF in von Willebrand Disease

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Background: Thiol oxidoreductases are critically involved in the production of von Willebrand factor (VWF) multimers. In the endoplasmic reticulum (ER), intra-monomeric disulfide bonds are formed which mediate protein folding. Formation of disulfides between the C-termini of two VWF monomers is catalyzed by protein disulfide isomerase isoform A1 (PDIA1) to produce VWF dimers, and multimerization is realized in the Golgi apparatus by formation of N-terminal inter-dimer disulfide bonds catalyzed by the VWF propeptide.

Mutations in the VWF gene can lead to von Willebrand disease (VWD); the most common congenital bleeding disorder. A variety of these VWF mutants exhibit defects in multimer biosynthesis, storage and secretion and are intracellularly retained.

Aims: The aim of this study is to determine whether PDIA1 is involved in cellular retention of VWD mutants.

Methods: We started the study with eleven VWD type 3 mutants, which were investigated by immunofluorescence after transient expression in HEK293 cells. Parallel staining of endogenous PDIA1 was employed to visualize VWF-PDIA1-association and measurement of fluorescence intensities revealed influence of the VWF mutant expression on PDIA1 levels.

Results: Our preliminary data indicate three different modes of mutant-VWF-PDIA1-association: 1) Most propeptide mutants exhibited normal PDI co-localization compared to wildtype VWF, 2) Expression of mutants p.Gly39Arg, p.Leu129Arg and p.Tyr271His was associated with a slight increase in PDIA1 expression level, 3) The cysteine mutants p.Cys2431Tyr and p.Cys2533Arg induced an alteration in PDIA1 localization that was associated with formation of big, ER-localized clusters containing VWF and PDIA1.

Conclusions: PDIA1 might be involved in different mechanisms underlying VWD. We are currently conducting studies to elucidate the connection between PDIA1 and VWF misfolding and degradation pathways in VWD type 3.

PB 2057 | Mutational Screening of a Type III VWD Cohort from Cohort Reveals 19 Novel Mutations in the VWF Gene

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Background: von Willebrand disease type III (Type III VWD) is a rare congenital autosomal bleeding disorder. It is characterized by quantitative defect in von Willebrand factor (VWF) secondary to a mutation in *VWF* gene. The incidence rate of this disorder in Pakistan is quite high owing to the cultural practice of consanguinity.

Aims: To screen *VWF* gene for mutations in a type III VWD Pakistani cohort and to study their genotype-phenotype correlation.

Methods: A total of 48 patients with type III VWD were enrolled in this study and informed consent was taken. Blood and plasma samples were collected from the patients using routine procedures. DNA was extracted from peripheral blood leucocytes. Mutational analysis was performed by direct gene sequencing on automated ABI-3130 Genetic Analyzer. Phenotypic investigations comprising FVIII activity (FVIII:C), VWF antigen (VWF:Ag) and VWF ristocetin cofactor activity, were carried out at National Institute of Blood Diseases and Bone Marrow Transplantation (NIBD). Impact of missense mutations were assessed by missense prediction tools (Polyphen-2, SNPGo, Provean, SIFT and Mupro). The 13 novel missense mutations were analyzed by molecular modeling tools to predict their putative impact on the structure of the protein.

Results: We identified a total of 31 mutations in the 46 index patients. These included 17 missense (13 were novel), 7 nonsense, 2 small deletions (both novel), 2 insertions (one was novel) and 3 splice site mutations (all 3 novel). All mutations were predicted to be causative using the *in silico* and protein modeling tools.

Conclusions: We found a very heterogenous spectrum of mutations in the Pakistani type III VWD cohort. A majority of them (62%; 19/31) were novel. Using *in silico* tools we were able to prove the causality of the 13 novel missense mutations.

PB 2058 | Effect of Elongational Flow on von Willebrand Factor Cleavage by ADAMTS13

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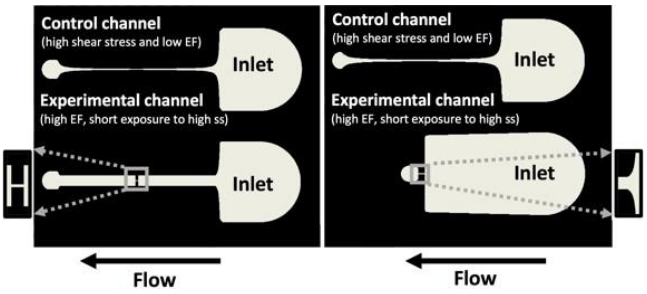

FIGURE 1 a) Abrupt 90° angle with varying EF magnitude b) Hyperbolic function region with constant EF magnitude

Background: Acquired von Willebrand Syndrome (aVWS) is characterized by loss of high molecular weight multimers (HMWM) of von Willebrand factor (VWF). Loss of HMWM of VWF in aVWS is commonly attributed to the presence of high shear stress (SS) that causes VWF to unravel, exposing cleavage sites to ADAMTS13. However, sufficient SS to cause VWF conformational changes is only present close to the vessel wall. Conversely, abrupt constrictions such as those in severely stenosed vessels or vascular assist devices (VADs) cause elongational flows (EF) across the majority of the vessel/channels. Our hypothesis is that EF will have a greater effect on VWF cleavage than SS alone.

Aims:

i) Evaluate the effect of EF magnitude on VWF cleavage and

ii) Identify the contribution of EF relative to SS for VWF cleavage

Methods: Flow simulation software was used to design microfluidic devices with either high SS and low EF (control channel) or high EF with minimal exposure to high SS (experimental channel) to evaluate relative contributions to cleavage. Recombinant ADAMTS13 was added to 1.5 µl of citrated human plasma diluted in buffer. The samples were placed in channel inlets and allowed to flow forward-back through the channel for various times (1, 3, 5 and 14 hours). Reaction products were analyzed by western blotting and multimer analysis. This assay was repeated at varying SS and EF conditions.

Results: Two sets of flow chambers were designed and optimized (Figure 1). Under high EF conditions cleavage was confirmed by western blot with the characteristic 176 kDa and 140 kDa fragments as well as loss of HMWM.

Conclusions: Judiciously designed flow chambers allowed to evaluate the effect of EF on VWF cleavage. Multimer analysis showed higher loss of HMWM in the EF channel when compared to SS alone. Results from these studies could provide guidance for the design of blood-contacting VADs to prevent aVWS.

PB 2059 | Wound Healing is Not Delayed in Mice with Reduced von Willebrand Factor in Spite of the Presence of a Severe Bleeding Tendency

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Background: von Willebrand Factor (vWF) recruits platelets to sites of injury by binding between exposed collagen and platelet GPIbα, particularly at sites of high shear. vWF is necessary for hemostasis, but can also play a key role in arterial thrombosis and vascular disease. Our group is currently testing an RNA aptamer that blocks vWF binding to GPIbα as an antithrombotic. We have previously shown that cutaneous wound healing is impaired in hemophilia B mice.

Aims: The Aim of the current study was to test the hypothesis that reduced vWF activity similarly impairs healing.

Methods: We studied C57BL mice injected with vehicle or vWF aptamer, as well as vWF knockout (KO) mice. The maximal effect of aptamer was 2-5 hemostasis events in 30 min in a saphenous vein (re)bleeding assay, compared to 20-25 in controls. vWF KO mice did not stop bleeding in 30 minutes. Healing was assessed in mice given a dose of aptamer that impaired hemostasis for >10 days. A skin punch biopsy model was used, as in our previous studies. Wound size was measured daily. The wound area was harvested for histology. Inflammation without tissue injury was induced by applying cantharidin, followed after 24 hr by sacrifice and tissue harvesting.

Results: There was no difference in time to healing between control and aptamer-treated mice, with all wounds healed at 10 days. There was also no statistically significant difference in wound size at any time. Wounds on vWF KO mice were also healed by 10 days. Cantharidin induced a similar level of leukocyte influx in treated, vWF KO and control mice. Aptamer-treated and vWF KO mice showed a small amount of inflammation-induced hemorrhage, though much less than that seen in thrombocytopenic mice.

Conclusions: Surprisingly, vWF does not play a major role in cutaneous wound closure. Lack of vWF led to only a modest degree of inflammation-induced hemorrhage, despite the presence of a severe hemostatic defect in a large vessel bleeding model.

PB 2060 | Variability in von Willebrand Factor Parameters in Blood Outgrowth Endothelial Cells from Healthy Controls

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Background: Blood outgrowth endothelial cells (BOECs) are mature endothelial cells derived from peripheral blood. BOECs are a powerful tool to study pathophysiological mechanisms of vascular diseases. We use BOECs to study von Willebrand factor (VWF), an endothelial protein of which defects relate to von Willebrand disease (VWD). However, to understand the pathophysiology of VWD, a detailed overview of VWF parameters in BOECs from healthy controls is necessary.

Aims: Determine the normal range of VWF parameters in BOECs from healthy controls.

Methods: Cultures of separate colonies (n=16) of BOECs derived from six donors were established. Experiments were performed on BOECs confluent for four days and at passage four. Additionally, two clones were followed during increasing passage numbers. Surface marker expression was determined by FACS to confirm endothelial lineage and to define the normal range of several markers. Storage of VWF in Weibel-Palade bodies (WPBs) was visualized by immunofluorescent staining. VWF:Ag secretion in 24 hours was measured in medium by ELISA and plotted against cell density determined by ImageJ (ITCN plugin).

Results: All BOECs were CD14/CD45 negative and CD31/CD146 positive and stored VWF in WPBs, confirming endothelial lineage. High variability was observed in the percentage of CD34, CD133 and VEGFR2 positive cells, cell density and VWF:Ag levels. VWF:Ag levels significantly correlated with cell density (R^2 = 0.90, P< 0.0001). Also, per increase of passage number the cell density decreased, which correlated with VWF:Ag levels as well (R^2 = 0.92, P< 0.0001).

Conclusions: High variability in cell surface markers, secreted VWF:Ag levels and cell density was observed in BOECs from healthy controls. Interestingly, VWF:Ag levels measured in confluent cells, significantly correlated with cell density, suggesting that smaller cells secrete more VWF. These results highlight the importance of understanding VWF parameters in BOECs from healthy controls.

PB 2061 | A Cell-based Assay to Quantify αIIbβ3 Integrin Binding of von Willebrand Factor Mutants

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Background: Integrin $\alpha_{IIb}\beta_3$ is a major constituent of the platelet membrane that plays an essential role in platelet adhesion and aggregation. It specifically recognizing the arginine-glycine-aspartic acid (RGD) sequence present in several adhesive proteins. Upon platelet activation the complex undergoes a conformational change that permits binding to fibronectin, vitronectin, thrombospondin, fibrinogen, and von Willebrand factor (VWF).

VWF is a large multi-domain plasma glycoprotein essential to primary hemostasis. VWF can directly interact with platelets, as it exhibits binding sites for GPIbα, which is part of the platelet membrane receptor GPIb-IX-V (binding site located in the VWF A1 domain), and for $\alpha_{IIb}\beta_3$ (via the RGD sequence in domain C4).

Aims: We strive to investigate the influence of von Willebrand disease-associated mutations in the C-domains of VWF on interaction with platelets via $\alpha_{IIb}\beta_3$.

Methods: We developed a cell-based binding assay by stably co-transfecting HEK293 cells with the two integrins α_{IIb} and β_3. To mimic activation of the complex a constitutively active mutant was generated by introducing a single missense mutation in β_3. Binding was determined by addition of the $\alpha_{IIb}\beta_3$-presenting cells to immuno-adsorbed VWF. The complex was detected by anti-$\alpha_{IIb}\beta_3$ and an HRP-coupled secondary antibody. After addition of the HRP substrate 3,3',5,5'-tetramethylbenzidine), optical absorbance at 450 nm was measured using an ELISA reader for quantification of $\alpha_{IIb}\beta_3$ binding.

Results: We validated our assays by using mutant p.Asp2509Gly, in which the RGD sequence was inactivated, as a negative control. As expected no binding was observed. We further investigated binding of the C-domain mutants p.Cys2257Arg, p.Arg2464Cys, and p.Cys2671Tyr. All of them exhibited decreased binding with the strongest effect by exchange of Cys2257.

Conclusions: We successfully established and validated a cell-based assay that allows quantification of binding of RGD-motif containing proteins to membrane incorporated $\alpha_{IIb}\beta_3$.

PB 2062 | Heterozygous Splicing Mutations Associated with Severe Type 1 von Willebrand Disease

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Background: A large proportion of type 1 von Willebrand disease (VWD) cases are associated with missense mutations scattered over the entire von Willebrand factor gene (VWF). A few mutations are caused by stop codon or splice site mutations. In these patients usually VWF levels of 30 - 50 U/dL are observed and bleeding history is mild.

Aims: To report four novel heterozygous splice site mutations associated with a full penetrance and expressivity pattern.

Methods: Whole VWF gene and intron-exon boundaries were sequenced by Sanger methodology. FVIII coagulant activity, von Willebrand factor antigen and ristocetin cofactor activity were assessed by routine methods. Bleeding history was assessed by the ISTH-BAT.

Results: Four different families were evaluated including 6 patients. FVIII ranged from 30 to 48 U/dL, VWF antigen and activity were always below 20 U/dL. Bleeding history was significant in all (BS > 6). A novel c.3538+1 G>T in intron 26, two novel changes at the same splice site in intron 33
(c.5657+1 G>T and c.5657- 1 G>C) and a novel deletion between intron 28 and exon 29 (c.5054-18_5063delTCCTGGTTGTCTTTGCAG ACTGCAGCCA) were detected. No other mutations or possible functional polymorphisms were identified. The reduction of FVIII and VWF levels were homogeneous within the families.

Conclusions: Rare splice site mutations in *VWF* can be associated with clear penetrance and expressivity suggesting possible skipping

of exons resulting in dominant-negative effect on the normal VWF allele. mRNA studies are undergoing to elucidate the pathophysiology of these splicing abnormalities.

PB 2063 | Single Centre Audit of Laboratory Testing in Sub-classification of Type 2 von Willebrand Disease

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Background: Laboratory tests in the diagnosis of von Willebrand Disease (VWD) consists of assessment of von Willebrand Factor (VWF) function. Patients with type 2 VWD display heterogeneity in phenotype and treatment response, with treatment options including plasma derived VWF concentrates or desmopressin (DDAVP). Trial of efficacy of DDAVP in those without contraindication as recommended by the British Committee for Standards in Haematology (BCSH) is important as lower response rates have been reported in those with type 2 (2A, 2M and 2N) VWD.

Aims: To audit compliance with laboratory testing in the sub-classification of patients with type 2 VWD.

Methods: Retrospective single centre (The Royal London Hospital Haemophilia Comprehensive Care Centre) review of laboratory testing and DDAVP trial against recent BCSH guidelines of patients registered with type 2 VWD.

Results: Of the 334 patients currently registered with VWD, 79 were classified as type 2. The median age was 37 years (1-96 years) with a slight female preponderance (M:F 1:1.3). Although, 76% (n=60) of type 2 VWD patients were registered as being sub-classified (2A, 2B, 2M or 2N), multimer testing had been performed in 21 patients (35%). A FVIII binding assay to confirm a diagnosis of type 2N was performed in 2 patients (33%, 2/6). No patient registered with type 2B VWD or with loss of high molecular weight multimers had testing of Ristocetin-Induced Platelet Aggregation. One patient had genotype testing and none a trial of DDAVP. Changes in guidelines for VWF activity:antigen (< 0.6) resulted in reclassification of 5 patients from type 2 to type 1 VWD.

Conclusions: Despite national guidance there was low uptake of secondary testing for type 2 VWD subclass confirmation. No patient received testing of DDAVP response which may relate to loss to follow up or contraindication. Increased awareness of this treatment option may reduce VWF concentrate exposure. Re-classification due to changes in recent guidelines was seen in a small number of patients.

PB 2064 | Von Willebrand Factor Testing through the Ages: A Review of Recent Results from the ‚Royal College of Pathologists of Australasia Quality Assurance Program'

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder but due to its heterogeneity its diagnosis can be difficult. Six types of VWD are characterised on the basis of quantitative or qualitative defects in von Willebrand factor (VWF). A panel of tests are therefore required. Such tests include both antigenic (VWF:Ag) and functional (e.g., ristocetin co-factor (VWF:RCo); collagen binding (VWF:CB)) assays.

Aims: To evaluate performance of laboratories enrolled in the RCPAQAP Special Haemostasis program for identification of VWD. To identify and characterise errors associated with incorrect results and/or interpretation to analytical or post-analytical event.

TABLE 1 Sources of error in VWD identification

Sample ID	Sample type	Total Errors	Total Errors Errors comprised	Analytical errors: N (% of errors)	Limited test panel: N (% of errors	Misinterpretation: N (% of errors)
VW13-03a	Normal	3	Type 1 (1) Type 2A/2B (2)	0	2 (67)	1 (33)
VW13-03b	VWF deficient (~10U/dL)	6	Type 1 (3) Type 3 (3)	0	4 (67)	2 (33)
VW13-08a	Mild/Mod type 1	10	Type 2A/2B (6) Type 2M (4)	5 (50)	5 (50)	0
VW13-08b	high molecular weight VWF deficient 'type 2'	4	Type 1 (4)	0	3 (75)	1(25)
VW14-08a	Type 2M VWD	19	Normal (17) Equivocal (2)	5 (26)	10 (53)	4 (21)
VW15-08a	Type 2M VWD (duplicate of VW14-08a)	16	Normal (15) Equivocal (1)	5 (31)	10 (63)	1 (6)
VW15-08b	high molecular weight VWF deficient 'type 2'	10	Type 1 (4) Type 3 (2) Other (4)	1 (10)	3 (30)	6 (60)
VW16-03b	high molecular weight VWF deficient 'type 2'	10	Type 1 (8) Type 3 (2)	6 (60)	3 (30)	1 (10)
VW16-08a	Acquired (type 2) VWD	9	Normal (1) Type 1 (8)	1 (11)	3 (33)	5 (56)

Methods: Data was collected from over 60 participants and analysed using robust statistics (median, mean and CV) and interpretations reviewed for incorrect interpretation of correct results, limited test panels and poor performance of testing.

Results: A review of recent challenges is summarised in table 1 and identified: (a) a VWF deficient sample (VW13-08a) resulted in nearly 20% incorrect interpretations of type 2 VWD; half of which were attributed to use of a limited test panel. (b) A type 2M VWD patient sample sent twice in separate years (VW14-08a; VW15-08a) yielded ~25% of participants failing to identify the qualitative defect and discordant functional/antigenic ratio, with limited test panels being the main cause of error. (c) Several high molecular weight (HMW) VWF deficient samples (VW15-08b; VW16-03b) yielded nearly 20% incorrect identifications of type 1 or type 3 VWD.

Conclusions: From this data, supported by other published data, laboratories are recommended to perform a VWF:Ag assay plus preferably several functional assays representing different activities (ie, glycoprotein Ib and collagen binding) in order to avoid errors in VWD identification. Limited test panels excluding this combination were a cause of significant error and impede laboratories from correctly diagnosing VWD.

PB 2065 | Comparing Platelet-dependent von Willebrand Factor Activity Assays in 661 Patients with von Willebrand Disease - from the WiN Study

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Background: Measuring von Willebrand factor (VWF) activity is crucial for the diagnosis and classification of von Willebrand disease (VWD). Historically, ristocetin co-factor activity is used, but new commercial assays are now also available. Large side-by-side comparisons between these assays in VWD patients are urgently needed.

Aims: To compare three widely used VWF activity assays in a large cohort of VWD patients.

Methods: We included 661 VWD patients (historically lowest VWF ≤30 U/dL) from the nationwide "Willebrand in the Netherlands" (WiN) Study. We compared VWF activity assays based on: 1) binding of VWF to a monoclonal antibody directed against the GP1b binding domain of VWF (VWF:Ab, HemosIL® von Willebrand Factor Activity, Instrumentation Laboratory), 2) induction of platelet agglutination by ristocetin (VWF:RCo, BC von Willebrand Reagent, Siemens) and 3) binding of VWF to a recombinant gain-of-function mutant platelet glycoprotein 1b fragment (VWF:GP1bM, INNOVANCE VWF Ac, Siemens).

Results: All assays were highly correlated (r >0.95). Bland-Altman plots showed a small bias between assays (fig 1).

An absolute difference >10 IU/dL between assays was found in 16% to 24% of cases. In 248/643 patients (38.6%), VWF:RCo was below the detection limit of 12 IU/dL; 111 of these had VWF:Ag < 20 IU/dL and therefore an incalculable VWF:RCo/VWF:Ag ratio. Clinical agreement, defined as matching VWD type (using VWF activity/antigen ratio 0.6), was 89% for VWF:RCo and VWF:GP1bM, 92% for VWF:RCo and VWF:Ab, and 83% for VWF:GP1bM and VWF:Ab. In 8/21 patients classified as type 3 VWD based on VWF:Ag < 5 IU/dL and propeptide < 4 U/dL, VWF:GP1bM results were between 5 and

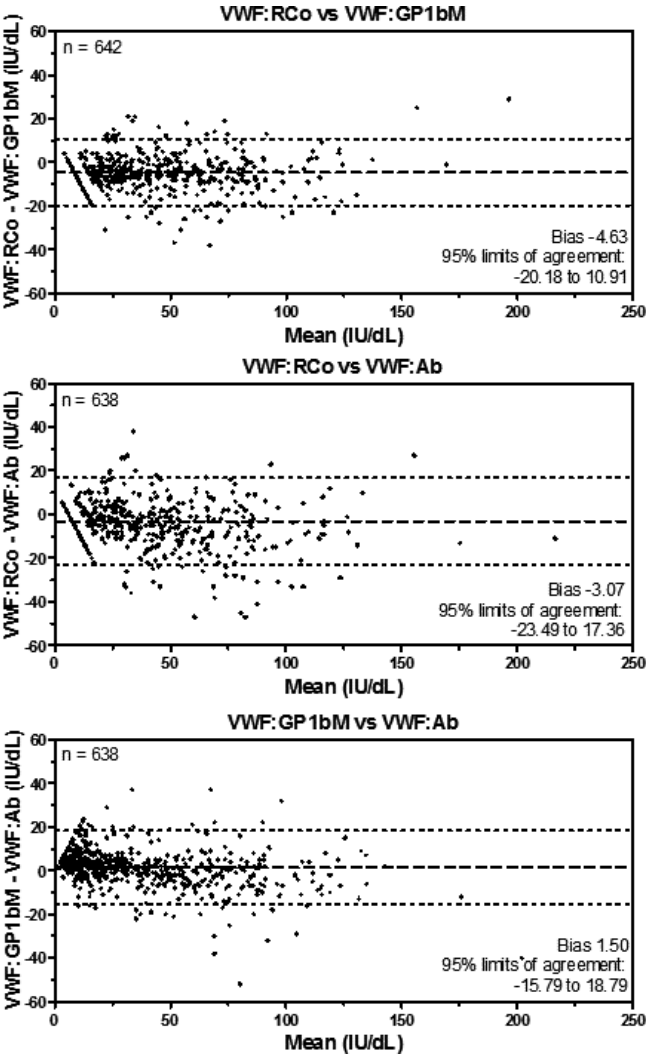

FIGURE 1 Bland-Altman plots of VWF:RCO, VWF:GP1bM and VWF:Ab

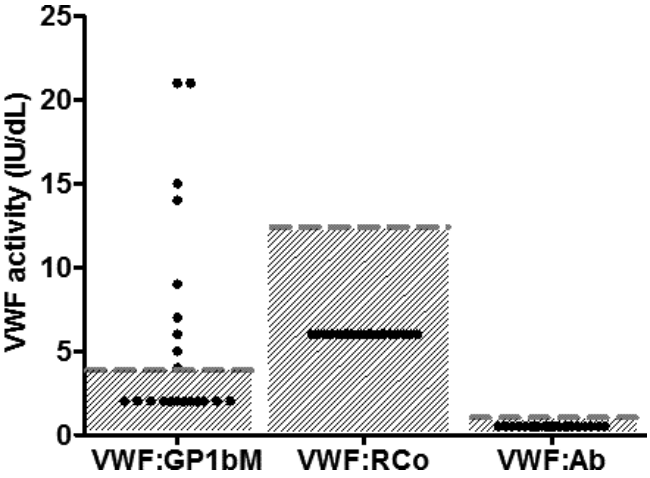

FIGURE 2 Assay results in type 3 VWD patients (VWF:Ag <5 IU/dL and VWFpp <4 U/dL) (----- limit of detection for each assay)

21 IU/dL whereas for both VWF:RCo and VWF:Ab all results were below the detection limit (fig 2).

Conclusions: In 17% of VWD patients VWF:RCo could not differentiate type 1 and 2 VWD. Almost 40% of type 3 VWD cases were missed by VWF:GP1bM. The choice between platelet-dependent VWF activity assays has a significant impact on the classification of VWD.

PB 2066 | Occurrence of Subclinical and Late Arthropathy in Patients with von Willebrand Disease

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Background: Joint bleeds (JB) are reported in a minority of patients with von Willebrand disease (VWD) but may lead to structural joint damage. Prevalence, severity and impact of JB in VWD are largely unknown. A recent study in the Netherlands (WIN study) showed that about 25 % of VWD patients with VWF level < 30 U/dL self-reported JB. Type 3 VWD may develop chronic arthropathy by clinical-radiologic evidence. Interestingly however a large proportion of patients with type 3 VWD do not show clinically evident joint bleeding. The role of ultrasound joint evaluation has not been systematically evaluated in VWD and the role of previous unrecognized minimal joint bleeding has not been investigated.

Aims: To evaluate clinical and ultrasound manifestations of joint bleeding in an Italian population of patients with type 3 VWD compared with a reference sample of type 1 and 2 VWD patients.

Methods: Ultrasound evaluation of knee, elbow and ankle was performed by the same investigator and scored according to the HEAD-US score (Thromb Haemost 2013; 109:1170). Fifteen patients with type 3 VWD (6 M and 9 F, age 20 - 61) and 26 patients with type 1 and type 2 VWD (11 M and 15 F) were evaluated.

Results: FVIII was < 5 U/dL in all type 3 VWD patients and ranged from 7 to 32 U/dL (median 13 U/dL) in type 1 and 2 VWD. All had VWF levels < 10 U/dL. Six patients (40 %) with type 3 VWD had US evidence of arthropathy (score ranged from 5 to 21) and all had radiological evidence of joint disease. All had the first joint bleeding < 15 years of age. No clinical and US evidence of joint disease was detected in all the other patients with type 1 and 2 VWD.

Conclusions: Joint disease is frequent in type 3 VWD but not invariably linked to the FVIII level. Joint disease associated with subclinical joint bleeding appears unlikely in patients with type 1 and 2 VWD. These results again emphasize the role of low FVIII in inducing clinical and instrumental evidence of joint disease in type 3 VWD.

PB 2067 | The Effect of Thioredoxin-1 on von Willebrand Factor Function

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Background: Von Willebrand Factor contains a significant number of cysteine residues with some of these in the free-thiol form. However it is not understood how & where these become unpaired. Thioredoxin-1 (TRX) is a ubiquitously expressed enzyme that catalyses the formation & reduction of disulphide bonds. We hypothesised that TRX may be responsible for the formation of free-thiols in VWF.

Aims: To determine the effect of TRX on the free-thiol content of VWF.

Methods: VWF was purified from Haemate P by gel filtration. Plasma was obtained from healthy donors. Recombinant TRX was activated with thioredoxin reductase before use. Static collagen binding, multimers gel analysis & GPIb binding assays & free-thiol analysis were performed using standard methods. Assessment of VWF function under shear stress was performed in a flow chamber assay.

Results: TRX induced the formation of new free-thiols in a concentration and time dependent manner & TRX addition to plasma resulted in an increase in VWF free thiols. Functional assays demonstrated a loss of collagen binding and GPIb binding function. Multimer analysis showed a loss of multimers with higher concentrations of TRX, however despite collagen binding being affected at low concentrations of TRX, the multimeric pattern was not affected sufficiently to cause this. Mass spectroscopy analysis of TRX treated VWF highlighted the formation of new thiols in the D4 & C-domains of VWF and significantly highlighted C1142 (responsible for multimers formation) & C1872 (forms part of the A3 disulphide loop) as being unpaired. Consistently, TRX treated VWF perfused over collagen demonstrated reduced platelet capture & the addition of TRX to whole blood before perfusion significantly reduced thrombus formation.

Conclusions: TRX can specifically attack the disulphide bonds responsible for multimer formation & maintaining the structural integrity of the A3 collagen binding domain & thus may therefore be a novel regulator of VWF function at sites of injury.

PB 2068 | The Prevalence of Bleeding Disorders in Women with Histologically Proven Endometriosis

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Background: Mild bleeding disorders affect 1% of the population. An increased prevalence of heavy menses in women with endometriosis has been known, but the rate of bleeding disorders in women with histologically proven endometriosis has never been studied.

Aims: To establish the rate of bleeding disorders (von Willibrands disease and platelet dysfunction) in women with histologically proven endometriosis.

Methods: Women with proven endometriosis were recruited from outpatient clinics. All women underwent screening using a modified international bleeding score.(The modification to the score included family history and mittleschmerz pain, both factors shown to be predictive of bleeding disorders).This modification increased the likelihood of women being tested; nontested women were presumed to be normal. Subjects with a history of excessive bleeding tendency (considered evident if bleeding score was >4 if nulliparous/>5 if parous or if ≥3 symptoms present) underwent tests for detection of a bleeding disorder. Evaluation included platelet function and relevant clotting factors utilising: Prothrombin Time, activated Partial Thromboplastin Time, von Willebrand Factor (Antigen, Ristocetin Cofactor, Collagen Binding Activity), Factor VIII, Platelet Function Analyser-100® - Collagen/Epinephrine, Collagen/Adenosinediphosphate.

Results: Of 56 women (with histopathological confirmation of endometriosis) aged 18-48 years (mean 32.8 years, standard deviation 7.3 years), 7.4% had results outside expected range on initial vWF screening and 11.5% had prolonged closure times for PFA-100® (excluding prolonged results due to known NSAID use < 14 days prior to testing).

Conclusions: This is the first study to demonstrate an elevated rate (17.7%)of abnormal bleeding tests on testing for both VWD and platelet dysfunction in women with endometriosis and suggests a potential new avenue of intervention for endometriosis management.

PB 2069 | Report of the ISO15189 Accreditaion of the Hydragel 5 von Willebrand Multimers Assay. A within-day Method

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Background: The electrophoresis of the vWF multimers allows to assess the distribution of vWF multimers in the plasma and is required to type von Willebrand disease (VWD). Until now, multimers assay was performed using home-made, time consuming and non-standardized methods. In 2016, Sebia has launched a new kit (Hydragel 5 von Willebrand Multimers) on the Hydrasis 2 Scan system. The purpose of this work is to evaluate its performances.

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Methods: VWD-typed plasma samples were used for testing the specificity of the Hydragel test. Intra-day and inter-day (3 consecutive days) reproducibility have been tested using normal and pathological controls and patients. 3 different ECAT samples belonging to independent surveys where employed for exploring the accuracy of the test. Absence of inter-sample carry-over was assessed with a VWD type 3 sample. Multimers test was carried following manufacturer's instructions.

Results: Hydragel test displayed an excellent reproducibility; electrophoretic patterns were conserved in their distribution as well as in their intensity. An arbitrary fractioning of patterns allowed calculating the relative concentration (%) of each multimers subset. Type 2A and type 2B yielded complete and/or partial loss of HMW and IMW. We did not observe any inter-sample carry-over.

Conclusions: Based on the obtained results, we achieved to get the ISO15189 accreditation (June 2016) of the Hydragel 5 von Willebrand multimers and its now introduced in our lab's routine. The excellent reproducibility of the test may help to reach a good homogeneity in results reporting.

PB 2070 | Comparison of Three Different Factor VIII Assays in 154 Patients with Type 3 von Willebrand Disease: Results from the 3WINTERS-IPS Cohort

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Background: Patients with type 3 VWD have undetectable VWF levels. Usually the FVIII:C level is less than 5 IU/dL although higher levels (10-15 IU/dL) have been reported.

Aims: The aim of the study was to measure FVIII in a well-defined cohort of patients with type 3 VWD by using 3 different assays: one-stage clotting (FVIII:C), chromogenic (FVIII:am), antigen (FVIII:Ag) to establish their baseline FVIII concentration.

Methods: Patients diagnosed as type 3 VWD were recruited in the course of the 3WINTERS-IPS study. The phenotypic profile was checked in central laboratories. For this study only the patients with VWF:Ag < 1 IU/dL were selected. FVIII was determined by 3 assays 1) FVIII:C with FVIII deficient plasma (Siemens) and APTT reagent Triniclot (Stago) 2) FVIII:am (Hyphen BioMed) 3) FVIII:Ag by ELISA Asserachrom (Stago).

Results: In the 154 selected patients, FVIII measurements showed variation which was dependent on the type of assay (p< 0.001): FVIII:Ag > FVIII:C > FVIII:am (see Table).

TABLE

	FVIII:am (IU/dL)	FVIII:C (IU/dL)	FVIII:Ag (IU/dL)
Mean ± SD	1.23 ± 0.72	2.30 ± 0.73	3.97 ± 1.16
Median	1.3	2.2	3.8
IQR	< 1 - 1.7	1.8 - 2.8	3.2 - 4.7
Range	< 1 - 3.0	1 - 4.4	< 1 - 7.5

All patients had measurable FVIII:C (≥1 IU/dL). About 20% patients had no detectable FVIII:am, but only 1 had undetectable FVIII:Ag. Nineteen % of the patients had > 5 IU/dL FVIII:Ag, whereas none of them had FVIII:C or FVIII:am > 5 IU/dL. There was a good correlation between FVIII:C and FVIII:am (r:0.825; p< 0.001) and between FVIII:C and FVIII:Ag (r:0.318; p< 0.001) but FVIII:am and FVIII:Ag did not correlate.

Conclusions: In this study reporting the measurement of FVIII by using 3 different assays in a large cohort of patients with type 3 VWD, we observed strongly decreased FVIII:Ag concentration due to the increased clearance of FVIII in the absence of VWF. In addition, functional activities of FVIII are lower than FVIII:Ag due to the role of VWF in FVIII activation/inactivation. The presence of VWF in the FVIII deficient plasma reagent used for FVIII:C or the longest incubation time in the chromogenic assay could explain their differences. The 3 assays are not equivalent in type 3 VWD.

PB 2071 | Treatment of Gastrointestinal Bleeding Episodes with Recombinant von Willebrand Factor (rVWF) in Patients with Severe von Willebrand Disease (VWD): Sub-analysis from Pivotal Phase III On-demand Study

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Background: Gastrointestinal (GI) bleeds occur in up to 20% of patients with VWD, most closely associated with the absence of ultra-large VWF multimers (ULM), generally observed in Type 2A and 3 VWD. Higher doses and longer duration of VWF replacement therapy is usually needed to resolve GI bleeds compared to bleeds at other sites and may even be unsuccessful. rVWF is a VWF concentrate in which ULM, the most hemostatically effective VWF multimers, are preserved, due to lack of exposure to ADAMTS13 during manufacturing.

Aims: A prospective, phase 3, randomized, clinical trial (NCT01410227) has been conducted to assess the efficacy and safety of rVWF-rFVIII and rVWF in the treatment of bleeds in subjects with severe VWD. The present sub-analysis was conducted on patients who experienced GI bleeds and were treated with rVWF.

Methods: The initial dose of rVWF was co-administered with rFVIII at a ratio of 1.3:1 and rVWF was administered alone after a hemostatic FVIII:C level was achieved. Efficacy was rated on a nominal 4-point scale (none=4 to excellent=1).

Results: 4 subjects (all with Type 3 VWD) experienced 6 GI bleeds (2 mild, moderate and severe). The median age of patients who experienced GI bleeds was 32.5 years (26-42). 100% of GI bleeds treated with rVWF had a hemostatic rating of excellent (83%) or good (17%) (Table 1). A total of 67% (4/6) required only 1 infusion of rVWF to successfully treat the GI bleed, with a mean of 1.3 infusions. The median

TABLE 1 GI Bleed Sub-Analysis: Bleed Characteristics and Efficacy

Patient #	# of Bleeds Treated on Study	Severity of GI Bleed	# of Days Treated with rVWF †	Clinical Efficacy Rating	# of rVWF Infusions to Resolution	Time to Resolution (hours)*
1	4	Major/Severe	0	Excellent	1	Unknown
2	6	Moderate	3	Excellent	1	1.75
2	6	Moderate	7	Excellent	1	2.67
3	2	Minor	3	Excellent	1	18.58
3	2	Minor	0	Good	2	Unknown
4	1	Major/Severe	3	Excellent	2	14

†Days from bleeding onset to first infusion, *Time from 1st infusion of rVWF to resolution of bleeding episodes

time to resolution was 8.3 h (1.75-18.6 h) in 4 of the 6 bleeds where it was known. The median total dose of rVWF per bleed was 60 IU/kg (53.6-121.0 IU/kg), and median dose per infusion of rVWF was 58.7 IU/kg (53.5-60.5 IU/kg). 2 possibly related non-serious AEs occurred in 2 patients (tachycardia & infusion site paraesthesia).

Conclusions: In this small case series of 4 subjects, rVWF was safe and effective at achieving hemostatic efficacy in the on-demand treatment of 6 GI bleeds in patients with severe VWD.

PB 2072 | Type of Renal Replacement Therapy, Access and von Willebrand Factor (VWF) Function

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Background: Acquired von Willebrand syndrome occurs in native cardiovascular disorders with intravascular turbulence, with blood pumps and prosthetic valves. Anemia and gastrointestinal bleeding from angiodysplasia also occur in dialysis patients, but the prevalence of VWF abnormalities based on dialysis modality and access is unknown.

Aims: Patients undergoing dialysis, or scheduled for surgical arteriovenous fistula (AVF) creation were targeted for enrollment as follows: hemodialysis (HD) with tunneled catheter (CATH), 10, HD with AVF, 10, peritoneal dialysis (PD), 5, and pre- and post-AVF creation, 10.

Methods: Blood tests measured included VWF activity and antigen, platelet function analyzer-100 collagen adenosine diphosphate closure time (PFA-CADP), and VWF multimers. Results after2/3 enrollment are presented.

Results: VWF high molecular weight multimer loss in the range of severe aortic stenosis (AS) was seen in 5 /13 patients with an AVF, but none of the CATH or PD patients, p=0.13, figure. The VWF multimer ratio trended lower in HD patients, median (IQR) 0.13 (0.12-0.16), versus 0.16 (0.14-0.19), p=0.09. Additional trends included abnormal PFA-CADP in 56% of HD versus 17% of PD patients, p=0.16, and trends toward higher activity and antigen in PD versus HD. PFA-CADP was elevated in 64% of AVF patients versus 20% of non-AVF patients, p=0.047. VWF multimers were abnormal in 21% of AVF compared to 10% of non-AVF patients (NS). A history of gastrointestinal bleeding was present in 4 patients, 18%, 2 with abnormal multimers. The normalized multimer ratio in the bleeding patients was (median IQR) 0.72 (0.64-0.80) compared to 0.86 (0.77-0.92) in the non-bleeding patients, p=0.15.

Conclusions: Laboratory findings of acquired von Willebrand syndrome may be present in 1/4-1/3 of HD patients with AVF but not in HD with CATH or in PD. Thus, AVF may be a source of acquired von Willebrand syndrome in some HD patients.

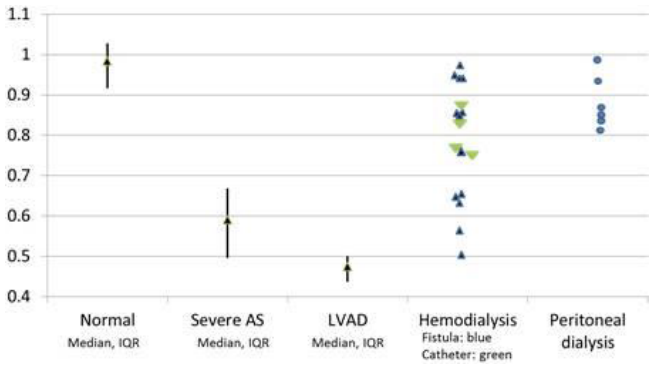

FIGURE 1 Normalized multimer ratio >15/2-15. AS=aortic stenosis, LVAD=left ventricular assist device

PB 2073 | Retrospective Single Centre Study of On-demand and Treatment Prophylaxis in 24 Patients with Type 3 von Willebrand Disease

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Background: Prophylaxis with coagulation factor concentrate is the standard of care for patients with haemophilia, but few studies have assessed prophylaxis in patients with severe forms of von Willebrand disease (VWD). The present study determines patterns of prophylaxis use and clinical outcomes in patients with type 3 VWD.

Aims: Rates of bleeding, joint damage and factor concentrate consumption were compared in patients who received prophylaxis and those who received on-demand therapy.

Methods: Data of 24 patients with type 3 VWD were retrospectively reviewed. Bleeding frequency, joint damage, and factor concentrate consumption were assessed.

Results: The median age was 34 years (range: 10-66), the median age at time of presentation was 10.5 years (range: 0-46), and the total observation period was 496.5 years (mean 20.69 years per patient). Fifteen patients received prophylaxis for a period of time and nine received only on-demand therapy. Patients who received continuous prophylaxis had fewer bleeding events per observation year (2.7) than those who received episodic on-demand therapy (5.0) or permanent on-demand therapy (4.6). Thirteen patients (54.2%) had arthropathy in at least one joint; eight of these patients had received prophylaxis. Joint status was substantially better in patients who had received primary prophylaxis than in those who had received secondary prophylaxis (total Petterson score 1.4 vs. 24.5). Factor concentrate consumption was greater in patients who received secondary prophylaxis (3146 U/kg BW/year) than in those who received primary prophylaxis (1761 U/kg BW/year).

Conclusions: Long-term prophylaxis is beneficial in most patients with type 3 VWD. Early initiation of prophylaxis may provide optimal protection against bleeding events and joint damage.

FIGURE 1 VWF Act/Ag ratio (normal >/=0.80), left and Normalized mutlimer ratio (normal=1.0), right

Results: Four women and 15 men were tested after HM II implantation 1.6±1.1 years after implant, and 9 women and 22 men 0.3±0.3 years after HW implantation (p< 0.001 for difference in time of testing). We found no difference in levels of either VWF:Act or VWF:Ag levels. However, VWF:Act / VWF:Ag trended higher in the HW patient, 0.76±0.14 versus HM II, 0.69±0.07, p=0.05 (figure, left panel). VWF multimer ratios showed similar trends with, HW versus HM II, multimer ratio >15/2-15, 0.9±0.03 versus 0.8±0.02, p=0.16, multimer ratio >10/2-10, 0.27±0.07 versus 0.25±0.05, p=0.15, normalized multimer ratio >15/2-15, 0.51±0.09 versus 0.44±0.05, p=0.06 figure, (right panel), and normalized multimer ratio >10/2-10, 0.61±0.13 versus 0.57±0.05, p=0.24).

Conclusions: In this relatively small study limited by differences in time of acquisition of samples, we see evidence of favorable trends in severity of AVWS by two independent tests of von WIllebrand factor function in the lower rotational speed LVAD.

PB 2074 | Trends in Severity of Acquired von Willebrand Syndrome with Lower Rotational Speed Left Ventricular Assist Device

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Background: Bleeding related to acquired von Willebrand syndrome (AVWS) is a major source of morbidity and mortality in patients with left ventricular assist device (LVAD) support. New pump designs with reduced rotational speed and axial versus centrifugal flow may reduce blood shear stress and consequently lessen the severity of AVWS.

Aims: We sought to compare quantitative measures of AVWS in patients implanted with Heartmate II ™ (HM II) with usual rotational speeds of 9400-9600 RPM to patients implanted with Heartware ™ (HW) with usual rotational speeds of 2600 RPM.

Methods: After implantation, von Willebrand factor antigen (VWF:Ag), and activity (VWF:Act) and VWF multimers by gel electrophoresis were done on all patients. As measures of AVWS severity, we calculated VWF:Act / VWF:Ag, VWF multimer ratio >15/2-15 monomers, VWF multimer ratio >10/2-10 monomers and the two multimer ratios normalized to the plasma control sample from the electrophoresis run.

PB 2075 | Prevalence of von Willebrand Disease among Saudi Students: from Saudi Screening Program for Bleeding Disorders (SSBD)

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Background: The Saudi Screening Program for Bleeding Disorders (SSBD) is a national screening program for bleeding disorders among students.

Aims: The aim of this analysis of the SSBD study was to estimate the prevalence of Von Willebrand disease (VWD) among students (male / female) in Saudi Arabia.

Methods: Over 2 years trained interviewers surveyed 2,000 students (male /female) at a university in Riyadh, Saudi Arabia using a detailed questioner based on MCMDM1 - VWD. Any students answered one or more of the 11 questions had been subjected to sample collection and detailed coagulation factor measurements. Initial evaluation consists of (CBC, PT, PTT, ABO and PFA-100) and followed by testing for all coagulation factors including testing for VWF:Ag, VW: RCof and FVIII) assay according to standard techniques.

Results: Out of 2000 surveyed students (age were from 17 to 22 years), 730 had answered yes for more than one bleeding questions,

but only 326 students had agreed for blood sampling. After initial hemostasis laboratory testing, 117 were suspected to have VWD. A confirmatory VWD assays were carried out and identified 9 students with low level of vWF /FVII. The estimated prevalence of VWD among Saudi Adolescents in general population was 2.7%.

Conclusions: This is initial results of the first epidemiological survey of bleeding disorders in Arab ethnicity. Our results suggest that the prevalence of VWD might be higher than reported in other populations. We are continuing to survey more areas in the country and increase the sample size of this survey.

PB 2076 | Molecular Caracterization of Type 2 von Willebrand Disease

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Background: von Willebrand disease (VWD) is the common inherited bleeding disorder. Type 2 VWD is characterized by qualitative deficiency of VW factor (VWF) and is classified in subtypes 2A, 2B, 2M and 2N.

Aims: The purpose of this study is to characterize the molecular basis of type 2 VWD in a cohort of Brazilian patients.

Methods: Eligible patients presented bleeding symptoms and ≥ 2 tests revealing vWF:Rco/vWF:Ag ratio below 0,7. Five pairs of primers were designed to amplify exons 17, 18, 19, 20 and 28 of the VWF gene. The amplified fragments were sequenced in equipment Genetic Analyzer 3130 (Applied Biosystems). The sequences obtained were compared to wild-type allele (CCDS8539.1 from NCBI) using the BioEdit and Sequence Alignment program (version7.2.5). ISTH database of VWD mutations was checked when a base change was found.

Results: Samples from 32 patients, belonging to 28 families with type 2 VWD were included, median age of 37 years (interquartile range, 22,5 - 48,5), 65% female. So far, exon 28 was sequenced 29 patients (90,6%), of whom exon 18 and 20 were sequenced in 5 (15,6%) and 7 (21,8 %), respectively. A total of 8 mutations were identified in association with type 2 VWD and all are registered in the ISTH database (Table 1).

The bleeding score (BS, ISTH 2011) was applied in 27 patients (84,3%), of whom only 3 patients (9,3%) presented value BS > 3 (2 male) and > 5 (1 female). Another 10 base changes were found, all of which are reported in ISTH or NCBI databases (Table 2).

Conclusions: So far, we identified 7 different mutations, associated with type 2 VWD and 10 polymorphisms, all changes reported in published databases. We confirm that the molecular characterization of type 2VWD sequencing is useful in the definition of the diagnosis of the disease. This is an ongoing study and additional molecular defects will be reported upon completion of the work.

TABLE 1 Molecular characterization of eight patients with type 2 VWD

Patient	Exon	Nucleotide Number	Amino acid Change	SNP ref. No	VWD type	Registered
09	28	4790	Arg1597Gln	rs61750577	2A	ISTH database
14	28	4738	Leu1580Val	rs61750114	2A	ISTH database
17	28	4789	Arg1597Trp	rs61750117	2A	ISTH database
05	28	4022	Arg1341Gln	rs61749403	2B	ISTH database
06	28	4022	Arg1341Gln	rs61749403	2B	ISTH database
10	28	3797	Pro1266Leu	rs61749370	2B	ISTH database
13	28	3922	Arg1308Cys	rs61749387	2B	ISTH database
08	28	3949	Arg1315Cys	rs61749395	2M, type 3 or unclassified	ISTH database

TABLE 2 Characteristics polymorphisms found in the VW gene

Exon	Nucleotide Number	Allele	Amino Acid Change	SNP ref. No	Record	Clinical influence	Reference
18	2365	A/G	Thr789Ala	rs1063856	ISTH database	elevated plasma VWF:Ag levels	Smith et al., 2011
20	2555	A/G	Gln852Arg	rs267607376	NCBI database	None	None
28	4141	A/G	Thr1381Ala	rs216311	ISTH database	variation on VWF levels	Burns et al., 2013
28	4414	G/C	Asp1472His	rs1800383	ISTH database	bleeding	Ezigbo at al., 2017
28	4693	G/T	Val1565Leu	rs1800385	ISTH database	None	None
28	4751	A/G	Tyr1584Cys	rs1800386	ISTH database	bleeding	Ezigbo at al., 2017
28	4304	A/G	Asn1435Ser	rs11063987	ISTH database	None	None
28	4138	A/G	Ile1380Val	rs11063988	ISTH database	None	None
28	4457	C/T	Ser1486Leu	rs149424724	NCBI database	low VWF levels	Johnsen et al., 2013

PB 2077 | Validation of a Rapid Diagnostic Approach with Automatic Tests in a Cohort of Chinese Patients with von Willebrand Disease: Results from the Chinese-Italian CReWilAct Study

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by quantitative and/or qualitative defects of von Willebrand factor (VWF). VWD correct diagnosis remains still problematic in many developed and developing countries.

Aims: A cross sectional study [Chinese Registry of von Willebrand disease with Instrumentation Laboratory approach using Automatic Tests (CReWILAcT)] was designed by the Centers of Suzhou and Milan to evaluate the appropriate approach to VWD diagnosis.

Methods: Chinese and Italian healthy subjects were used as controls versus Chinese VWD. The 3 main VWF activities (VWF:Ag, VWF:RCo, VWF:CB) were measured in citrated plasma by the automatic ACL-ACUSTAR (Instrumentation Laboratory, Bedford, USA) systems using commercially available reagent kits. The data of these 3 tests were expressed versus International Standard in IU/dL with their sensitivity and variability assessed. 3 steps were planned:

a) in lyophilized samples from 2 normal individuals and 6 cases with known VWD types available at NIBSC of London, UK;

b) in 70 Chinese and 65 Italian healthy subjects with O and non-O blood groups;

c) in 108 VWD followed in China.

Results: Sensitivity/Variability (CV) of VWF:Ag, VWF:RCo, VWF:CB were < 1 (5%), < 1 (7%) and < 1 (6%), respectively.

In step a, in 2 blind exercises, both labs could confirm diagnosis of normal controls or VWD2A, VWD2B and VWD2M.

In step b, VWF levels (Mean±SD) of O blood groups were lower than non-O in both Ch (34/36) and It (32/33) cases: VWF:Ag [Ch=89±51/102±38); It=70±26/102±38); VWF:RCo [Ch=97±36/114±33); It=74±29/101±31); VWF:CB [Ch=87±27/102±25); It=77±25/114±33).

In step c, in Ch VWD, the total mean±SD (range) of activities were: VWF:Ag [16±14(< 1-67)]; VWF:RCo [13±12(< 1-46); VWF:CB [13±13 (< 1-47)]. Using VWF:RCo/Ag VWF:CB/Ag ratios VWD3(24), VWD1/2N(43), VWD2A(21), VWD2B(8), VWD2M(13) could be confirmed.

Conclusions: These results support the use of such an automatic approach for VWD diagnosis in routine labs world-wide.

PB 2078 | The Self-association of Ristocetin

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Background: Ristocetin promotes platelet aggregation and agglutination in the presence von Willebrand factor. This phenomenon is the basis for the VWF ristocetin cofactor assay that is widely used to diagnose von Willebrand disease. NMR studies have revealed that ristocetin self-associates. Platelet function studies indicate that the self-association of ristocetin is required for promotion of VWF ristocetin cofactor activity.

Aims: The goal of this study was to characterize the self-association of ristocetin.

Methods: Ristocetin (Bio-Data) was characterized by HPLC and ESI mass spectrometry. The self-association of ristocetin was studied by sedimentation velocity analysis at 260,000 *g* and 20 °C in a Beckman XL-I analytical ultracentrifuge (AUC) using interference optics. Sedimentation coefficient (c(s)) distributions and weight-average sedimentation coefficient (s_w) isotherms were calculated using SEDFIT and SEDPHAT.

Results: Ristocetin was >95% pure by HPLC with a molecular mass of 2068 Da, consistent with its known structure. c(s) distributions produced s_w values increasing from 0.70 S to 1.00 S at concentrations of ristocetin ranging from 0.6 to 6.8 mM. The s_w isotherm fit a monomer-dimer self-association model yielding estimates of 1.3 mM (2.7 mg/mL) for the dimerization constant and $s_{20,w}$ values of 0.68 S and 1.18 S for monomeric and dimeric ristocetin. c(s) distributions displayed a single peak, consistent with a rapid monomer-dimer equilibrium.

Conclusions: SV AUC is a powerful method to study weakly self-associating systems and allowed characterization of the self-association of ristocetin, which has been difficult to characterize because its high self-association constant. The concentration of ristocetin used in clinical assays varies from 0.5-1.5 mg/mL. Our results indicate that this range is below the ristocetin dimerization constant. This predicts that the assay is sensitive to small variations in the concentration of ristocetin and may contribute to the problematic variability of the assay.

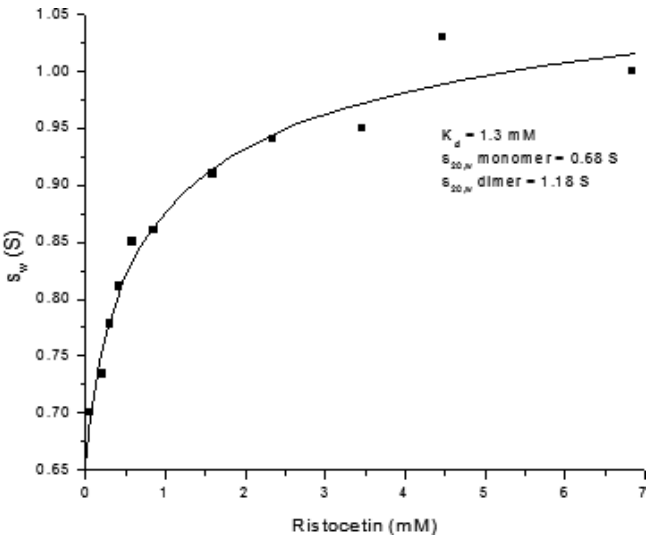

FIGURE 1 Weight-average sedimentation coefficient isotherm for ristocetin

PB 2079 | VWD2M Genotype Carrying Double Heterozygous Mutations with More Severe Phenotype than Classic VWD2M

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Background: We have characterized a group of VWD2M patients (2MC) by VWF:RCo/VWF:Ag (RCo/Ag)< 0,6, normal multimers, DDAVP test and mutations (Mt) in the A1 domain of VWF.

Aims: To describe the phenotype resulting from muco-cutaneous bleeding symptoms, in the presence of double heterozygous Mt: a novel missense Mt, and its association with a deletion in a 2M patient (P). To compare the phenotype of P with that of 2MC.

Methods: Tests considered for comparison between P and 2MC: FVIII:C (one-stage method), VWF:Ag (Ag) (ELISA), VWF:RCo (RCo) (aggregometry). DDAVP response, tested before and after 90min of intravenous infusion.

Exon 28 of *VWF* gene was analyzed in P and 100 healthy controls (HC).

Results: P: boy (8-yr) (blood O group); ISTH bleeding score (BS)=6; epistaxis requiring tamponades; bleeding after tooth eruption and extraction; easy bruising; gum bleeding; normal multimers and VWF:CB (collagen type 1).

2MC (n=31): BS=4.6±3.2. Laboratory tests in table 1.

P showed lower FVIII:C, VWF:Ag and the DDAVP response than those in 2MC. Two Mt were found in *VWF* gene: 1) c.4276C>T (p.R1426C) in A1 domain, and 2) c.4944delT (p.P1648fs*45) in A2 domain.

p.R1426C was absent in HC, predicted as damaging by in silico analysis (PolyPhen-2, Mutation Tester, SIFT), and submitted by us to the ISTH-SSC-VWF database.

Conclusions: We report here a novel Mt: p.R1426C, and its association with a deletion, in a severe VWD2M phenotype. The lower FVIII:C, and VWF:Ag would probably be by the combination of the blood O group and the presence of p.P1648fs*45 which was reported as type 1 (ISTH-SSC-VWF database). In these situations, we should be aware of the selection of appropriate treatment.

PB 2080 | The Occurrence of Prothrombotic Mutations in a Patient with von Willebrand Disease and Symptomatic Bleeding Disorder

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Background: VWD is genetically inherited and is clinically heterogeneous. Moreover, the coexistence of other hemostatic defects may enhance or weaken the predisposition for bleeding.

Aims: Assessment of the impact of prothrombotic mutations on the clinical picture of bleeding disorder in a patient with VWD.

Methods: Activity of FVIII (FVIII:C) and von Willebrand factor (VWF), ristocetin cofactor activity (VWF:RCo) as well as of VWF factor antigen (VWF: Ag) were determined using Siemens reagents (BCS Siemens). The method of Křížek et al. (2000) was used for analysis of von Willebrand factor multimers. The G20210A mutation in the prothrombin gene, the G1691A mutation in the V (factor V Leiden), and C677T methylenotetrahydrofolate reductase gene (MTHFR) mutation were determined using RFLP / PCR (EURx, Poland).

Results: A 22 year old woman was referred with symptoms of bleeding disorder. Bleeding episodes appeared in childhood and consisted in nose and mouth bleeds, extensive bleeding following extraction of milk teeth, and heavy menstrual bleeding. Medical history revealed occurrence of VTE and arterial thrombosis in the families of both mother and father as well aa bleeding episodes in the mother's family (CNS bleeding). The patient was diagnosed with von Willebrand disease type 1: FVIII:C - 108% ; VWF:RCo - 29%; VWF:Ag - 44% and VWF:RCo / VWF:Ag ratio 0.66; the VWF multimer structure was normal. Further tests revealed the presence of heterozygous factor V Leiden mutation, heterozygous G20210A mutation of prothrombin gene and homozygous C677T MTHFR mutation coexistent with hyperhomocysteinemia. Family studies showed heterozygotic factor V Leiden mutation and heterozygous C677T MTHFR gene mutation with hyperhomocysteinemia in the mother and G20210A prothrombin

TABLE 1 Phenotypic profile of P and 2MC

	FVIII:C (IU/dL)	Ag (IU/dL)	RCo (IU/dL)	RCo/Ag	CB (IU/dL)	CB/Ag
P pre DDAVP	24	18	<10	0.5	14	0.78
P post DDAVP	45	34	<10	0.26	not done	not done
P post/pre ratio	1.87	1.88	1			
2MC pre DDAVP	52.6±16.5	36±20	8.9±3.8	0.35±0.19	35.4±27.3	0.9±0.23
2MC post DDAVP	126.8±42.6	100.2±59.4	37.2±28.8	0.33±0.15	not done	not done
2MCpost/pre ratio	2.41±0.9	3.15±0.8	3.8±2.2			
Normal range	50-150	50-150	50-150	>0.6	60-130	>0.6

gene mutation (heterozygous) and MTHFR C677T mutation (homozygous) coexistent with hyperhomocysteinemia in the father and his brother.

Conclusions: The prothrombotic mutations does not show significant impact on hemostatic function in the patient with type 1 VWD.

PB 2081 | Von Willebrand Factor (VWF) Propeptide (VWFpp) and VWFpp to VWF Antigen (VWF:Ag) Ratio in Assessment of Inherited (VWD) and Acquired (AVWS) von Willebrand Disease

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Background: VWFpp is essential for proper biosynthesis of VWF. After cleavage from mature VWF, noncovalent complex of VWF and VWFpp is packed into Weibel-Palade bodies of endothelial cells and α granules of platelets. After release to plasma the complex dissociate and its elements circulate independly.

Aims: It has been assumed that the VWFpp/ to VWF:Ag ratio (VWFpp/VWF:Ag) is an important biomarker of VWF clearance.

Methods: 50 healthy persons; 108 with VWD and 21 patients with AVWS: 11 with essential thrombocythemia, 3 with antibodies to VWF, 7 with aortic valve stenosis. We determined Factor VIII (VIII:C), VWF ristocetin cofactor activity (VWF:RCo) and VWF antigen (VWF:Ag) by Siemens; VWFpp by Sanquin, collagen binding activity (VWF:CB) by Technoclone and VWF multimetr structure by Krizek et al., 2000.

Results: The following results were obtained: 1)control group: VWFpp/VWF:Ag 0,49 - 1,8. 2) patients: a) with VWF:Ag< 5% and VWF:RCo< 5% - 6/7 had VWFpp < 1 (type 3 VWD) and 1/7 - VWFpp/VWF:Ag - 16,0; b) with VWF:RCo< 10% and VWF:Ag>5% - 8/9 had VWFpp/VWF:Ag >2; c) with VWF:RCo 10-20% - in 8/29 -VWFpp/VWF:Ag was >2; d) with VWF:RCo 20-30% - VWFpp/VWF:Ag - in 1/11 was >2; e) with VWF:RCo 30-40% - VWFpp/VWF:Ag ratio was >2 in 2/28; f) with VWF:RCo 40-50% - in 24/24- VWFpp/VWF:Ag ratio was within normal range.

AVWS patients with essential thrombocythemia and aortic stenosis had normal VWFpp/VWF:Ag ratio. Patients with antibodies in a course of monoclonal gammapathy had pathological ratio of VWFpp/VWF:Ag 3,57; 6,54; 4,57 respectively.

Conclusions: Increased VWFpp/VWF:Ag ratio was determined more frequently in VWD patients with VWF:RCo < 20% with type VWD 2. In AVWS patients, VWFpp/VWF:Ag ratio was within normal range; except for patients with antibodies against VWF.

PB 2082 | Extensive Bilateral Mandibular Pseudotumor in a Boy with Type 3 von Willebrand Disease (VWD) Treated with Plasma Derived VW/Factor VIII Concentrate (FC): Diagnosis, Treatment and Outcome

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Background: Pseudotumor is a rare complication of hemophilia whereas it was exceptionally described in VWD.The pathogenesis and therapy of pseudotumor remain elusive.

Aims: To describe the diagnosis, therapy and outcome of a boy with type 3 VWD with bilateral mandibular pseudotumor treated with FC(*Humate-P*®).

Methods: Case report.

Results: A Peruvian 6-year-old boy was referred to our center. He had severe epistaxis, gingivorragia, bruising and anemia since the first year of age. He had no hemarthrosis. Laboratory results support the diagnosis of type 3 VWD: VWF:Ag: < 1 IU dl^{-1}, VWF:RCo: undetectable, FVIII:C: 4 IU dl^{-1}.His mother is dead and his father has VWF:Ag: 45 IU dl^{-1}, VWF:RCo: 31 IU dl^{-1}, FVIII:C: 88 IU dl^{-1}. Exon 28 (PCR/Sanger):no variant was detected, multiplex ligation-dependent probe amplification: no large deletion was found, next generation sequencing results are pending. At 13-year-old he developed progressive painless slow-growing tumefactions in the angles of both mandibles. CT scan detected bilateral osteolytic lesions. A surgery biopsy showed osseous trabeculae, blood clotting material and a cystic wall with intense hyalinization and collagenization. Moderate inflammatory infiltrates and marked osteoblast bone remodeling was also found. FC was given followed by prophylaxis with 50 IU VWF ristocetin cofactor/kg two times a week during 50 months.

Conclusions: We present a boy with extensive mandibular pseudotumor treated with FC followed by long-term prophylaxis who achieved a complete and sustained bone resolution. The role of VWF in osteoclast differentiation could be involved in this response.

PB 2083 | Laboratory Identification of von Willebrand Disease: A Contemporary Approach

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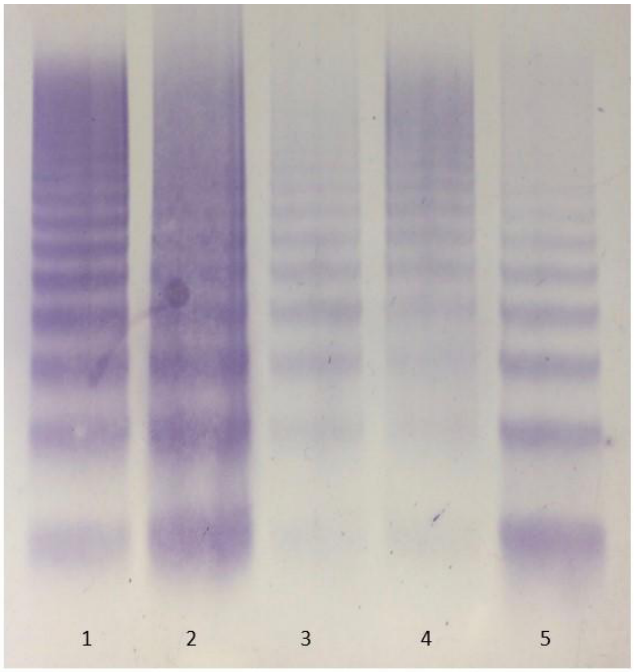
Background: von Willebrand disease (VWD) is reportedly the most common inherited bleeding disorder, with high rates of acquired events also documented. VWD arises from quantitative and/or qualitative deficiency/defects in von Willebrand factor (VWF), a plasma

TABLE 1 Summary of representative sample VWD results (refer Figure 1)

Lane ID	Method Group	VWD type	VWF:Ag	VWF:CB	VWF:RCo	CB/Ag	RCo/Ag	Lab Interp
2	ELISA/plt agg	2M	100	87	23	0.87	0.23	2M
3	ELISA/plt agg	2B	28	25	24	0.89	0.86	2A/B
4	ELISA/plt agg	2M	47	66	17	1.40	0.36	2M
5	ELISA/plt agg	2A	45	19	10	0.42	0.22	2A/B
2	AcuStar	2M	103	33	35	0.32	0.34	2A/B/M
3	AcuStar	2B	28	16	15	0.57	0.54	2A/B/M
4	AcuStar	2M	42	27	28	0.64	0.67	2A/B/M
5	AcuStar	2A	43	11	16	0.26	0.37	2A/B/M
Abbreviations:	Ag, Antigen	CB, collagen binding	RCo, ristocetin cofactor					

protein with multiple activities. VWD shows significant heterogeneity with six recognised types, representing quantitative deficiency of VWF (partial, Type 1; complete, Type 3) or diverse qualitative defects (Types 2A, 2B, 2N, 2M). However, recognition of VWD by labs, including correct assignment of type, suffers from high error rates, potentially due to analytical issues (eg, poor or inconsistent methodologies; limited test panels) or post analytical issues (eg, misinterpretation of test results).

Aims: To evaluate contemporary laboratory methods used for identification of VWD as an aid to diagnosis, and identify patterns that may most effectively facilitate diagnosis.



Lane		VWF:Ag	VWF:CB	VWF:RCo	CB/Ag	RCo/Ag
1	Norm Ctrl	91	100	93	1.10	1.02
2	VWD-2M	100	87	**23**	0.87	**0.23**
3	VWD-2B	**28**	**25**	**24**	0.89	0.86
4	VWD-2M	**47**	66	**17**	1.40	**0.36**
5	VWD-2A	**45**	**19**	**10**	**0.42**	**0.22**

FIGURE 1 Sample multimer gels (refer to Table 1)

Methods: Retrospective and prospective evaluation of test results from a large panel of samples comprising VWD, non-VWD, and therapy (eg, VWF concentrate, DDAVP) associated samples with a battery of contemporary methods.

Results: Sample results are shown in Table 1 and Figure 1. Different assays yield differential utility according to VWD type. For example, AcuStar **c***hemiluminescence* methods were best at identification of low level VWF, and thus best facilitated early identification of Type 3 VWD, and best discrimination of Type 1 vs 2A/2B/2M VWD. However, ELISA methods were better at discriminating Types 2A vs 2M VWD. VWF glycoprotein Ib binding results approximated ristocetin cofactor results.

Conclusions: Laboratories should select the best methods and test panels able to identify VWD. A composite and sequential approach provides the best opportunity for effective diagnosis of VWD.

PB 2084 | Importance of Classification and Genetic Tests in the Diagnosis of von Willebrand Disease Type 2B and 2N

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Background: Von Willebrand disease (VWD) is the most frequent inherited bleeding disorder caused by a dysfunction of primary hemostasis. Type 2 VWD includes a wide range of qualitative abnormalities of von Willebrand factor (VWF) resulting in different subtypes. Type 2B VWF variants have increased affinity for platelet GPIb. Type 2N is characterized by markedly decreased VWF affinity for coagulation factor VIII (FVIII).

Aims: We present three cases with these VWD subtypes to demonstrate the importance of thorough laboratory investigation for the exact diagnosis.

Methods: Coagulation screening tests, immunological and functional assays of VWF, FVIII activity and classification VWD tests (including ristocetin-induced platelet aggregation; RIPA, VWF:FVIII binding assay) and DNA sequencing were performed.

Results: Patient1 is a 13 year old girl who had prolonged menstrual bleeding. Among hemostasis tests the only abnormality was enhanced low-dose RIPA, which suggests type 2B VWD. With molecular genetic test a heterozygous p.P1266L mutation was found in exon 28 of the VWF gene. Patient2 and patient3 are 9 and 10 year old girls without bleeding symptoms, in their cases hemostasis screening tests were carried out before tonsillectomy and prolonged APTT was detected due to low FVIII activity (22% and 19%, respectively). VWF levels and VWF activities were in the reference range. Their extremely low VWF:FVIII binding ELISA assay results verified type 2N VWD. DNA sequence analysis showed that patient2 carries homozygous p.R854Q mutation in exon 20, while this mutation was identified in the heterozygous state in patient3 who also displayed the p.Y795C mutation in heterozygous form in exon 18 of the VWF gene.

Conclusions: These cases show that despite normal immunological and functional assay results the patient may suffer from rare VWD subtypes, therefore classification VWD tests followed by molecular genetic investigation have to be performed.

MANAGEMENT OF THROMBOEMBOLISM

PB 256 | Relevance of Polypharmacy for the Clinical Outcome of Patients Receiving Oral Anticoagulation Therapy - Results from the thrombEVAL Study

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Background: Polypharmacy (PP) is associated with negative clinical outcome in various settings and commonly observed in patients receiving oral anticoagulation (OAC) therapy.

Aims: To investigate the effect of PP on OAC-specific outcome (i.e. thrombotic and bleeding events) and non-specific outcome (i.e. hospitalization, all-cause mortality) among patients receiving vitamin K-antagonists (VKA).

Methods: Information on 2,011 individuals from the prospective, multi-center thrombEVAL study were available for analysis. Data were obtained from clinical visits, computer-assisted personal interviews, self-reported data and laboratory measurements. Information on study endpoints were validated by medical records and subsequently adjudicated. PP was defined as the intake of ≥5 drugs (including VKA).

Results: In total, the prevalence of PP was 84.1% (n=1,691) in the study sample, with 44.7% (n=899) of subjects receiving 5-8 drugs (moderate PP) and 39.4% (n=792) ≥9 drugs (marked PP). Time in therapeutic range was lower in individuals with moderate and marked PP (70.8%, IQR 53.5%/85.2% and 65.1%, IQR 45.9%/81.9%, respectively) in comparison to subjects without PP (74.2%, IQR 50.0%/90.5%). During the 2-year follow-up, the incidence of bleeding, hospitalization and all-cause mortality was higher in PP individuals and increased significantly with severity of PP (Table). In Cox regression analysis with adjustment for age, sex, cardiovascular risk factors and comorbidities, clinically-relevant bleeding (HR 1.71; 95%CI 1.05-2.78; P=0.032) and all-cause mortality (HR 2.25; 95%CI 1.41-3.59; P< 0.001) were independently associated with marked PP, whereas moderate PP ($HR_{bleeding}$ 1.44;95%CI 0.90-2.32 and $HR_{mortality}$ 1.37;95%CI 0.85-2.19, respectively) did not pass the threshold of statistical significance for both endpoints.

Conclusions: The presence of PP affects quality of OAC therapy and the incidence of clinical outcome of anticoagulated patients, which

TABLE 1 Incidence of anticoagulation-specific and non-specific outcome according to polypharmacy status

	Incidence of adverse events			
	No polypharmacy	Moderate polypharmacy	Marked polypharmacy	
	[no./100 patient-years] (95%CI)	[no./100 patient-years] (95%CI)	[no./100 patient-years] (95%CI)	P for trend
Thromboembolic events	5.3 (3.4/8.0)	3.5 (2.6/4.8)	4.5 (3.3/6.0)	0.67
Clinically-relevant bleeding	5.3 (3.4/8.0)	10.0 (8.3/12.0)	14.4 (12.1/16.9)	<0.0001
Major bleeding	3.7 (2.1/6.0)	7.0 (5.6/8.7)	9.5 (7.7/11.6)	0.003
Hospitalization	54.5 (47.7/61.9)	71.0 (66.3/76.0)	97.2 (91.2/103.5)	<0.0001
All-cause mortality	5.1 (3.2/7.7)	8.2 (6.6/10.0)	15.1 (12.8/17.7)	<0.0001

might be clinically-relevant for risk stratification and management of OAC therapy.

PB 257 | Thromboembolic and Bleeding Risk of Patients with Left-sided Mechanical Heart Prosthesis: The FCSA-START-Valvole Study

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Background: Patients (pts) who have received a mechanical prosthetic heart valve implantation need to be treated with a vitamin K antagonist (VKA).

Aims: Aim of the study was to evaluate adverse events during treatment.

Methods: In the frame of the START Register (Italian Survey on Anticoagulated Patients Register), we conducted an observational retrospective multicenter study among 33 centers affiliated to the Italian Federation of Anticoagulation Clinics (FCSA). FCSA centers follow pts for the management of anticoagulant treatment, perform INR test and prescribe written daily VKA dosage. Patients were followed-up with the aid of specific dedicated softwares; quality of anticoagulation, bleeding and thrombotic events occurring during the follow-up were recorded. Centers were asked to provide information on each patient in whom a left-sided mechanical prosthetic heart valve was implanted after 1998, with follow-up data.

Results: We enrolled 3027 pts; in this sub-study we analyze 2357 pts (77.9%) with mechanical prosthesis, 1928 (81.8%) on warfarin and 429 (18.2%) on acenocoumarol, followed for a median of 9.7 yrs. More time was spent below the intended therapeutic range in mitral than aortic prosthetic valve (**Table 1**).

TABLE 1 Characteristics of patients

Males, N (%)	1301 (55.2)
Median age at implantation, yrs (IQR)	59.0 (49.7-65.7)
Total follow-up (x 100 pt-years)	24080
Aortic, N(%)	1382 (59.7)
Mitral	666 (28.8)
Mitro-Aortic	33 (1.20)
Median time spent below TR (%) (IQR)	24 (12-36)
Median Time spent within TR (%) (IQR)	60 (47-74)
Mitral: median time spent below TR (2·5-3·5 INR) (%) (IQR)	26 (15-38)
Aortic: median time spent below TR (2·0-3·0 INR) (%) (IQR)	14 (5-25)

TABLE 2 Bleeding and thrombotic events in relation to site of implantation

Site of implantation	Major Bleeding, N (rate % pt-yrs)	Thrombosis,N (rate % pt-yrs)
Aortic	144 (1.01)	72 (0.5)
Mitral	62 (0.89)	74 (1.07)
Mitro-aortic	33 (1.20)	17 (0.62)

Relative risk for thromboembolism of mitral vs aortic prosthetic valve was 2.39 (95%CI 1.70-3.36; p< 0.0001). No difference for bleeding risk was found in relation to valve position.

Conclusions: In this cohort of patients with a mechanical heart prosthesis the rate of adverse events was low. No differences were recorded for bleeding in relation to the site of implantation. The rate of thrombotic events was higher in pts with mitral valves, probably due to their known higher thrombogenicity, but also to a higher rate of inappropriately low INR in anticoagulation management of these patients.

PB 258 | Warfarin Binds to a Hydrophobic Pocket within the Oxidized Open State Human VKORC1 Conformation

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Background: Vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1) catalyzes the reduction of vitamin K quinone (K) and vitamin K 2,3-epoxide (K>O). This process is essential for further modification of vitamin K dependent coagulation factors leading to their biological activity. VKORC1 is also a drug target of 4-hydroxycoumarins, as warfarin is used in therapy and prevention of thrombosis. Over-anticoagulation results in serious bleedings but can be reversed by administration of K.

Aims: To identify the preferred oxidized/reduced state of human VKORC1 (*hVKORC1*) to which warfarin binds.

Methods: Models of *hVKORC1* in different oxidized/reduced states were generated based on the X-ray crystallographic structure of a prokaryotic VKOR enzyme and with energy minimization techniques. Warfarin was docked on all these models. All these models were also submitted to the Encom and ANMpathway server for conformational sampling. Warfarin dose responses of mutated VKORC1 residues of cysteines in the loop or the active centre were measured in CRISPR/cas9 engineered VKOR deficient *HEK293T* cells.

Results: Best docking poses for warfarin were generated for an open loop conformation of the oxidized form of hVKORC1 (Cys43-Cys51 and Cys132-Cys135 disulphide bonds). Conformational sampling of hVKORC1 models suggests a conformational switch between the

open loop fully oxidized and closed loop partially oxidized state during reduction of K vitamers. This is blocked by warfarin binding to the open oxidized state. Shifted warfarin dose responses were measured when loop cysteines were mutated to alanine or serine.

Conclusions: Our results challenge the current concept of non-competitive warfarin inhibition since we demonstrate that K vitamers and warfarin share binding sites with F55 as central residue binding either the substrate or inhibitor. We suggest a competitive slow tight binding inhibition where warfarin binds VKORC1 in the oxidized form and is outcompeted by vitamin K when loop cysteines get reduced by oxidoreductase partners.

PB 259 | The Contribution of Genetic Variants that Are Associated with Venous Thrombosis or with Vitamin K Antagonist Dosage, alone or Combined, to the Risk of Major Bleeding in Patients Treated with Vitamin K Antagonists

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Background: Genetic variants in several genes are associated with venous thrombosis and the required dosage of vitamin K antagonists (VKA). However, data on the association between these variants and major bleeding in VKA-treated patients is limited.

Aims: To estimate the risk of major bleeding in VKA-treated patients in relation to 3 genetic variants that are associated with venous thrombosis or with VKA dosage.

Methods: The BLEEDS cohort comprises 16,570 patients who started VKA therapy between 2012 and 2014. Patients were followed until a major bleed, the end of VKA therapy, death, or December 31st 2014, whichever came first. From the cohort, we assembled a case-cohort study including all 326 cases with a major bleeding and a random sample of 978 patients at baseline (subcohort). Risk estimates of major bleeding based on all 3 genetic variants were estimated separately and in a risk score comparing the presence of 1 and 2-3 genetic variants versus those who had 0 genetic variants. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by means of weighted Cox regression.

Results: DNA was available from 246 cases and 834 subcohort patients. The mean age was 71 years and 54% were male. Allele frequencies were 93% for rs6025 in Factor V, 39% for rs505922 in ABO and 16% for rs9934438 in VKORC1. Individual genetic variants were associated with an increased risk of major bleeding, with hazard ratios (HRs) of 1.27 (95%CI 0.66 to 2.44) for rs6025, 1.05 (95%CI 0.78 to 1.41) for rs505922 and 1.39 (95%CI 0.95 to 2.03) for rs9934438. When considering genetic variants concomitantly with 0 variants as a reference, 1 variant was associated with a HR of 1.73 (95%CI 0.59 to 5.10) and 2 or 3 variants with a HR of 2.18 (95%CI 0.74 to 6.38).

Conclusions: Multiple genetic variants associated with venous thrombosis or VKA dosage increase the risk of major bleeding in VKA-treated patients.

PB 260 | Assessment of the Efficacy of a Novel Tailored Vitamin K Dosing Regimen in Lowering INR in Over-anticoagulated Patients

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Background: Current guidelines advocate the use of fixed-doses of oral vitamin K for the reversal of excessive anticoagulation in warfarinized patients (either asymptomatic or with minor bleeds). Over-anticoagulated patients present with a wide range of International Normalized Ratio (INR) values and response to fixed doses of vitamin K varies. Consequently, a significant proportion of patients remain outside their target INR 24 hours after vitamin K administration making them prone to either haemorrhage or thromboembolism.

Aims: To compare the performance of a novel tailored vitamin K dosing regimen to that of a fixed-dose regimen with the primary measure being the proportion of over-anticoagulated patients returning to their target INR 24h later.

Methods: The study was approved by the National Research Ethics Service-Committee North East, Newcastle & North Tyneside. After obtaining informed consent, 181 patients with an index INR >6.0 (asymptomatic or with minor bleeding) were randomly allocated to receive orally either a tailored dose (calculated according to index INR and body surface area), or a fixed-dose (1 or 2mg) of vitamin K. The patients' INR was checked 24h after vitamin K dosing.

Results: Compared to the fixed-dose regimen, the tailored dose resulted in a significantly greater proportion of patients returning to within target INR range (68.9% v 52.8%; p=0.026), whilst a significantly smaller proportion remained above target INR range (12.2% v 34.0%; p< 0.001). There was no significant difference between the two dosing regimens in the proportion of patients (18.9% v 13.2%) with over-corrected INR at 24 h.

Conclusions: Individualisation of vitamin K dosing is simple to implement in the clinic and is significantly more accurate than fixed-dose regimen in lowering INR in excessively anticoagulated patients.

PB 261 | Clinical Usefulness of the SAMe-TT2R2 Score: A Systematic Review and Meta-analysis

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Background: It has proven hard to predict the time in the therapeutic range for vitamin K antagonist therapy. The SAMe-TT2R2 risk score was developed to predict therapy outliers before start of treatment. It is now also used as a proposed decision rule to provide extra care, or to indicate DOAC therapy for those at high risk of poor VKA control. However, the studies supporting this use assessed diverse cutoffs for outcome and their predictive value has not been well evaluated.

Aims: To evaluate the predictive value and clinical usefulness of the SAMe-TT2R2 score.

Methods: We performed a systematic review in Embase and PubMed for original research papers or abstracts assessing the SAMe-TT2R2´s performance to predict low TTR. A meta-analysis was done with cutoffs for score (≥ 2 and ≥ 3) and TTR (< 70%), such that a high score predicts low TTR. Different cutoffs were harmonised by multiple simulations with patient characteristics from the individual studies.

Results: 18 studies were identified and used in the meta-analysis (4 and 2 times directly, 9 and 10 times harmonised for scores ≥ 2 and ≥ 3, respectively). A score ≥ 2 yielded a sensitivity of 42% (95% CI 29-54%) and specificity of 66% (54-78%) to predict TTR < 70%. A score ≥ 3 had a sensitivity and specificity of 34% (12-55%) and 70% (48-92%), respectively. The positive likelihood ratios were 1.26 (1.16-1.36) for a score ≥ 2, and 1.28 (1.12-1.43) for a score ≥ 3; the negative ones were 0.88 (0.83-0.93) and 0.97 (0.90-1.03), respectively. This shows that the post-test probabilities hardly differ from the prior probability (prevalence) (see also Figure 1).

Conclusions: The SAMe-TT2R2 score does predict low TTR, but the effect is small. Its effect on individual patients is too limited to be clinically useful. Therefore, the evidence does not support the use of the aforementioned decision rules.

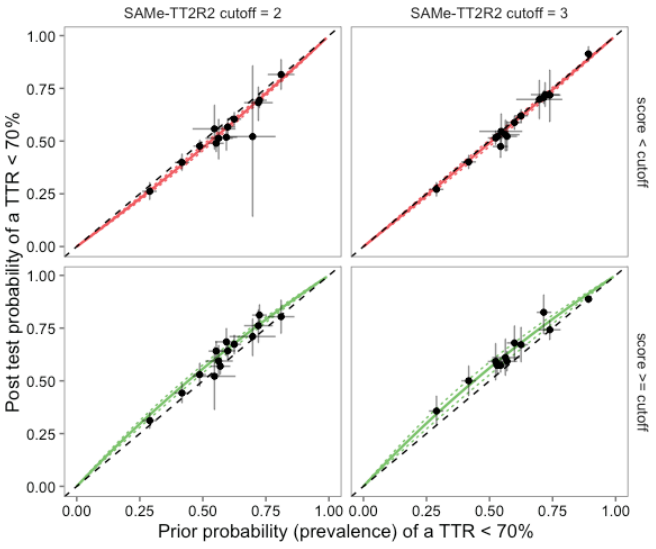

FIGURE 1 Benefit of the SAMe-TT2R2 score for different prevalences. Based on likelihood ratios from meta-analysis and results of individual studies

PB 262 | Safety of Long Term Warfarin Therapy in Real Clinical Practice: Predictors of Major and Non-Major Clinically Relevant Bleedings

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Background: In spite of available of novel anticoagulant, warfarin (W) is still remained most prescribed medicine in different clinical cases. The main complication of W is bleeding (Bl).

Aims: To estimate the frequency of major Bl and non-major clinically relevant Bl (definition as in GARFIELD Registry) and separately gastrointestinal bleedings (GIB) in patients, received warfarin as mono therapy and in combination with one or two antiplatelets.

Methods: Our prospective study involved 315 pts (181 males), aged 63,1±10,1 years with AF or VTE, prescribed W :among those as monotherapy (n=227), double antithrombotic (n=58) and triple antithrombotic therapy (n=30). Follow up periodwas 6,9 ±4,75 years.

Results: The overall incidence of all Bl (major, non-major clinically relevant and minor) was 8,66/ 100pts-year among all pts with W. The incidence rate of major Bl in W -monotherapy, double and triple antithrombotic therapy was 2,44; 3,63;8,0/per 100 person-years respectively. The incidence rate of non-major clinically relevant Bl in W -monotherapy, double and triple antithrombotic therapy was 1,96; 4,84; 16,0per 100 person-yearsrespectively. By multifactor discriminate analysis four parameters were specified as predictors of combination major Bl and non-major clinically relevant Bl: omeprazole intake (F=14,1, p=0,0002), labile INR(F=9,25, p=0,0025), history of recurrent minor bleedings (F=6,42, p=0,011) and amiodarone therapy (F=4,46, p=0,035). Relative risk of GIB was significant higher in triple antithrombotic therapy (RR 3.02, CI 1.2-9.1,p=0.029), but not on double therapy (RR 1.42, CI 0.52-4.8,p=NS).

Conclusions: In our study four parameters: omeprazole intake, labile INR, history of recurrent minor bleedings and amiodarone therapy,-were specified as predictors of combination major Bl and non-major clinically relevant Bl on long term W therapy. Triple antithrombotic therapy is relevant risk factor gastrointestinal bleedings.

PB 263 | Decreased Bone Mineral Density in Fontan Patients Receiving Long-term Warfarin

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TABLE 1 Comparison of BMD of patients receiving aspirin versus warfarin (**P Value ≤0.001)

Warfarin Vs Aspirin						Age-adjusted Z-Scores			
	N	M:F	Age (years)	Height (cm)	Weight (kg)	Whole Body	Right Hip	Left Hip	AP Spine
Warfarin	42	23:19	**24 ± 8	165 ± 11	63 + 15	**-1.47 ± 1	**-1.08 ± 1.16	-1.1 ± 1.17	**-1.26 ± 1.16
Aspirin	24	14:10	18 ± 6	160 ± 34	64 ± 14	-0.48 ± 1.19	-0.3 ± 0.92	-0.55 ± 1.22	-0.47 ± 1.09

Background: Patients who undergo Fontan procedure are at increased risk of thrombosis. To mitigate this risk, they frequently receive life-long thromboprophylaxis, usually with warfarin or aspirin. Warfarin, a vitamin K antagonist, may be associated with decreased Bone Mineral Density (BMD). Aspirin, via prostaglandin modulation, may also have a negative impact. However there is no previous comparative data. Reduced BMD is of particular concern when medication is commenced prior to peak bone mass acquisition, such as in the Fontan population.
Aims: To determine if long-term warfarin use is associated with lower BMD compared to aspirin use in Fontan patients.
Methods: This cross-sectional study utilized the Australian & New-Zealand Fontan registry to recruit participants, who were required to have been on their current thromboprophylaxis medication for at least 5 years. BMD was assessed using DXA (Hologic Discovery W, University of Melbourne; Lunar Prodigy 2000, Children's Hospital at Westmead). BMD and subsequent age-adjusted z-scores were calculated for whole body, hips and anteroposterior (AP) spine. Z-scores of participants on warfarin were compared to those on aspirin using an unpaired student's t-test.
Results: Table 1 shows that participants receiving warfarin had reduced BMD compared to age-matched reference population, and significantly lower BMD than their aspirin counterparts at 3 of the measured sites.
Conclusions: This study demonstrates that long-term warfarin use is associated with decreased BMD compared to that of patients on aspirin following the Fontan procedure. Early detection of patients at risk of osteoporosis is important as early intervention may improve outcomes. Therefore, patients on long-term warfarin should be considered for regular BMD screening. These findings are an important consideration in the risk benefit analysis of whether warfarin or aspirin is the better method of anticoagulation for young people with Fontan circulation.

PB 264 | Predicting Recurrent Venous Thromboembolism in Patients with Deep-vein Thrombosis: Development and Internal Validation of an Simply Applicable Prediction Model (Continu-8)

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Background: Existing prediction models for recurrent thromboembolism (VTE) are complicated to apply in clinical practice, mainly because of the need to stop anticoagulant treatment for D-dimer measurements.
Aims: To develop and internally validate a simply applicable prediction model for recurrent VTE that can be used without stopping anticoagulant treatment.
Methods: Cohort data of 497 patients treated between 2003 and 2013 in a clinical care pathway at Maastricht University Medical Center were used. Variables in the model (D-dimer, unprovoked DVT, male gender, factor VIII levels) were derived from literature and multivariate logistic regression analysis. For reasons of practicality d-dimer was removed from the model. This set of predictor variables was added to a cox proportional hazards regression model. Resulting regression coefficients were converted to integers to provide a scoring rule. The scoring rule was internally validated using bootstrapping techniques.
Results: Patients were followed for a median of 3.12 years (IQR 0.78, 3.90). The overall recurrence rate was 3.7/100 patient-years (95% CI 2.9, 4.8); 64/ 479 (13%) patients developed recurrent VTE. The

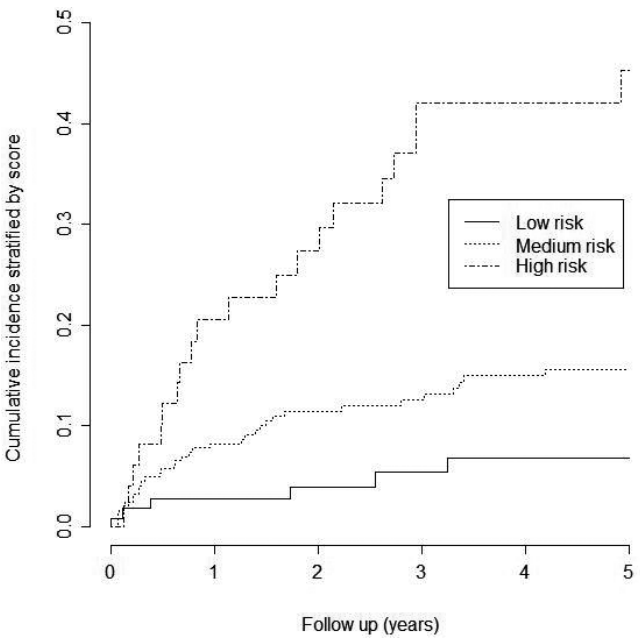

FIGURE 1

scoring rule consisted of unprovoked DVT (yes: 2 points), male sex (yes: 2 points), and factor VIII > 211 % (yes: 1 point). After converting to integers, the score ranged from 0 to 5 and was categorized into three groups (i.e. low risk [score 0], medium risk [scores 1, 2, or 3] and high risk [scores 4 and 5]). Cummulative incidences are illustrated in; the concordance statistic was 0.68.

Conclusions: Internal validation of a proposed prediction model for recurrent VTE ("continu-8 score") that can be potentially used in all patients (provoked and unprovoked) during anticoagulant treatment, demonstrated a moderate distriminative ability in patients with DVT. If externally confirmed in other settings, the continu-8 score provides a easy applicable assessment tool for all patients with DVT in clinical practice.

PB 266 | Anticoagulation Control in Premenopausal Women with Mechanical Heart Valves Using Vitamin K Antagonists: Room for Improvement

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Background: High quality of anticoagulation control is essential for safe and effective treatment with vitamin K antagonists (VKA) in patients with mechanical heart valves (MHV), as these patients are at high risk of bleeding and thrombotic complications. Variability of coagulation factors during the menstrual cycle may cause differences in quality of anticoagulation control between premenopausal women and other patient groups.

Aims: To investigate anticoagulation control in women with MHV on VKA.

Methods: We included a cohort of patients treated with VKA for a MHV, who were monitored by a Dutch Anticoagulation Clinic and started VKA therapy between 2005 and 2015. Quality of anticoagulation control in premenopausal women with MHV, defined as percentage of time in therapeutic range

(TTR, Rosendaal method) and as cross-sectional proportion (CSP), was compared with that of postmenopausal women and age-matched men. A waiver for informed consent was obtained by the local ethics committee.

Results: In total 1348 patients were eligible for inclusion, of whom 41% were female. Of these, 118 patients were ≤40 years at inclusion, 47% were female. In women ≤40 years, more INR-measurements were performed during follow-up than in age-matched men (55,7% vs. 44,3% of total measurements;

p< 0.001). Median TTR was significantly lower in women compared to men (70.1% vs. 73,3%; p=0.022). Furthermore, premenopausal women had a significantly lower TTR compared to postmenopausal women (64,4% vs. 70,6%; p=0.015). Overall, CSP was similar in males and females (p=0.66). However, premenopausal women had a lower CSP (60,7%) compared to postmenopausal women (67,5%; p=0.31) and age-matched men (67,2%; p=0.46).

Conclusions: This study shows that menopausal status is associated with anticoagulation control. Anticoagulation control is worse in premenopausal women compared to postmenopausal women and age-matched men. As these patients are at significant risk of complications, optimization of VKA therapy in young women is of great importance.

PB 267 | Involvement of Antithrombotic Therapy in Intracranial Bleeding: A Tertiary Hospital Experience

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Background: Intracranial bleeding (IB) is a major cause of death and is the most feared complication of antithrombotic therapy (AT). Little data is available on the clinical course and prognosis of these patients.

Aims: To describe the clinical profile, etiology and management in patients diagnosed with IB in a tertiary hospital.

Methods: A retrospective analysis was performed in a consecutive series of patients older than 18 years with the diagnosis of IB from the 1st of January to the 30th of June 2015. The demographic characteristics, clinical presentation, etiology, treatment strategies, and outcome were studied.

Results: A total of 213 patients [median age 72 yo (29-96), 57% male] were included in the analysis; The most frequent localization of the IB was intraparenchymal (35.6%), followed by subdural (30.9%) and subarachnoid (24.4%). Initial clinical presentation was heterogeneous, headache being the most common finding in the subarachnoid cohort, loss of consciousness in the subdural cases, and focal neurological signs in the intraparenchymal subgroup. Additionally, less than half (46%) received antithrombotic treatment, of which 38% were with acetylsalicylic acid 100 mg (ASA), 34% with a vitamin K antagonist (two-thirds of which were within therapeutic range) and 5% with DOACs. We would like to highlight that only 28% of these patients received antithrombotic reversal agents. With regards to the etiology, 35% were due to head trauma, 10% to aneurysm or vascular malformation, 29% to unknown causes and 7% due to AT without previous trauma. Mortality in patients on AT was 25% and 20% in non AT cases.

Conclusions: In just 6 months, our cohort reflected a high number of IB, mostly due to head trauma injuries. Half of them were on AT, out of which, the majority received ASA and VKA. Although reversal agents are one of the mainstays of the treatment, only 30% received them Thus elucidating the possible impact of AT decision and management in this patient set.

PB 268 | One-year Medical Costs and Predictors of Costs of Pulmonary Embolism: A Prospective Study

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Background: Pulmonary embolism (PE) is a frequent and severe disease and an important driver of costs. Available medico-economic studies are retrospective. Most of them were conducted in North America and only focused on initial in-hospital costs. Clinical and social predictors of costs have not yet been assessed.

Aims: To prospectively assess long-term medical costs and social and clinical predictors of these costs of acute PE in France.

Methods: Prospective, monocentre, observational medico-economic study. From May 2012 to May 2013, all patients hospitalized for an objectively confirmed PE were included and followed up one year. Hospital-acquired PE patients were excluded. In-hospital costs were estimated from the French hospital discharge database and we prospectively collected after patients were discharged all relevant PE-related costs. We assessed the influence of demographics, of social and clinical characteristics of patients at baseline, of initial severity of PE and of rehospitalisation on these costs.

Results: 204 patients were included. Their mean age was 67 years. Proportion of patients with low-, intermediate- and high-risk PE was respectively of 48% (n=97) 47% (n=95) and 6% (n=12). Mean total cost was 10,886€ (US$ 11,452) (Table 1) and was mainly driven by total (81%) and initial (62%) hospitalization costs. Initial severity of PE (p=0.01), index PE occurring while on therapeutic anticoagulation (p=0.04) and likely low compliance to treatment (assessed at baseline) (p=0.01) independently predicted one-year PE costs. A lower weight, an initial anaemia and regular visit to primary care physician or nurse prior to index PE event also increased one-year costs but results failed to reach statistical significance (0.05< p< 0.10).

TABLE 1 Description of one-year Pulmonary Embolism-related costs of patients hospitalized for a symptomatic Pulmonary Embolism

	Mean costs 2016 € (SD)	Mean Costs 2016 US$ (SD)
In-hospital Costs		
Initial hospitalisation	6,758 (6,455)	7,109 (6,791)
Post-acute care & readmission (bleeding, recurrence, cancer screening)	2,031 (5,654)	2,136 (5,948)
Total in-hospital costs	8,819 (8573)	9,277 (9,019)
Total ambulatory costs	2,070 (1,970)	2,177 (2,072)
1-year Total costs	10,886 (8,905)	11,452 (9,368)

Conclusions: One-year direct medical costs of PE are high and hospitalizations represent most of this cost. Initial severity of PE, occurrence of PE while on anticoagulants and likely low compliance to treatment significantly increased these costs.

PB 269 | The Impact of Antiphospholipid Syndrome and Related Comorbidities in the Quality of Oral Anticoagulation

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Background: Oral anticoagulation with vitamin K antagonists (AVK) is the mainstay in the management of Antiphospholipid Syndrome (APS). An adequate monitoring of anticoagulation is important to reduce morbi-mortality in APS patients, since they may still present with recurrent thromboembolic events despite proper treatment.

Aims: This study aimed to compare the characteristics and quality of oral anticoagulation between patients with different indications of VKA use.

Methods: The quality of anticoagulation was evaluated by Time in Therapeutical Range (TTR). Clinical data was obtained from patients' electronic medical records. The differences among groups were determined by one-way ANOVA and the association between variants was obtained by linear regression. The hazard ratio for recurrence of thrombosis and bleeding was assessed by Cox Regression.

Results: We evaluated 517 patients assisted from January, 2014 until January, 2016 - the patients´ and treatment´s data are described in Tables 1 and 2. Among APS patients, we found a lower mean value of TTR, when compared to the atrial fibrillation/prosthetic valve (AF/PV) cohort (61±19% *vs* 67±16%, p= 0.028), even though the APS group received higher doses of AVK (64.9±35.2mg *vs* 41±17.5mg, p= 0.001).

TABLE 1 Clinical characteristics of patients under anticoagulation

	AF/PV	APS	DVT
Number of patients	144 (27.5%)	141 (27.3%)	232 (44.7%)
Age (y)	60±10.6	43.1±12.1	53±12.2
Gender (M:F)	1:1.3	1:2.3	1:1.2
Atrial fibrillation:metallic prosthetic valve	1:1.8	-	-
Venous thrombosis:arterial thrombosis	-	1:0.4	1:0.1
Comorbidities*	4 (2.8%)	54 (38.3%)	84 (36.2%)

The continuous variables above are expressed by mean ± standard deviation and the categorical variables are expressed by number and percentage.

*Main comorbidities were systemic auto-immunity, cancer, chronic kidney and liver disease and benign hematologic pathologies.

TABLE 2 Anticoagulation therapy data

	AF/PV	APS	DVT
TTR	67±16	61±19	63±19
TTR ≥ 65%	83 (57.6%)	65 (46.1%)	116 (50%)
Recurrence of thrombosis	0	7 (5%)	3 (1.3%)
Bleeding events	31 (21.5%)	15 (10.6%)	48 (20.7%)
Maximum AVK weekly dose (mg)	41±17.5	64.9±35.2	54.3±26.1
Mean time between visits (days)	58±15.5	53±21.9	61±25.5

The continuous variables above are expressed by mean ± standard deviation and the categorical variables are expressed by number and percentage.

No differences were observed in mean TTR values between the APS and venous thromboembolism (VTE) cohorts. There was a higher frequency of comorbidities in the APS and VTE groups in comparison to the AF/PV group (38.3% and 36.2% *vs* 2.8%; $p < 0.0005$). The presence of comorbidities was associated with lower TTR values and accounted for 6.3% of its variability along with age and gender ($F(4, 512) = 8.62$, $p < 0.0005$). Despite the higher AVK doses and lower TTR values, the APS cohort presented with a lower number of bleeding events (HR 0.33; $p=0.003$) with similar incidences of recurrent thrombosis.

Conclusions: APS patients still represent a clinical challenge nowadays and the quality of AVK therapy in this group may differ from other AVK users.

PB 270 | Predicting Recurrent Venous Thromboembolism in Patients with Deep-vein Thrombosis: External Validation of a Prediction Model

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Background: Predicting the risk of recurrent venous thromboembolism (VTE) remains challenging, and existing prediction models are still incompletely evaluated.

Aims: To externally validate an existing prediction model for recurrent VTE in a contemporary cohort of patients with proximal deep vein thrombosis (DVT).

Methods: The DASH score (D-dimer, Age, Sex, Hormonal therapy), a prediction model developed in patients with unprovoked VTE only, was applied to data of patients with provoked as well as unprovoked proximal DVT. All patients treated between 2003 and 2013 in a clinical care pathway at Maastricht University Medical Center were prospectively followed for up to 11 years from cessation of anticoagulation treatment. Analyses were stratified for provoked and unprovoked DVT, and c-statistics were calculated as a measure of predictive performance. Differences between risk groups (i.e. low risk, intermediate risk and high risk) were assessed by Kaplan-Meier plots and log-rank test.

Results: Sixty-four of 479 patients, followed for a median of 3.12 years (IQR 0.78, 3.90), developed recurrent VTE (13%); the overall recurrence rate was 3.7 per 100 patient-years (95% CI 2.9, 4.8). The c-statistic of the DASH score was 0.68 (95% CI 0.61, 0.74). After stratification the c-statistic was 0.73 (95% CI: 0.61, 0.86) for patients with provoked, and 0.63 (95% CI: 0.54, 0.71) for patients with unprovoked DVT.

Conclusions: External validation confirmed the predictive value of the DASH score in patients with unprovoked as well as provoked proximal DVT. However, the discriminative capability is only moderate and the implementation in clinical practice is hampered by the need to stop anticoagulation treatment prior to D-dimer testing.

PB 271 | Clinical Characteristics Associated with Diagnostic Delay of Pulmonary Embolism in Primary Care: A Retrospective Observational Study

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Background: Pulmonary embolism (PE) is listed among the diagnoses most frequently missed, or delayed, in clinical practice. Current evidence on diagnostic delay comes largely from studies performed in emergency departments (EDs), whereas evidence from primary care is scarce.

Aims: To evaluate the extent of delay in the diagnosis of PE in primary care, and to identify determinants that are associated with such diagnostic delay.

Methods: We performed a retrospective observational study in six primary care practices across The Netherlands. Patients with an objectively confirmed diagnosis of pulmonary embolism (ICPC-code K93) up to June 2015 were extracted from the electronic medical records. For all these PE events we reviewed all consultations with their general practitioner (GP) and scored any signs and symptoms that could be attributed to PE in the 3 months prior to the event. Also, we documented actual comorbidity and the diagnosis considered initially. Delay was defined as a time gap of >7 days between the first potentially PE-related contact with the GP and the final PE diagnosis. Multivariable logistic regression analysis was performed to identify independent determinants for delay.

Results: In total 180 incident PE cases were identified, of whom 128 patients had one or more potential PE-related contact with their GP within the three months prior to the diagnosis. Based on our definition, in 33 of these patients (26%) diagnostic delay was observed. Older age (age >75 years) (OR 5.1 (95%CI 1.8-14.1)) and the absence of chest complaints (OR 5.4 (95%CI 1.9-15.2)) were independent determinants for diagnostic delay. A respiratory tract infection prior to the PE diagnosis was reported in 13% of cases without delay, and in 33% of patients with delay (p=0.008).

Conclusions: Diagnostic delay of more than seven days in the diagnosis of pulmonary embolism is common in primary care, especially in elderly and if chest complains, like pain on inspiration, are absent.

PB 272 | Variability in Calculation of Time in Therapeutic Range for Quality Control Measurement of Warfarin

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Background: Time in therapeutic range (TTR), a widely recognized quality control metric of warfarin anticoagulation, measures the time a patient's international normalized ratio (INR) is within the desired range. The TTR value can vary depending on the method used (cross-sectional, Rosendaal, or traditional) and can thus be a skewed indicator of anticoagulation control. However, the different quantification approaches have been inadequately evaluated in literature for the clinical setting.

Aims: The aim of this study is to investigate variance and bias between the different TTR methods.

Methods: We conducted a 21-week retrospective study of patients on warfarin in an anticoagulation clinic. The clinic's TTRs were calculated using each method and then repeated with added tolerances per CHEST guidelines; the results are listed in Table 1. The wide range in values obtained is indicative of the dependence of TTR on the quantification method used and should be taken into careful consideration prior to analyzing provider performance. The following tests were conducted to quantify differences between the traditional and Rosendaal methods: paired t-test, correlation between size of the TTR and bias, and Bland-Altman plots.

Results: The traditional method resulted in significantly higher TTR values than the Rosendaal method, with high variability between the methods in both positive and negative directions. There was also a lack of independence between the size of the TTR and bias with the addition of tolerance (Table 2 and Figure 1). Thus the different methods are not measuring the TTR similarly and the addition of tolerance can further alter results predicting control of anticoagulation achieved.

Conclusions: The method used to calculate TTR can distort the provider´s perception of anticoagulation achieved. The impact of this perception could falsely imply control that is absent from practice. More evaluation is needed to determine the impact on clinical care.

TABLE 1 Results for TTR Methods with Added Tolerances

Method	TTR Result (n=612)
Rosendaal	55.1
Traditional	56.5
Traditional +/-0.2	71.4
Traditional +/-0.5	85.7
Cross-sectional	66.0
Cross-sectional +/-0.2	81.8
Cross-sectional +/-0.5	91.5

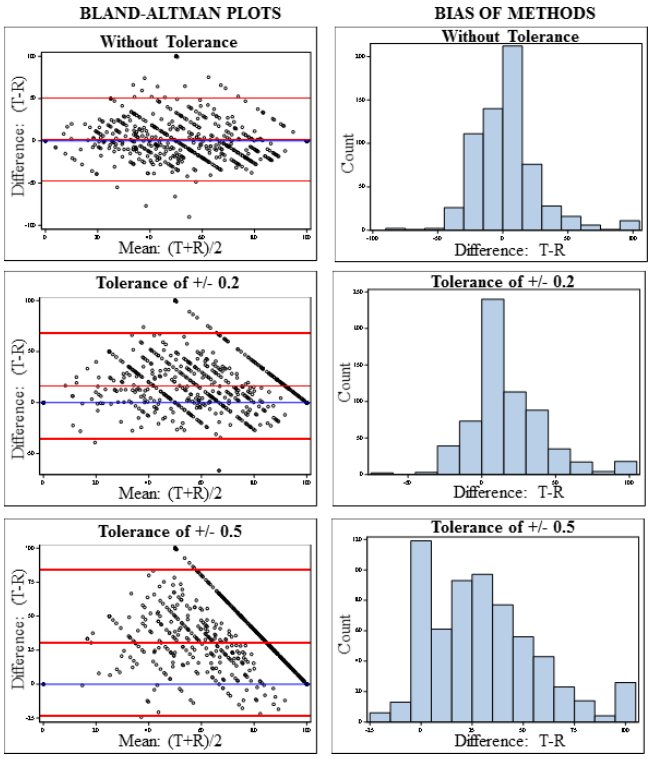

FIGURE 1 Bland-Altman Plots (Difference vs Mean) with Corresponding Bias Distribution Graphs of Traditional and Rosendaal Methods

PB 273 | D-dimer Levels Predict Clinical Outcomes in Patients with Mechanical Heart Valve Replacement during Oral Anticoagulation Therapy

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Background: Thrombosis and bleeding are main outcomes of patients after mechanical heart valve replacement (MHVR), however, there are still no useful marker to predict these outcomes.

Aims: To investigate whether D-dimer can predict outcomes in patients with MHVR during oral anticoagulation therapy.

Methods: Patients suffered MHVR in Wuhan Asia Heart Hospital between 2013 January and 2014 June were screened. Patients were

assigned to abnormal D-dimer group and normal D-dimer group according to d-dimer levels measured three months after the oral anticoagulation beginning. All patients were followed up for 24 months or until the observation of end points, which included thrombotic events, bleeding events and all-cause deaths. The anticoagulation therapy was monitored once per 1-2 months by the international normalized ratio (INR), and the target was INR 1.8-3.0.

Results: A total of 640 patients were included in analysis, 88 of whom had abnormal d-dimer levels and 552 of whom have normal d-dimer levels. During a follow-up period of 24 months, a total of 47(7.3%) events were observed. The incidence of total events among patients in abnormal d-dimer group, was significantly higher than that of normal d-dimer group (15 vs 32,17.0% vs 5.8%, p< 0.01, HR:5.6); There were 23(3.6%) thrombotic events totally, the incidence of thrombotic events among patients in abnormal d-dimer group was significantly higher than that of normal d-dimer group (10 vs 13, 11.4% vs 2.3%, p< 0.01, HR:15.7). There were 18 (2.8%) all-cause deaths totally, the all-cause mortality among patients in abnormal d-dimer group was significantly higher than that of normal d-dimer group (7 vs 11, 8.0% vs 2.0%, p< 0.01, HR:10.4). Total 19 bleeding events were observed, there was no significant difference in the incidence of bleeding events between two groups (2 vs 17, 2.1% vs 3.3%, p=0.77, HR:0.8).

Conclusions: D-dimer could be a useful marker to predict thrombotic events and all-cause deaths in patients with MHVR.

PB 274 | Prevention of Recurrent Gastrointestinal Bleeding in Anticoagulated Patients with Vitamin K Antagonists: Randomized Study Comparing Warfarin with Bemiparin

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Background: Acute digestive haemorrhage (DH) in anticoagulanted patients is highly prevalence of unknown etiology and considerable rate of haemorrhagic recurrence. Low molecular weight heparins offer a better pharmacokinetic profile and more stable anticoagulation levels, so they may be safer than coumarins

Aims: Evaluate if bemiparin at adjusted anticoagulant doses, decreases DH recurrences compared to warfarin

Methods: A prospective, open, controlled, and randomized study was carried out between 2012-2016. We were included patients with anticoagulant treatment and severe DH of unknown etiology or with the presence of multiple lesions. Patients were randomized in 2 groups: warfarin (INR range suitable for its indication) vs bemiparin (0.4-1.0 Uanti-Xa / ml peak)

Results: We included 46 patients (24 Bemiparin vs 22 warfarin). Mitral valve prothesis (5 p), multiple prothesis (4), atrial fibrillation (AF) embolic (6), AF + valvulopathies (18), venous thromboembolism(4), isolated AF with CHADS2> 3 (5). The diseases were homogeneously distributed between both groups.

The mean follow-up was 1,123 days (range 434-1,689). DH were of diverticular origin in 19 (41.3%), angiodysplasia 4 (8.7%) and unknown 23 (50%). The groups were comparable in age, sex, and other baseline characteristics. There was no difference in the severity of the DH. INR at time of index event DH was supratherapeutic in similar percentage in both groups. The recurrences were 6 (27%) warfarin vs 0% bemiparin (p = 0.004). No embolic events were seen. Complications related to their basal cardiovascular disease were 14% warfarin vs 17% bemiparin. Survival was similar (11% vs. 15% at 12 months, respectively, HR = 2.5, 95% CI = 0.5-12.7, p = 0.27).

Conclusions: The use of bemiparin (dose-adjusted anti-Xa levels) decreases long-term DH recurrence compared to warfarin. No thromboembolic complications were observed despiteof the high thromboembolic risk.

PB 275 | Has the Introduction of the DOACs Moved Patients with Labile INRs from Warfarin Therapy?

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Background: Warfarin therapy is first line for long term anticoagulation but patients with labile INRs, measured by TTR, should be considered for DOAC (NICE UK, MMP Ireland). If patients with low TTRs are switching to DOAC therapy, the mean TTR of the patients remaining on warfarin therapy should improve.

Aims: This study investigates if the mean TTR of the warfarin clinic at University Hospital Limerick has been affected by the introduction of the DOACs to the market in 2011.

Methods: A retrospective study of all INR tests performed by the clinic from June 2008 to July 2016 was conducted. The data was divided into years. TTR Rosendaal method was calculated for all patients with > 2 months anticoagulation and ≥3 INR tests. The patients were divided into two groups short term (≤4 months) and long term (>4 months) anticoagulation. A one-way ANOVA of the yearly TTR was performed. The patients achieving TTR of >=70% were identified.

Results: There is an overall reduction in patients on warfarin therapy since 2013.

The numbers of patients on short term warfarin therapy has reduced (101-52) since 2013, although the percentage achieving target TTR has decreased (59-33%).

The numbers of patients on long term warfarin therapy has reduced since 2013 (895-864). The mean TTR of the long term group has increased (74.9% to 76.3%), a one way ANOVA showed a statistical difference p=0.04).

The mean of the combined group increased from 74.5-75.3% since 2013 (ANOVA p=0.001).

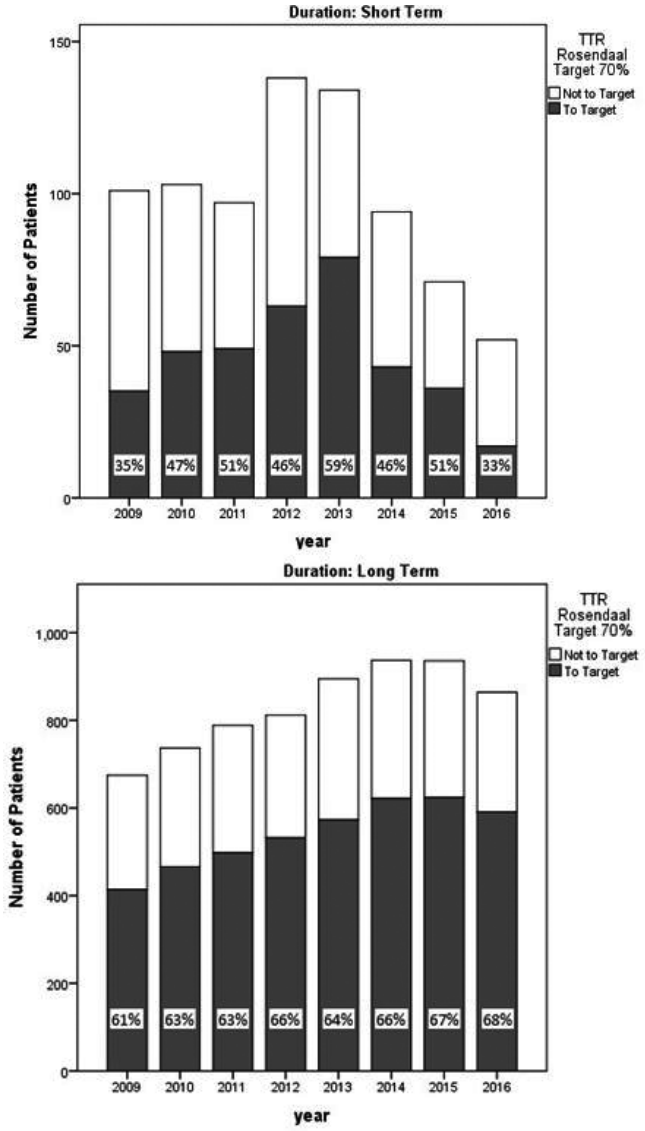

FIGURE 1 Long and Short term anticoagulation patients and percentage of patients achieving TTR target of 70%

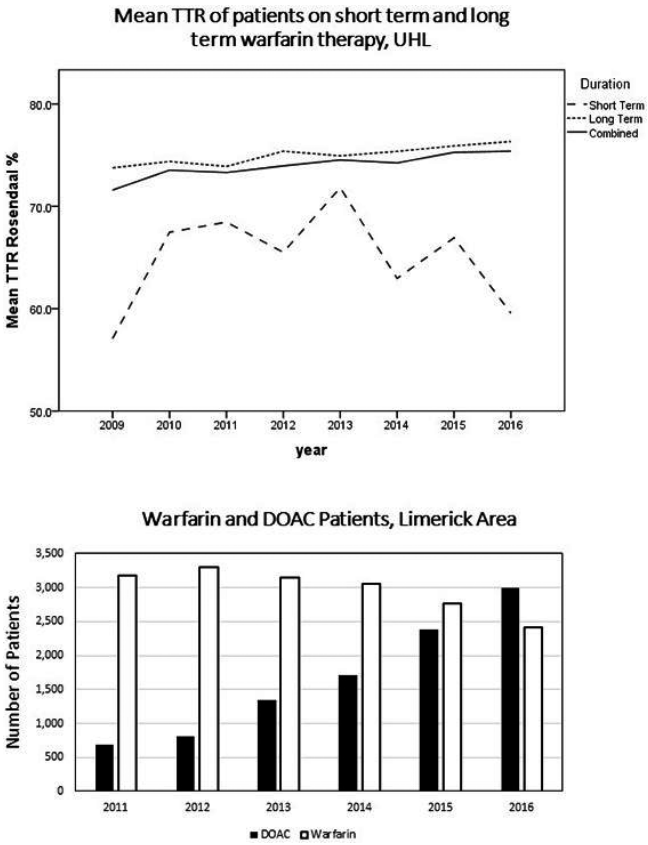

FIGURE 2 DOAC and warfarin use shown with mean TTR of the long and short term anticoagulation groups

Conclusions: The reduction of patients on warfarin therapy, is likely due to the introduction of the DOACs. The cohort on short term therapy is relatively more affected.

There is a small increase in mean TTR for those on long term therapy which may reflect some patients, with labile INRs, actively switching to DOAC therapy.

The combined TTR of the clinic has increased due to improved TTR in the long term group and a reduction in numbers in the short term group. There is clearly potential for improvement; 32% of patients in the long term group have TTRs below target.

PB 276 | Risk of Recurrence after Stopping Anticoagulation in Patients with a First Episode of Unprovoked Venous Thromboembolism: A Systematic Review and Meta-Analysis

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Background: For patients with a first unprovoked venous thromboembolism (VTE), the optimal duration of anticoagulation is a crucial clinical dilemma which has yet to be resolved. The decision to stop anticoagulant therapy (AT) after the initial 3-6 months or to continue

TABLE 1 Study and Patient Characteristics

Study	Design	Number of Patients	Gender (% Male)	Mean Age (SD)	Initial VTE Event	Definition of Unprovoked VTE (VTE occurring in the absence of)	Types of Thrombophilia Excluded
Kearon et al. (1999)	Randomized controlled trial (RCT)	83	53.0	58 (16)	61 DVT; 22 PE	Known cancer; Known Thrombophilia; Prolonged immobilization; Recent trauma; Recent surgery	Protein C/Protein S/ Antithrombin deficiency
Agnelli et al. (2001)	RCT	133	61.2	67.7 (7.3)	All proximal DVT	Known cancer; Known Thrombophilia; Prolonged immobilization; Recent trauma; Recent surgery; Pregnancy; Exogenous estrogen	Currently Not Available
Couturaud et al. (2015)	RCT	174	42.4	58.7 (17.9)	All PE	Known cancer; Known Thrombophilia; Prolonged immobilization; Recent trauma; Recent surgery; Pregnancy	Antiphospholipid antibodies; Factor V Leiden; Protein C/Protein S/Antithrombin deficiency
Rodger et al. (2016)	Prospective observational study	663	51.4	53.2 (range 18-95)	194 isolated PE; 346 isolated DVT; 123 DVT+PE	Known cancer; Known Thrombophilia; Prolonged immobilization; Recent trauma; Recent surgery	Antiphospholipid antibodies; Protein C/ Protein S/Antithrombin deficiency; Combined/"Double Hit"

TABLE 2 Risk of Recurrence After Stopping Anticoagulant Therapy (AT)

Study	Number of recurrent VTE events	Number of Patient-Years of Follow-up	Risk (%) per patient-year (95% Confidence Interval)	
In the first year after stopping AT				
Agnelli et al. 2001	11	89.431	12.30 (5.03 - 19.57)	
Rodger et al. 2016	62	600.110	10.33 (7.76 - 12.90)	
Pooled	73	689.541	10.55 (8.13 - 12.97)	
In the first 2 years after stopping AT				In the second year after stopping AT
Kearon et al. 1999	17	62.044	27.40 (14.38 - 40.42)	Currently Not Available
Couturaud et al. 2015	25	268.817	9.30 (5.65 - 12.95)	Currently Not Available
Rodger et al. 2016	96	1115.520	8.61 (6.88 - 10.33)	6.60 (4.38 - 8.82)
Pooled	138	1446.381	10.92 (6.11 - 15.73)	Currently Not Available

AT indefinitely, is primarily governed by the long-term risk of recurrence when treatment is discontinued. This risk however, is not well established, hindering decision making.

Aims: We sought to more precisely quantify the absolute, long-term risk of recurrent VTE over standardized time intervals of 1, 2, 5, 10, and 20 years after stopping AT in patients with a first unprovoked VTE.

Methods: We performed a systematic review and a meta-analysis of randomized controlled trials and prospective cohort studies involving unprovoked VTE patients who had completed at least 3 months of initial AT; and who were followed-up for the standardized time intervals of 1, 2, 5, 10 and 20 years (± 3 months) after stopping AT. The primary outcome of the rate of recurrent VTE was calculated for each study from the total number of recurrent events and the corresponding number of patient years of follow-up. We used a random-effects model to pool study results and reported a weighted estimate of the absolute risk per patient-year.

Results: We included 22 eligible studies in our analyses, and contacted the primary investigators of each study for additional data. Based on currently available data from 4 studies (1,053 patients; **(Table 1)**, the pooled absolute risk of recurrence during the first year was 10.55% per patient-year (95% CI, 8.13-12.97), and 10.92% per patient-year (95% CI, 6.11-15.73) during the first 2 years after stopping AT **(Table 2)**.

Conclusions: In unprovoked VTE patients, the risk of recurrence when AT is discontinued appears to be high. Final/updated numbers and conclusions will be provided once final data clarifications are supplied from all investigators. A clear-cut estimate of this risk will help guide the optimal duration of AT for such patients.

PB 277 | Outcome of Vitamin K Antagonist Associated Bleeding in Patients Treated with Prothrombin Complex Concentrate: The ROVAP Study

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Background: Vitamin K antagonists (VKA) are widely used for the treatment and prevention of thromboembolism. In patients with severe VKA-associated bleeding, haemostasis can be restored swiftly by prothrombin complex concentrate (PCC).

Aims: To evaluate clinical outcome of patients treated with PCC for VKA-associated bleeding.

Methods: We performed a retrospective cohort study of consecutive patients who received PCC for VKA-associated bleeding in five Dutch hospitals. Data was collected by chart review on medical history, VKA-related bleeding event, procedures and interventions to treat the bleed, international normalized ratio (INR), haemostatic efficacy, thromboembolic (TE) complications, and mortality. Haemostatic efficacy was classified as excellent, good, or poor for different bleeding localizations based on haemoglobin decrease over 24h (gastrointestinal [GI] bleeding), hematoma expansion (intracranial haemorrhage [ICH]), or cessation of visible blood loss (Sarode, *Circulation* 2013).

Results: One hundred patients were included. The mean age (±SD) was 74±12 years, 54% were male and 79% received VKA for atrial fibrillation. Most patients presented with ICH (41%) or GI bleeding (36%). Almost 80% of the patients received 10 mg vitamin K in addition to PCC. Median baseline INR was 3.9 (IQR 2.9-5.8). One hour after start of infusion, the INR was available for 50 patients and of these, 35 (70%) had an INR ≤1.4. Effective haemostasis was not assessable in 34 patients with ICH, since repeat CT was not performed routinely. Effective haemostasis was achieved in 47 of 66 (71%) patients (35 excellent, 12 good efficacy). TE complications were reported in 5 patients and 22 deaths (60% bleeding-related) were observed within 30 days after PCC administration.

Conclusions: PCC was shown to achieve effective haemostasis in 71% of patients with VKA-associated bleeding. TE complication rates were low, but mortality rates rather high, probably due to the large number of patients presenting with ICH.

PB 278 | Association of 5'-untranslated Region / Exonic VKORC1 Mutations with Vitamin K Antagonist Resistance: Results of the GFHT Cohort Study

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Background: Rare patients (pts) require unusual very high doses of vitamin K antagonist (VKA) and may not achieve therapeutic INR despite proper dose escalation. Relationships between vitamin K oxydoreductase gene *(VKORC1)* variations and their impact on VKA resistance are poorly known in clinical practice.

Aims: To address this issue, we conducted a multicenter cohort study to identify new *VKORC1* causes of VKA resistance and to evaluate their impact on resistance.

Methods: Patients were eligible if they received a VKA daily dose ≥ 2-fold mean maintenance dose (D) given in normal responders with combined *g.-1639GGVKORC1/CYP2C9*1/*1* genotype. A standardized questionnaire was prospectively fulfilled. *VKORC1* exons and 5´-untranslated region (UTR) on 2 kb were sequenced. Patients were also genotyped for *CYP2C9 (2*/*3)* and *CYP4F2* (p.Val433Met) SNPs. A resistance index (IRes) was defined as (effective dose/D)x100. Potential influence of patient characteristics on IRes was analysed wirh univariate and multivariate statistical models.

Results: Of the 278 analyzed pts, 94 (33.8%) carried ≥ 1 *VKORC1* mutations. At least one exonic mutation was identified in 76 pts (27.8%), corresponding to 18 distinct mutations (8 new ones); 57 pts (20.9%) presented ≥ 1 mutations corresponding to 13 5'-UTR variations (4 new ones). To be non-Caucasian was the only non-genetic variable associated with a higher IRes (p=0.0123). Carriers of an exonic mutation had a higher IRes ($p< 10^{-4}$). Interestingly, carrying both exonic and 5'-UTR mutations (excluding *-1639AVKORC1*) was associated with a lower IRes than carrying an isolated exonic mutation (p=0.005). Finally, mutations located in warfarin binding domains or in the reticulum lumen according to Czogalla *et al.* (2016) were associated with higher IRes, $p=10^{-4}$ and $p< 10^{-4}$, respectively.

Conclusions: Our study provides new data on the impact of *VKORC1* mutation location on VKA resistance. *VKORC1* status knowledge may help physicians in VKA escalation strategies or to propose alternative oral anticoagulant.

PB 279 | Repeated Serial D-Dimer (DD) Measurement after Anticoagulation Therapy (AT) withdrawal to Identify Patients (PTS) at Risk for Venous Thromboembolism (VTE) Recurrence

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Background: The DULCIS study (Palareti et al, Blood 2014) has shown that serial DD measurements (during and after AT withdrawal) is suitable in clinical practice for identification of pts in whom AT can be safely discontinued.

Aims: Since a number of pts who resumed AT subsequently to a first positive serial DD assessment repeated this procedure after a second period of AT, aim of this study was to evaluate the results of the second serial DD measurement procedure.

Methods: 107 outpts [71 males; age: 66 years (20-84)] with a first VTE [prox. deep vein thrombosis (DVT): n=52, DVT + pulmonary embolism (PE): n= 35, and isolated PE: n=20] were investigated. They had resumed AT after a positive result in a first serial DD measurement; after a median period of 7 months they repeated the serial DD procedure as previously performed (before stopping AT and after 15, 30, 60 and 90 days). The same age/sex specific DD cut-offs adopted in the first serial measurement (those proposed by the DULCIS study) were used. All pts were followed for a median period of 25 months and VTE events (proximal DVT, with or without PE, or isolated PE) recorded.

Results: At the second serial DD evaluation, DD was persistently negative in 18 (16.8%) pts who stopped AT, whereas DD resulted positive in 89/107 (83.2%) pts who were advised to resume AT; however, only 70 resumed AT while 19 refused. Primary outcomes occurred in: 1/18 pts [5.6% (95%CI: 0.1-27.3); 1.6% pt/y (95%CI: 0.01-8.5)] with persistently negative DD who stopped AT and in 7/19 pts [36.8% (95%CI: 16.3-61.6); 17.1% pt/y (95%CI: 7.1-32.0)] who had positive DD results but refused to resume AT. In the 70 pts with positive DD who resumed AT no VTE events occurred.

Conclusions: These data indicate that a second serial DD measurement after a first positive procedure is mainly confirmatory of positive results in the large majority of pts (83.2%), and cannot be recommended as routine.

PB 280 | Safety and Efficacy of Warfarin in Patients with Thrombocytopenia

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Background: Patients with moderate thrombocytopenia and comorbidities requiring anticoagulation are currently sub-optimally treated because of bleeding concerns. Guidance on anticoagulating such patients is currently lacking because of limited data on safety and efficacy of anticoagulation in such patients.

Aims: To evaluate the safety and efficacy of warfarin in patients with moderate thrombocytopenia.

Methods: This retrospective study compared the incidence of bleeding and thrombosis in a cohort of warfarinized patients with sustained platelet counts below 100 x 109/L against a cohort with normal platelet counts (>140 x 109/L). Primary outcomes of safety and efficacy were determined by incidence rate ratios (IRR) of bleeding and thrombotic events. International Normalized Ratio (INR) and platelet counts during adverse events in thrombocytopenic arm were secondary outcomes.

Results: 137 thrombocytopenic patients (104,985 patient-exposure days) were compared against 939 normal patients (715,193 patient-exposure days). IRR of minor, major bleeding and thrombosis amongst thrombocytopenic patients were 3.03 (95% CI: 1.57- 5.60), 1.48 (95% CI: 0.44-3.98), and 0.807 (95% CI: 0.09 - 3.43) respectively. Median INR and platelet count readings during minor and major bleeds were 3.60 (IQR: 2.70 - 4.12) and 3.12 (IQR: 2.82 - 4.22), and 99 ×109/L (IQR: 77.0 - 147.0 ×109/L) and 115 x109/L (IQR: 107.5 - 169.5 ×109/L) respectively.

Conclusions: Warfarinized thrombocytopenic patients are at higher risk of minor bleeding complications with a higher tendency for major bleeding but derive similar benefits against thrombotic events compared to normal patients. Bleeding events are associated with higher INRs. A narrow INR target with an upper limit below 2.5 together with closer anticoagulation monitoring may improve safety of patients.

PB 281 | AF Patients on VKAs: Heart Failure is Associated with Worst Quality of Anticoagulation and Higher Bleeding Risk. Results from the START Register

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Background: In patients with atrial fibrillation (AF) heart failure (HF) is a risk factor for stroke and its frequency is higher when AF is associated with coronary artery disease (CAD). HF is associated with volume overload, pulmonary congestion, peripheral edema and systemic venous congestion involving the liver. Warfarin metabolism can be reduced with prolonged half-life leading to overanticoagulation.

Aims: Aim of this study was to evaluate if HF is associated to a worst quality of anticoagulation expressed as time in therapeutic range(TTR) and to higher bleeding risk in a cohort of AF patients enrolled in the prospective multicenter START Register.

TABLE 1 Characteristics of patients

	Heart Failure	No Heart Failure	p
Patients	506	3010	
Males (%)	298 (58.9)	1623 (53.9)	0.04
Median Age, yrs(range)	78 (41-91)	76 (28-98)	0.000
Follow-up (x 100pt yrs)	764	5143	
Antiplatelet drugs	103 (20.4)	462 (15.3)	0.006
Median (IQR) Time above therapeutic range (%)	11 (4-19)	9 (4-16)	0.02
Median (IQR)Time in therapeutic range (%)	64 (52-75)	67 (55-77)	0.001
Median (IQR)Time below therapeutic range (%)	21 (12-33)	20 (12-30)	ns
Major Bleeds (rate x100pt-yrs)	20(2.6)	66(1.3)	0.01

Methods: Patients' demographic and clinical data were collected as electronic file in anonymous form in the web site of START-Register (ClinTrials Gov Identifier: NCT02219984). Here we present data on 3516 patient-streated with Vitami K antagonist (VKA) for AF. Quality of anticoagulation was evaluated, major bleeds occurred during follow-up was recorded.

Results: We evaluated 3516 AF patients on vitamin K antagonists, 506 of whom (14.7%) with a history of HF. Clinical characteristics of patients are reported in table 1. Among HF patients CAD is more frequent and the quality of anticoagulation is significantly worst with respect to patients without HF. In particular time in therapeutic range (TR) is significantly lower and time above TR longer. During follow-up 86 major bleeding were recorded , 20 occurred in patients with HF. Patients with HF have higher bleeding risk in comparison to patients without HF: RR 2.0(95% CI 1.17-3.40); p=0.01.

Conclusions: AF patients with HF patients show a less good quality of anticoagulation, versus those without HF, with longer time spent above the TR and carry a higher risk for bleeding.

PB 282 | Thrombotic Complication Rates Following Anticoagulation Reversal: A Retrospective Evaluation of Prothrombin Complex Concentrates

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Background: Effective reversal of anticoagulants for life-threatening bleeding events and emergent procedures can be challenging. In Canada, patients on vitamin K antagonists (VKAs) receive prothrombin complex concentrates (PCCs), with or without vitamin K. PCCs have also been used for direct oral anticoagulant (DOAC) associated bleeding, before specific antidotes were available.

Aims: To describe the clinical outcomes of PCC reversal in patients with VKA and DOAC-associated life-threatening bleeding or needing emergent surgery.

Methods: A retrospective chart review of consecutive patients receiving either activated PCC for reversal of DOAC anticoagulation or PCC for reversal of VKAs between January-June 2014 at The Ottawa Hospital was performed. Thrombotic complications and associated mortality were evaluated up to 30 days post-PCC.

Results: A total of 11 patients received activated PCC for reversal of DOAC anticoagulation

(Table 1, Table 2). There were 5 patients on dabigatran, 3 patients on rivaroxaban and 3 patients on apixaban. Activated PCC dosing ranged from 1812 IU to 6000 IU. A single patient experienced a transient ischemic attack (TIA) within hours of aPCC administration (30-day thromboembolic complication rate = 9.1%). 30-day overall mortality was 36.4% (4 deaths). There were 3 fatal bleeding events, and no fatal thromboembolic events.

A total of 194 patients received PCC for reversal of warfarin anticoagulation (Table 1, Table 2). PCC doses ranged from 1000 IU to 2500 IU. The 30-day thromboembolic complication rate was 5.2% (4 deep veins thromboses, 2 myocardial infarctions, 2 TIAs, 2 strokes). The 30-day overall mortality rate was 16.0%

(31 deaths), with 8 deaths attributable to fatal bleeding and 2 deaths attributable to thrombotic events.

TABLE 1 Patient Demographics

Characteristic	*aPCC-DOAC Cohort (n=11)*	*PCC-Warfarin Cohort (n = 194)*
Age (Mean, Years)	83.9	76.3
Male (%)	72.7	54.1
CHADS2 (Mean)	3.1	2.5

TABLE 2 Indications for Anticoagulation and Reversal

	aPCC-DOAC Cohort (n=11)	PCC-Warfarin Cohort (n = 194)
Anticoagulation Indication		
Atrial Fibrillation	9	123
VTE	2	34
Atrial Fibrillation + VTE	0	17
Mechanical Valve	0	16
Other	0	20
Reversal Indication		
Major Bleeding	8	87
Pre-Operative Reversal	3	107

Conclusions: Administration of aPCC for DOAC reversal appears to be associated with a relatively similar thromboembolic complication rate as compared to PCC administration for warfarin reversal. Larger prospective studies are required to confirm these findings.

PB 284 | Assessment of the Quality of Oral Anticoagulation Control at Two Hospital-based Clinics in Brazil

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Background: Warfarin remains widely used by patients with cardiovascular diseases. When using warfarin, the quality of oral anticoagulation control is a critical determinant to minimize the risk of bleeding and thromboembolic events. Anticoagulation control is usually assessed by the time in therapeutic range (TTR) which is recommended to be above 60%.

Aims: To assess the quality of oral anticoagulation control at two pharmacist-managed anticoagulation clinics (AC) using two different measurement methods.

Methods: This study included adults with indication of continuous warfarin use. Patients were recruited at two AC of public hospitals in Brazil (2014-2015). Anticoagulation control was assessed by time in therapeutic range (TTR) and by the proportion of International Normalized Ratio (INR) values in range. These methods were compared by the Spearman correlation coefficient ($p < 0.05$). The study has been approved by the Research Ethics Committee, and all patients signed an informed consent.

Results: A total of 554 patients were studied, 57.4% female. The median age was 63.7 (Quartile 1 (Q1) 54.3; Quartile 3 (Q3) 73.6) years. The most frequent indication for warfarin therapy were AF/flutter (n=427; 77.1%) (Table 1). TTR variation was 12.7-99.5% and 344 (61.6%) patients had TTR ≥60%. The proportion of INR values in range was 53.5% (Q1 43.5%; Q3 62.5%) with a variation of 14.0-92.3%. The proportion of INR values in range was 53.5% (Table 2). There was a high correlation (0.88; $p < 0.001$) between these methods.

TABLE 1 Sociodemographic data of studied patients at two hospital-based anticoagulation clinics

Sociodemographic data	AC 1 (n=242)	AC 2 (n= 312)	Total (n=554)
Age, median (Q 1; Q3)	68.4 (58.7; 76.0)	61.0 (51.25; 70.1)	63.7 (54.3; 73.6)
Female, n (%)	131 (54.1)	187 (59.9)	318 (57.4)
Race, n (%)			
Non-White	172 (71.1)	204 (65.4)	376 (67.9)
White	70 (28.9)	108 (34.6)	178 (32.1)
Montly income - US dollars, median (Q 1; Q3)	221.4 (221.4; 284.0)	184.5 (110.7; 221.4)	221.4 (137.0; 221.4)

Currency conversion, $1.00 US dollar=R$ 3.27 Brazilian Reais (12/26/2016).

Abbreviations: AC, anticoagulation clinic; Q1, Quartile 1; Q3, Quartile 3.

TABLE 2 Clinical data of studied patients of two hospital-based anticoagulation clinics

Clinical data	AC 1 (n=242)	AC 2 (n= 312)	Total (n=554)
Indications for oral anticoagulation, n (%)			
Atrial fibrillation/flutter	196 (81.0)	231 (74.0)	427 (77.1)
Mechanical heart valves	20 (8.3)	117 (37.5)	137 (24.7)
DVT/pulmonary embolism	3 (1.2)	9 (2.9)	12 (2.2)
Stroke/transiente ischemic attack	90 (37.2)	18 (5.8)	108 (19.5)
TTR, median (Q 1; Q3)	66.8 (57.0; 76.7)	61.5 (52.2; 72.0)**	64.3 (54.0; 74.0)
Fraction of INR in therapeutic range, median (Q 1; Q3)*	53.9 (44.4; 63.22)	53.0 (43.2; 62.0)**	53.5 (43.5; 62.5)

Abbreviations: AC, anticoagulation clinic; DVT, deep venous thrombosis; TTR, time in therapeutic range; Q1, Quartile 1; Q3, Quartile 3.

*TTR and fraction of INR in therapeutic range calculated for 546 patients.**No access to INR for eight patients at AC 2.

Conclusions: The studied pharmacist-managed AC have achieved an adequate oral anticoagulation control in Brazilian patients with low socioeconomic status. This study enables us to contextualize the performance of two hospital-based public AC in the context of internationally used parameters to predict warfarin-related events. Pharmacists may be important as part of multidisciplinary teams, to improve patient education and the quality of warfarin use in low- and middle-income countries.

PB 285 | Factors Associated with Postoperative Hemorrhage in Patients Undergoing Dental Surgery

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Background: Anticoagulant and antiplatelet therapies are widely used to prevent and treat thromboembolism. Dental surgery in patients under antithrombotic therapy is of concern. Anticoagulant therapy may increase the risk of peri/post-operative bleeding.

Aims: To investigate factors associated with postoperative bleeding in patients under therapy with anticoagulant or antiplatelet drugs undergoing dental surgery.

Methods: This cohort employed a retrospective chart review of patients on antithrombotic therapy and undergoing dental surgery (2010-2015) at a hospital-based dentistry service. We surveyed sociodemographic and clinical data, including peri/postoperative bleedings. INR values were used to calculate the time in therapeutic range (TTR). We investigated the factors associated with postoperative bleeding using odds ratios (OR) calculations with 95% confidence intervals (CI). The study was approved by the

Research Ethics Committee, and patients signed an informed consent.

Results: The mean age was 57 (±14.2) years, 57% male. A total of 179 patients underwent 293 dental surgeries. Eight cases of perioperative and 12 of postoperative bleeding were recorded. The complications were generally managed with local measures and did not require hospitalization. The mean TTR was 64% for patients without bleeding complications and 67% for those with postoperative bleeding. We found association of postoperative hemorrhage with increased perioperative bleeding (OR 8.88; 95% CI 1.51-52.20), therapeutic-INR 2.5-3.5 (OR 3.76; 95% CI 1.03-13.73) and the combination of anticoagulant and antiplatelet therapies (OR 1.85; 95% CI 1.43-2.50).

Conclusions: Dental surgery in patients under antithrombotic therapy might be carried out without altering the regimen because of low risk of peri and postoperative bleeding. However, patients with increased perioperative bleeding or taking the combination of anticoagulant and antiplatelet therapies should be closely follow up because of increased risk of postoperative complications.

PB 287 | Thrombin Generation Test: A Reliable Tool to Evaluate the Pharmacodynamics of Vitamin K Antagonist Rodenticides in Rats

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Background: Vitamin K antagonist (VKA) rodenticide pharmacodynamics (PD) has been extensively studied in rodents with traditional laboratory tests based on clotting time which are insensitive to physiological inhibitors and intrinsic amplification of the clotting cascade.

Aims: We wondered if thrombin generation test (TGT) could add value to PD study of VKA in rats.

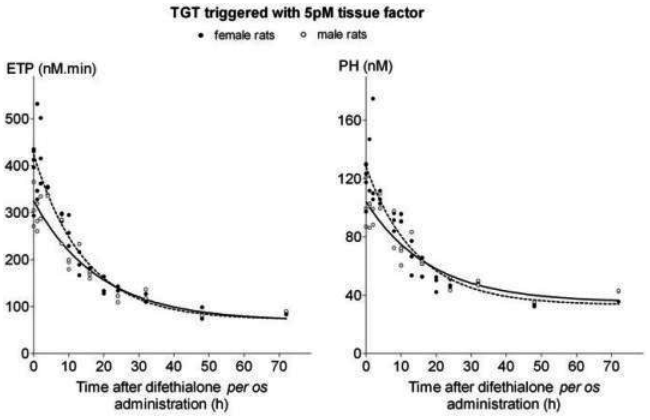

FIGURE 1 Kinetics of thrombin generation parameters (ETP, PH) after difethialone intoxication

Methods: Difethialone (10 mg/kg) was administered *per os* to 78 OFA-Sprague Dawley rats, and its PD was studied over a 72h-period using TGT (Calibrated Automated Thrombogram) on platelet poor plasma along with prothrombin time (PT) and vitamin K dependent factor activities (FII, FVII, FIX and FX), before (T0) and 1, 2, 4, 8, 10, 13, 16, 20, 24, 32, 48 and 72 hours after intoxication.

Results: Triggered with either 5 or 20 pM tissue factor, thrombin generation was fully inhibited at T24. To obtain reliable TGT parameters, samples were complemented with pooled normal rat plasma (3/1, v/v). Complementation allowed obtaining peak height (PH) and endogenous thrombin potential (ETP) all along the study-period. PH and ETP as a function of the intoxication time fitted well exponential decay models (Figure). TGT results confirmed the known significantly more pronounced procoagulant basal level in females compared to males for ETP and PH ($p< 0.0001$ and $p=0.0015$, respectively). ETP and PH decrease were observed very early after VKA intoxication, with comparable half-lives: 10.5h (CI95% [8.2; 13.6]) for ETP and 10.4h (CI95% [7.8; 14.1]) for PH. In contrast, PT prolongation was observed 2 hours post-intoxication in males and even 8 hours in females. Finally, we observed that FVII and FX decreased firstly, followed by TGT parameters (ETP, PH), while FII and FIX decreased later on.

Conclusions: We demonstrated that TGT run on samples from intoxicated rats previously complemented with pooled normal plasma is a reliable tool to evaluate VKA rodenticide PD, allowing exploration of both hyper- and hypo-coagulability states in rodents.

PB 288 | The Efficacy of 3-mg versus 5-mg Warfarin Initiating Dose and Corresponding Dosing Schedule in Thai Patients Diagnosed with Venous Thromboembolism

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Background: Currently, the initiation of warfarin with a dose between 5 and 10 mg was recommended for the treatment of venous thromboembolism (VTE). However, variation in warfarin requirement has been reported with a lower maintenance dose required by the Asians. Presently, the optimal initiating dose in patients of different ethnic groups is unknown.

Aims: To evaluate the efficacy of a lower warfarin initiating dose and a corresponding dosing schedule in Thai patients with VTE.

Methods: Patients diagnosed with VTE were randomized to receive warfarin 3 mg/d or 5 mg/d for the first 2 days (day 1-2). Subsequent dose was adjusted according to the INR and the corresponding dosing schedule. The INRs were measured at baseline, on day 3, 5 and 8. Blood for *CYP2C9* and *VKORC1* SNPs were collected. The primary outcome was the number of patients who achieved the target INR (2.0-3.0) within 8 days. The study was approved by the ethic committee.

Results: A total of 56 patients were enrolled, 28 patients in each group. The mean age was 46.9±16.3 years. Thirty-six patients (64.3%)

had deep vein thrombosis. There were no significant differences of baseline characteristics between the groups. Seventeen patients (60.7%) in the 3-mg group and 22 patients (78.6%) in the 5-mg group achieved the target INR within 8 days (p=.146). However, there were significantly more patients in the 5-mg group who achieved the target INR earlier on day 5 comparing with those in the 3-mg group (53.6% vs 25%, p=.029). None of the patients had INR>5.0.The prevalence of *CYP2C9* *1/*1 and *VKORC1*-1639G>A was 92.9% and 53.6%, respectively. In addition, *VKORC1*-1639G>A was associated with the likelihood to achieve the target INR within 5 days (OR 3.81, 95%CI 1.19-12.16, p=.021).

Conclusions: The efficacy of 3-mg warfarin starting dose with subsequent dose adjustment was similar to that of 5-mg on day 8 after warfarin initiation. However, 5-mg initiating dose resulted in more patients who achieved therapeutic INR earlier on day 5.

PB 289 | Barriers to Implementing an Extended INR Testing Interval Policy for Stable Warfarin Patients: A Mixed Methods Study in Five Anticoagulation Clinics

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Background: Patients on chronic warfarin therapy require regular INR monitoring. Recent studies have challenged the need for monthly INRs in the most stable patients, suggesting the safety of extended INR testing intervals (up to 12 wk).

Aims: To explore barriers to implementing an extended INR testing interval for stable warfarin-treatment patients managed in nurse/pharmacist-run anticoagulation clinics.

Methods: Using a mixed-methods approach, we conducted semi-structured interviews with eight anticoagulation nurse or pharmacist staff members at five participating anticoagulation clinic sites to assess barriers to implementing a new extended INR testing interval policy. Interview guides were based on the Tailored Implementation for Chronic Disease framework. Informed by these interviews, we surveyed all anticoagulation clinical staff (n=62) about their self-reported utilization of extended INR testing intervals and specific barriers to implementing this practice.

Results: From the interviews, four themes emerged:

(1) staff overestimating their actual use of extended INR testing intervals,

(2) barriers to electronically reviewing existing INR values and the need to provide additional education for eligible patients,

(3) broad support for an electronic medical record flag to identify potentially eligible patients, and

(4) the importance of personalized nurse/pharmacist feedback.

In the survey (65% response rate), staff report offering extended INR testing intervals to 56% (46%-66%) of eligible patients. Most survey responders (24; 60%) agreed that an eligibility flag in the electronic medical record would be very helpful. Twenty-four (60%) of respondents agreed that periodic, personalized feedback on use of extended INR testing would also be helpful.

Conclusions: Leveraging information system notifications, reducing additional work load burden for participating patients and providers, and providing personalized feedback are strategies that may improve adoption and utilization new policies in anticoagulation clinics.

PB 290 | Vitamin K Antagonists Compared to Low-molecular-weight Heparins for Treatment of Cancer-associated Venous Thromboembolism: An Observational Study in Routine Clinical Practice

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Background: Since several trials have demonstrated that low-molecular-weight-heparin (LMWH) is superior to vitamin-K-antagonist (VKA) in preventing recurrent VTE in patients with cancer-associated VTE, guidelines now recommend LMWH mono-therapy in this setting.

Aims: We evaluated whether this shift resulted in improved outcomes in routine clinical practice.

Methods: We performed a cohort study of consecutive patients with cancer-associated VTE during 2001 and 2010. We compared the risks for recurrent VTE, major bleeding and mortality between patients diagnosed before and after 2008 during 6-month routine follow-up.

Results: A total of 381 patients were included, of which 234 (61.4%) were diagnosed before 2008. Before 2008, 23% of the patients were treated with LMWH, thereafter this percentage was higher: 67%. The 6-month cumulative incidence for recurrent VTE was 10.6% in patients diagnosed before 2008 versus 9.3% for patients diagnosed after 2008 (risk difference (RD) -1.1%, 95%CI -6.3, 5.3). The respective risks for major bleeding were 8.0% versus 6.3% (RD -1.6%, 95%CI -3.8-5.8), and 40% versus 42% (RD 1.8%, 95%CI -8.8, 12) for overall mortality (Table 1). The mean time in therapeutic range of patients treated with VKA was 61%.

TABLE 1 Patient outcomes

Outcome	Diagnosed before 2008 (n=234)	Diagnosed since 2008 (n=147)	Difference (95% CI)
Recurrent VTE	8.6%	7.5%	-1.1% (-6.3-5.3)
Major bleeding	6.4%	4.8%	-1.6% (-3.8-5.8)
Mortality	40%	41%	+1.8% (-8.8-12)

Conclusions: Despite a clear shift towards LMWH as agent of choice for cancer-associated VTE, we did not observe a clear improvement in terms of recurrent VTE and bleeding complications.

PB 291 | External Quality Assessment of the POCT Prothrombin Time Determination - the 6-Year Experience with the Evaluation of the Postanalytical Phase

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Background: The accuracy of the International Normalized Ratio (INR) determination is important for the anticoagulation care, just as proper interpretation of the INR is. The evaluation of the postanalytical phase as a subject of external quality assessment (EQA) has been added to the EQA of Point of Care Testing (POCT) prothrombin time determination in Czech Republic.
Aims: To analyze the results of the postanalytical phase evaluation as a part of 22 EQA cycles.
Methods: Each participant received two brief case histories with 3 questions (Q1: Is the INR below, within, or above the therapeutic range? Q2: Should the dose of warfarin be reduced, unchanged, or increased? Q3: What interval to the next INR measurement do you recommend?) The answers were classified as correct (leading to optimal treatment) acceptable (leading to suboptimal, but not harmful treatment) and incorrect (leading to potentially harmful modification of warfarin treatment). Correct and acceptable results were considered as successful. The Mann-Whitney test was used for the statistical analysis.
Results: 7378 triplets of answers were evaluated. The answers of (mean±SD) 85.5±13.4%, 85.2±14.6% and 79.9±15.7% of participants to Q1, Q2 and Q3, respectively, were successful and the answers of 85.5±13.4%, 80.4±17.8% and 66.9±18.8% of participants to Q1, Q2 and Q3, respectively, were correct. The answers to Q1 were more frequently successful ($p=0.03767$) and correct ($p < 0.00001$), than answers to Q3 and the answers to Q2 were also more frequently successful ($p=0.04253$) and correct ($p= 0.00037$), than answers to Q3.
Conclusions: The results of this study are in concordance with the real word clinical experience; the inappropriately long interval to next INR determination in an unstable patient is the most frequent mistake in the anticoagulation care, leading often to bleeding complications.

PB 292 | The Incidence of Thromboembolic and hEMorrhagic (ITEM) Complications in Patients on Anticoagulant Therapy. A Dynamic Parallel-group Cohort Study

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Background: Different healthcare models have been proposed to manage patients undergoing anticoagulant therapy but there are no studies comparing strategies and the effectiveness of Hemostasis and Thrombosis Centers (H&TC) work organization.
Aims: To evaluate:
1) Any difference in use of anticoagulant drugs (prevalence and type) in the two healthcare models and
2) the incidence of major bleeding and thromboembolic complications in patients on VKA and DOAC in two areas using different management strategies.
Methods: The ITEM study is a dynamic cohort study of patients receiving oral anticoagulant therapy performed in two districts, Cremona and Vicenza, North Italy. While these two areas share comparable health resources, the Cremona Anticoagulant Clinic (AC) offers a highly organized telemedicine system connecting with the peripheral health care units (general practitioners, nursing homes, home patients) managing nearly 90% of anticoagulated patients. The Vicenza AC operates only about 5% of all anticoagulated residents. All patients presenting at the local Emergency Department with a major complication(either thrombotic or hemorrhagic) of anticoagulant treatment are prospectively enrolled. The study is seized to demonstrate, after a 24-months follow-up, a 35% reduction of major bleeding and thrombotic events in the Cremona cohort.
Results: Follow-up started on February 1st, 2016 in Cremona and in March 1st, 2016 in Vicenza. At an 8-months interim analysis, use of anticoagulant was greater in Cremona than in Vicenza (2.1 vs. 1.4%, $p< 0.001$). The rate of thromboembolic events in patients on VKA was 1.5% in Vicenza and 0.9% in Cremona (corresponding to a 60% reduction of events by the telemedicine model). The incidence of bleeding complications was 0.2% in Cremona vs. 0.1% in Vicenza.
Conclusions: These preliminary results suggest that the availability of a telemedicine model may promote the use of anticoagulant drugs, with a possible relevant reduction of thromboembolic manifestations.

PB 293 | Specific Inhibition of Endogenous VKOR Enzymes by Oral Anticoagulants Reveals Lower VKORC1L1 Susceptibility due to Different Binding Site

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Background: Coumarins like warfarin are widely used as oral anticoagulants (OACs) that are prescribed to treat and prevent thromboembolism. In addition, coumarins are used as rodenticides since the 1950s. OACs inhibit vitamin K epoxide reductase (VKOR) activity, thereby reducing the availability of vitamin K (K) hydroquinone, thus leading to reduced γ-carboxylation of vitamin K dependent proteins. Vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1) and its paralog VKORC1-like1 (VKORC1L1) are enzymes which catalyze the reduction of K 2,3-epoxide to K hydroquinone.

Aims: Half-maximum inhibitory concentrations (IC_{50} values) of different 4-hydroxycoumarins and 1,3-indandiones were determined. Furthermore, warfarin binding on both VKOR enzymes were investigated by means of *in silicoand in vitro* analysis.

Methods: *VKORC1* and *VKORC1L1* gene expression was knocked out in HEK 293T cells using the CRISPR/Cas9 gene editing technique. *VKORC1* and *VKORC1L1* knockout HEK cells were transfected with human *F9* cDNA and incubated with vitamin K and various OACs. The resulting FIX activity served as a marker for VKR activity. In a second approach, *in silico* binding studies were performed to identify warfarin binding site in VKORC1L1. *In vitro* experiments were performed in double knockout cells for expression of *F9* and VKOR variants.

Results: OACs inhibited VKORC1 more efficiently compared to VKORC1L1. Enzyme specificity was even more pronounced when using the 1,3-indandiones. In contrast, rodenticides showed no marked difference in IC_{50} values of VKORC1 and VKORC1L1. To elucidate different inhibition pattern, *in silico* and *in vitro* studies were performed. Loop swapping indicated that this region is responsible in OAC binding. *In silico* analysis further restricted warfarin binding to three arginines in VKORC1L1.

Conclusions: VKORC1 and VKORC1L1 are enzymes both susceptible to various OACs. The warfarin binding sites for VKORC1 and VKORC1L1 are located in the loop but are different with respect to position and interacting residues.

PB 294 | A comparison of management strategies for patients with warfarin-induced bleeding

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Background: While 4 factor prothrombin complex concentrate (4PCC) is recommended for management of warfarin-induced bleeding, many clinicians still use fresh frozen plasma (FFP) and vitamin K. Concerns with the use of 4PCC include inexperience, cost, and risk of thrombosis, while concerns with avoiding 4PCC include extended time needed for efficacy.

Aims: Compare the safety and efficacy of the management of warfarin-induced bleeding with 4PCC vs a strategy without 4PCC.

Methods: This retrospective study included patients at our hospital with a major bleeding event on warfarin from 11/12 to 10/16. Patients receiving other anticoagulants were excluded. Patient data and outcomes were collected from patient charts. All descriptive data are shown as mean ± standard deviation.

Results: We identified 88 patients with warfarin-induced major bleeding treated with 4PCC (n=27) and without 4PCC (n=61). Patient demographics were not significantly different (Table).

TABLE 1 Patient Demographics

	4 Factor PCC (n=27)	No 4 Factor PCC (n=61)	p-value
Age (years)	74 ± 12	71 ± 13	0.31
Male (%)	63%	62%	0.95
Weight (kg)	86.3 ± 27.6	88.1 ± 23.7	0.75
HAS-BLED Score	2.4 ± 1.0	2.4 ± 1.2	1.0
Atrial fibrillation	56%	48%	0.49
Venous thromboembolism	30%	15%	0.14
Left ventricular thrombosis	3.7%	16%	0.16
Valvular disease	7.4%	21%	0.13
Stroke	3.7%	0.0%	0.31

Patients with 4PCC vs without 4PCC had similar baseline INR values (4.1±2.6 vs 3.6±1.9). Patients receiving 4PCC were more likely to have cranial bleeds (74% vs 41%; p=0.01), less likely to have gastric bleeds (7.4% vs 57%; p< 0.01), and less likely to receive PRBCs (22% vs 51%; p=0.01) and FFP (19% vs 82%; p< 0.01). Vitamin K was similar in use (81% vs 77%) and dose (8.5±4.0 vs 8.3±3.7mg). While overall reversal success was similar between the groups (70% vs. 62%), 4PCC provided significantly faster INR normalization (2.1±2.0 vs 30.0±26.7hrs; p< 0.01) and time to bleeding cessation (20±27 vs 37±32hrs; p=0.02). Use of surgery to control bleeding was less common with 4PCC (19% vs 33%). Length of hospital stay and mortality (18% vs 13%) were not different. No thrombotic events were reported within 30 days.

Conclusions: Reversal of warfarin-induced bleeding with 4PCC provided faster resolution and reduced the use of other blood products. While patient outcomes were not different, this may be due to our small sample size.

PB 295 | Provision of External Quality Assurance for Post Analytical Assessment of Point of Care Haemostasis Tests

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Background: UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) has been providing External Quality Assurance (EQA) for Point of care POC tests for 20 years.

Aims: In this report, the aim was to increase awareness of the importance of post analytical assessment within our POC programmes.

Methods: For POC D-dimer testing users receive a sample with a brief clinical history including age and Wells score and using their result are asked to state whether or not the patient requires further investigation. Surveys are scored if there is 80% or more agreement. Over 3 years 11 samples have been distributed for users of Triage and Cobas

TABLE 1 Results of the anticoagulant dosing exercises 1-3

			Recommnedations from CDSS users		Recommendations from Non CDSS users	
Exercise	Past INRs	Previous Daily dose	New daily dose median (range)	Recall for testing median (range)	New daily dose median (range)	Recall for testing median (range)
1	INR increasing above target over 7 week period	7mg	6.4mg (2-8mg)	7 days (7-28)	6.4mg (2-7mg)	7 days (2-28)
2	INR decreasing below target over 12 week period	7mg	7.7mg (1-9.1mg)	7 days (2-42)	7.7mg (1-10mg)	7 days (1-42)
3	INR increasing to top of target over 10 week period	5mg	5mg (3-5mg)	21 days (3-70)	4.8mg (2.5-5mg)	14 days (1-70)

h232 devices. For Triage users 9 surveys have been scored. In only 5/9 there was 100% agreement on interpretation. For h232 users, 9/11 samples were able to be scored but in only 1/9 was there 100% agreement on interpretation.

Results: In an assessment of INR dosing using virtual patient results in which users are encouraged to input the data into their local computer dosing support system (CDSS), 3 exercises have been completed. In 2/3 exercises the CDSS recommended follow-on warfarin dose was within 1mg of the median recommended dose in ≥90% but in 1 exercise only 76% were in agreement. A small number of results were potentially dangerous.

Conclusions: There is growing acceptance that EQA should include assessment not only of the analytical phase but should include pre- and post-analytic phases. The data presented strongly supports the need for external assessment of operator ability to interpret the results which they generate.

PB 296 | Warfarin Toxicities: Risk Factors and Management - Data from a Developing Country

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Background: Vitamin K Antagonists (VKA) has been the cornerstone in management and prevention of thromboembolic diseases. With the advent of target specific anticoagulants having encouraging results for safety and efficacy clinical trials, there is a paradigm shift in the use of VKA as standard. However in developing countries, where there is cost restraint VKA is still widely used. Bleeding is the most serious complication associated with oral anticoagulant. We studied the spectrum of bleeding complications in patients presented with warfarin toxicity in our institute.

Aims: To evaluate bleeding complications, risk factors and management strategies in patients with warfarin toxicity.

Methods: This was a retrospective study. All patients on warfarin who presented with bleeding complications from January - December 2015 were included in the study. Age, gender, indications, risk factors for bleeding, severity and management were reviewed from patient's record. Data was recorded on a predesigned proforma.

Results: The cohort included 51 patients (mean age 59 +/- 10 years, 64% male). Majority of patients were taking oral anticoagulant for therapeutic purpose most common being Atrial fibrillation (n=32, 63%). 76% (n=39) patients had one or more of co-morbidities. Common risk factors included old age (n=31, 60%) and hypertension (n=22, 43%). Major bleeding as per ISTH criteria was observed in 13 individuals (25.4%). Most common site of bleeding was gastrointestinal (n=17, 33%) followed by mucosal bleeding (n=13, 25%). Intracranial bleeding leading of death was observed in one case. The drug was discontinued in all cases with transfusion of fresh frozen plasma and use of Intravenous vitamin K supplementation in 33% (n=17) and 94% (n=48) cases respectively.

Conclusions: The rate of major bleeding and fatal complication with oral anticoagulants in our population where VKA are still preferred over novel anticoagulants is low. With regular monitoring, patient education and prompt management, bleeding complications can be reduced.

PB 297 | Use of a 4-factor Prothrombin Complex Concentrate for Warfarin Reversal prior to Urgent Surgery

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Background: While 4 factor prothrombin complex concentrate (4PCC) is recommended for the management of warfarin-induced major bleeding, its role in reversal of warfarin prior to urgent surgery is not well defined. If safe, the rapid onset of action would make 4PCC an attractive option for these patients.

Aims: Assess the efficacy and safety of 4PCC in warfarin reversal prior to urgent surgery in real world practice.

Methods: This retrospective study included all patients who received 4PCC at our hospital for reversal of warfarin for urgent surgery from 11/14 to 11/16. Success was defined as achieving an INR ≤1.5 prior to surgery. Patients receiving other anticoagulants were excluded from the analysis. Patient demographics, medication use, laboratory values, and outcomes were collected from patient charts. All descriptive data are presented as mean ± standard deviation.

Results: We identified 27 patients who received 4PCC for warfarin reversal prior to urgent surgery. Patients were 68% male, age 63.4±12.9 years, weight 96.4±26.1kg, and BMI 32.8±8.7. Indications for warfarin and most common indications for surgery are in Table 1.

TABLE 1 Indications for warfarin and surgery

Indications for Warfarin, n (%)	
Atrial fibrillation	12 (44)
Valve disease	7 (26)
Left ventricular assist device	6 (22)
Venous thromboembolism	2 (7)
Indications for Surgery, n (%)	
Heart transplant	7 (26)
Craniotomy	4 (15)
Bowel resection	3 (11)
Other (1 or 2 of each)	13 (48)

The weekly warfarin dose was 33.0±16.8mg, baseline INR of 3.1±1.7 and HASBLED Score of 1.6±0.7. Aspirin was also used in 13 patients. The 4PCC dose was 2581±886 U at 28.1±10.5 U/kg. Vitamin K was used in 15 patients and FFP in 8 patients. INR normalized to ≤1.5 in 21 patients (78%) within 1.4±1.2 hours. Time from 4PCC to surgery was 5.6±8.3hrs. INR prior to surgery was 1.3±0.3. Surgery lasted 4.8±3.4hrs with and estimated blood loss of 546±779mL. There were 7 thrombotic events within the following 30 days. Seven patients died during hospitalization, 1 due to thrombosis. 50% of surviving patients were discharged on anticoagulation.

Conclusions: While 4PCC was not 100% successful, it did provide a rapid strategy for warfarin reversal prior to urgent surgery. The thrombotic risk of 4PCC in this setting will require analysis in larger studies.

PB 298 | Can Results Be Improved on Vitamin K Antagonists (AVKs) Therapy ?

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Background: Prolongation of life leds to a sustained increase in the number of patients undergoing anticoagulant therapy. Perhaps it is time to focus on the education of the medical community, and raise awareness that these drugs must be handled by experts doctors. As well as insulin or chemotherapy all are in the category of high risk drugs.

Aims: In order to evaluate if the Time in Therapeutic Range (TTR) could be improved by the experience of the Hematologist and not only with the use of Technology Point of Care (POC) two populations of patients were compared.

These two populations received attention in the same Anticoagulation Clinic and were controlled with POC. One population attended by the young medical staff with „Non-strict physician instructions" (NSI) and the other with „strict instruction"(SI) by older staff.

Methods: Strict instruction included an education program using a web page. The use of one pillbox,and quick report of changes in other drugs prescribed. Retrospective analysis of 242 patients INR tested with POC technology (Coagucchek XSPro-Roche Diagnostics) were analyzed,130 patients in attention with SI, and 112 with NSI.

Time in Therapeutic Range (**Rosendaal Method for % INR in range)** and, Percentage of Test in Range (TINR%) were calculated. Comparative analysis of independent samples with a T Test, Confidence Interval (CI 95%) and median was performed in both groups.

Software SPSS 18.0 version was used in Statistic Analysis.

Results: NSI population shows TTR 57.,2% and TINR 52,2% versus SI population TTR 66,6% and TINR 63,2%.

Conclusions: The outcome of anticoagulant therapy with antivitamin K antagonist depends not only on the technology used in the control tests but also on the experience of the treating physician,and patient motivation.We are convinced of the need to raise awareness in society and in the medical community that the success of Anticoagulant Therapy (AVKs or DOACS) depends, not only on the drug chosen but also, on patient and his / her environment education and motivation.

PB 299 | Genetically Determined Hypersensitivity to Vitamin K Antagonists Caused by a c.109G>A (p.Ala37Thr) Mutation in the Factor IX Propeptide: The First Case Identified in Poland

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Background: Hypersensitivity of coagulation factor IX to vitamin K antagonists (VKA) is a rare congenital bleeding disorder that manifests only during VKA therapy. The bleeding tendency is related to severe reduction of FIX clotting activity, the level of which is much lower than that of other vitamin K-dependent coagulation factors. Two genetic alterations at locus 37 (previous -10) in exon 2 of the *F9* are associated with hypersensitivity to VKA: c.109G>A (p.Ala37Thr) and c.110C>T (p.Ala37Val).

Aims: Up to now bleeding diathesis associated with c.109G>A (p.Ala37Thr) mutation in *F9* have been reported in very few cases only.

Methods: A 74-year-old male patient was admitted to our Centre with recently presented bleeding disorder. Few months earlier he received acenocoumarol due to mechanical heart valve implantation. Plasma coagulation studies comprised screening tests and assessment of coagulation factors activity with one-stage assay using BCSXP Coagulation Analyser and Siemens reagents. Mutation analysis of *F9* was performed by direct Sanger sequencing of all coding regions and exon/intron splicing sites on the ABI Genetic Analyzer.

Results: Laboratory tests revealed anemia (hemoglobin 6.9g/dl) and prolongation of PT with INR 1.95. APTT was significantly prolonged (61.2s n.25-33s), fibrinogen and thrombin time - within reference range. FIX:C was less than 2IU/dl (n.50-150IU/dl) while levels of other

vitamin K-dependent factors II, VII and X were 51.5IU/dl, 26.5IU/dl and 26IU/dl, respectively. Factor V,VIII, XI and XII activity was normal. *F9* genetic analysis revealed c.109G>A variant. VKA was discontinued and normalization of coagulation test results and bleeding was reported.

Conclusions: To our best knowledge, this is the first case of genetically determined FIX hypersensitivity to VKA identified in Poland. Although the genetic mechanism of congenital hypersensitivity to VKA was discovered about 20 years ago, such cases are still worth presenting to increase the awareness of physicians and laboratory diagnosticians.

PB 300 | Vkorc1 and cyp2c9 Gene Polymorphisms in the Healthy Russian and Buryat Respondents in Trans-Baikal Region

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Background: Prevalence of CYP2C9 and VKORC1 gene polymorphisms widely study in Russian population. These are necessary for effective therapy of chronic atrial fibrillation with warfarin.

Aims: The aim was to study the prevalence of CYP2C9 and VKORC1 gene polymorphisms in Russian and Buryat respondents in Trans-Baikal region.

Methods: The study included 72 healthy respondents aged 40 to 60 years (31 Russian and 41 Buryat subjects). Genetic polymorphism was detected by PCR.

Results: It was found the frequencies of genotypes Arg/Arg and Arg /Cys of CYP2C9*2 gene among the Russian respondents were 74% and 26% respectively. In Buryats the polymorphic variants of this gene were follow: 95% - Arg/Arg genotype and 5% - Arg/Cys genotype. No statistically significant difference in the frequencies of genotypes among the Russian and Buryat were found. Genotype Cys /Cys was not detected in any of the studied groups. The frequencies of genotypes G/G, G/A, A/A of VCORK1 gene (polymorphic marker G3730A) in Russian were 7%, 74% and 19% respectively, that is statistically significantly different from the prevalence of these genotypes in Buryats (76%, 24%, and 0% respectively).

Conclusions: It was found the frequency of allele Cys of gene CYP2C9*2 is 12% in Russian population in Trans-Baikal region, which corresponds to European sings. But the same allele occurs at 2.4 times more rarely in Buryat respondents in Transbaikalia. Distributions of VCORK1genotypes in Trans-Baikal region differ from those in Moscow region: the genotype GG follows at almost 5 times more rarely among the Russian population in Trans-Baikal region than in Moscow region, while the incidence of genotype G/A, and A/A of gene VCORK1 is approximately the same. The frequency of homozygotes for the G allele of the gene VCORK1 (G3730A) was significantly higher among the representatives of Buryats and it was 1/12 of the studied, than among the Russian, while heterozygotes of this gene at 3 times more common in the Russian compared with Buryats.

PB 301 | The Use of Prothrombinex Alone to Reverse Warfarin in Orthopaedic Patients Requiring Emergency Surgery

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Background: Warfarin, a vitamin K antagonist, requires reversal prior to surgery for patients with acute orthopaedic injuries. Prothrombinex (PTX) is a lypholyzed concentrate of mostly coagulation factors II, IX, and X that is used to reverse warfarin. However, its therapeutic use without the addition of vitamin K (VK) is not well established, and theoretically has the potential to increase the risk of haemorrhage and cost in these patients.

Aims: Establish the efficacy and safety of PTX alone in warfarinized orthopedic patients.

Methods: A retrospective audit was conducted on all orthopaedic admissions to a Tertiary Hospital who received PTX with or without VK to reverse warfarin prior to emergency surgery between 3rd February 2015 and 3rd February 2016. Outcomes included time to surgery, length of hospital admission, haemorrhagic and thrombotic complications, and mortality. Results were compared to a control group of patients who received only VK for warfarin reversal.

Results: Of 48 patients, 4 were excluded because they were on a NOAC rather than warfarin, leaving 44 for analysis. Of these, 17 patients (38.6%) received VK prior to surgery in addition to PTX. The mean time to theatre was 23.8 hours (1.3 - 57) for those who received VK and PTX, compared to 23.4 hours (5.4 - 51.8) for the PTX group. With the mean time to haemostatic support following initial INR being 15.8 hours (2.3 - 52.5) and 18.2 hrs (4.2 - 38.3) respectively. The average length of hospital stay was 8.4 days (2 - 27) and 8.8 days (3 - 43) respectively. Analysis of the control group is pending. The 30d mortality rate was 6 (13.6%), 3 in each group. The total number of thrombotic complications documented was 3 (11.1%) in the PTX only group, and 5 (29.4%) in the VK and PTX group.

Conclusions: This audit supports the use of prothrombinex alone in the emergency setting to reverse warfarin's anticoagulant effect. There was no increased rate of haemorrhagic complications or mortality associated with the use of prothrombinex alone.

PB 303 | Utility of a new Point of Care system, Coag S, for Vitamin K Antagonist Therapy in a Rural Family Practice

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Background: Vitamin K Antagonists (VKAs) are still the most widely applied anticoagulants in the prophylaxis of thrombotic diseases. In contrast with Direct Oral Anticoagulants VKA anticoagulation provides a real - although relatively narrow - Therapeutic Range (TR) and the intensity

of anticoagulation could be characterized by a simple, internationally approved figure, the INR. VKA anticoagulation requires regular laboratory determination of the INR offering a tool to determine the efficiency of the treatment, the Time in Therapeutic Range (TTR). TTR is the proportion of time a patient spent within a predetermined Therapeutic Range. In rural environments where the access to a laboratory could be restricted, Point of Care (POC) INR measurements from capillary blood offer a suitable alternative, especially in the case of elderly patients where appropriate collection of venous blood sample can also be difficult.

Aims:

(i) Calculation of patient TTRs referring to one year period of stable VKA treatment.
(ii) Evaluation of the utility of the Coag S POC system.
(iii) Identification of practice specific factors influencing the efficiency of the treatment.

Methods: The study covers a retrospective review of records of 1 year follow up (2016). TTRs were calculated with the Rosendaal method. The practice covers 1679 persons and POC VKA management were chosen by 49 patients. Patients who underwent short term or interrupted VKA treatment were excluded thus 25 patients were eligible with TR from 2 to 3 INR.

Results: Patient description and TTRs are summarized in Table 1.

TABLE 1 Patient description and TTRs

Patient Charateristics		Patient Number	TTR [%]	Average Number of Tests	Average Time Between Tests [day]
Gender	Male	12	68.69	13	28
	Female	13	74.53	10	34
Age	50-59	4	79.39	12	34
	60-69	7	78.74	11	33
	70-79	6	67.83	11	32
	80-89	8	64.68	13	27
Total		25	71.73	11	31

Conclusions: Coag S is a suitable POC tool to monitor VKA treatment providing i.) appropriate TTRs and ii.) convenient VKA management. iii.) Advanced age associated with lower TTR but in contrast with the literature patients till 70 years of age can maintain TTR above 75% and women show higher adherence than men requiring significantly less frequent monitoring.

PB 304 | Duration of Anticoagulation in First Venous Thromboembolism (VTE) in the Newcastle upon Tyne Hospitals NHS Foundation Trust (NUTH): A Quality Improvement Project

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Background: Anticoagulation duration after first VTE is based on multiple factors, including thrombosis site, bleeding/recurrence risk and patient choice. In NUTH, practice is to anticoagulate for a minimum of three months in provoked or distal VTE and six months in unprovoked proximal VTE. A risk/benefit analysis informs whether to discontinue or extended treatment. D-dimer monitoring and DASH scores inform recurrence risks following discontinuation.

Aims: To determine the proportion of patients with first VTE who have their anticoagulation duration assessed at the appropriate interval (three months in provoked or distal and six months in unprovoked proximal), who have a risk/benefit analysis documented, and who have D-dimer monitoring and a DASH score calculated in those who have discontinued anticoagulation.

Methods: The sample was extracted from a database of patients with a first VTE between 01/02/16 and 31/05/16 who were followed up in NUTH. Clinic letters were reviewed to determine when anticoagulant duration was assessed, whether a risk/benefit analysis was documented, and in those who had anticoagulation discontinued, whether D-dimers were monitored and a DASH score calculated.

Results: 40 of 69 patients who were followed up were assessed at the correct interval. 57 of 69 had a risk/benefit analysis documented. Of the 34 patients who had their anticoagulation discontinued, 27 had D-dimer monitoring and 2 had a DASH score calculated. Table 1 shows results breakdown by VTE subtype.

TABLE 1 Results by VTE subtype

	Distal DVT	Provoked proximal DVT	Unprovoked proximal DVT	Provoked PE	Unprovoked PE
3 month review	0/3	6/9	n/a	15/17	n/a
6 month review	n/a	n/a	10/19	n/a	9/21
Risk/benefit analysis	1/3	8/9	17/19	15/17	16/21
D-dimer monitoring	1/3	4/5	9/11	8/10	5/5
DASH score	0/3	0/5	1/11	0/10	1/5

PB 305 | Out of Range INR Values Increase Healthcare Interaction Time in Four Large Anticoagulation Clinics

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Background: The impact on anticoagulation staff work time and healthcare utilization of a single out of range (OOR) INR value not associated with a bleeding or thromboembolic complication among chronic warfarin-treated patients is not well described.

TABLE 1 Work Time by Phase of Interaction and Activity

	Pre-Interaction		Interaction		Post-Interaction	
	OOR INR	Control INR	OR INR	Control INR	OOR INR	Control INR
Total (57 OOR, 92 control)	1.2 (0.8-1.8) minutes	1.0 (0.7-1.4) minutes	2.3 (1.4-4.5) minutes	1.2 (0.7-2.3) minutes	1.5 (0.8-3.2) minutes	0.7 (0.2-2.0) minutes
Phone Call (34 OOR, 58 control)	0.9 (0.6-1.5) minutes	1.0 (0.7-1.4) minutes	4.0 (2.3-5.5) minutes	1.8 (0.9-3.3) minutes	1.9 (0.9-4.8) minutes	0.9 (0.3-2.5) minutes
Voicemail (19 OOR, 18 control)	1.4 (1.1-2.0) minutes	1.2 (0.5-1.6) minutes	1.1 (0.5-1.7) minutes	1.2 (0.9-1.2) minutes	1.1 (0.5-1.8) minutes	0.7 (0.2-1.4) minutes
E-mail (1 OOR, 5 control)	1.5 minutes	0.7 (0.5-1.1) minutes	0.1 minutes	0.1 (0.1-0.1) minutes	0.4 minutes	0.2 (0.1-0.2) minutes
Letter (0 OOR, 3 control)	n/a	0.6 (0.4-1.0) minutes	n/a	0.2 (0.2-0.3) minutes	n/a	0.1 (0.1-0.6) minutes
Other (3 OOR, 8 control)	3.0 (1.8-3.1) minutes	1.8 (1.1-3.2) minutes	n/a	n/a	n/a	n/a

Aims: To compare the anticoagulation staff time required to manage and document an OOR vs. in-range INR value.

Methods: At four large phone-based anticoagulation clinics in Michigan, warfarin-treated patients with atrial fibrillation (AF) or venous thromboembolism (VTE) were identified. Direct observation occurred during 8.5 full work days by trained observers. Work time was divided based on the phase of work (pre/during/post-interaction) and type of activity. Measurements are described as median and interquartile ranges (IQR) and compared using Wilcoxon rank-sum tests.

Results: Fifty-nine OOR INR values and 92 control INR values were observed during the study period. Most patients were known to the provider (79.7% and 88.0%, respectively) with similar length of warfarin treatment (mean±SD 3.2±3.4 years and 3.5±4.2 years, respectively) for OOR INR and control INR patients, respectively. Total median time involved for each OOR INR value was 5.1 minutes (IQR 3.7-9.5) vs. 2.9 minutes (IQR 1.8-5.8) for control INR values (p< 0.001).

Time spent on pre-interaction preparation was similar for OOR INR patients (1.2 min [0.8-1.8]) and control INR patients (1.0 min [0.7-1.4]; p=0.20). Time spent interacting with patients (2.3 min [1.4-4.5] vs. 1.2 min [0.7-2.3], p< 0.001) and post-interaction documentation (1.5 min [10.8-3.2] vs. 0.7 min [0.2-2.0], p=0.002) were longer for OOR INR patients versus control INR patients.

Conclusions: Warfarin-treated patients who experience OOR INR values without any bleeding or thromboembolic complication require more anticoagulation clinic staff time than control INR patients. A more complete understanding of patient management time requirements may improve anticoagulation clinic models.

PB 306 | Evaluation of Time in Therapeutic Range in Anticoagulated Patients with Venous Thromboembolism. A Single-center, Retrospective, Observational Study

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Background: Patients with venous thromboembolism (VTE) frequently require vitamin K antagonists (VKAs) to prevent recurrent events, but their use increases hemorrhage risk.

Aims: We performed a study to assess the quality of international normalized ratio (INR) control, using time in therapeutical range (TTR) and to identify predictors of poor control and to examine the relationship between INR control and adverse outcomes in VTE patients.

Methods: We performed an observational, retrospective study, including all patients hospitalized for VTE who attended the internal medicine department of a Moroccan hospital (2010-2013), whose target INR was 2.0-3.0. The primary outcome under investigation was the TTR calculated according to F.R. Rosendaal's algorithm.

Results: 171 VKA-treated patients were evaluated; 53.2% men. The average age of our patients at diagnosis was 49.8 ± 16.6 years [17-92].The most common location of VTE was the lower limbs with 91.8 % cases; proximal in 83.6%. Five etiological groups have been ddistinguished : thrombophilia (5.8%), Behcet disease (9.4%), neoplasia (14.6%), with transient risk factor (34.5%) and idiopathic (35.7%). Patients were followed for a mean period of 178 days. The mean TTR was 41.2% (SD 32.9%) and 74.3% the patients had a mean TTR < 65%. The rates of major bleeds and thromboembolic events were 0.5% and 2.2%, respectively. In the statistical analysis, having a cancer and a non idiopathic VTE were negatively associated with a lower TTR (p < 0.05). ¨The rates of major bleeds and thromboembolic events were 0.5% and 2.2%, respectively. In the statistical analysis, having a cancer and a non idiopathic VTE were negatively associated with a lower TTR (p < 0.05).

Conclusions: Anticoagulation control needs to be improved in our unit. These results are informative and may help to identify patients who will require closer monitoring or innovative strategies to optimize the outcomes of oral anticoagulant therapy.

PB 453 | Safety and Efficacy of Edoxaban Compared with Warfarin for the Treatment of Acute Symptomatic Deep-vein Thrombosis in the Outpatient Setting

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Background: Direct-oral-anticoagulants streamline outpatient treatment of venous thromboembolism (VTE). However, the registration trials have not reported outcomes separately for patients managed either as outpatients or in the hospital.

Aims: We performed a subgroup analysis of the Hokusai-VTE study comparing the efficacy and safety of edoxaban with warfarin in outpatients.

Methods: Hokusai-VTE was a randomized double-blind trial in 8292 patients comparing edoxaban with warfarin for the treatment of acute VTE. All patients received initial therapy with enoxaparin or unfractionated heparin for at least 5 days. Treatment as an outpatient was at the discretion of the treating physician. Data on hospital admission was recorded for the last 5223 consecutively enrolled patients. Edoxaban or warfarin was given for at least 3 months and up to 12 months. The incidences of recurrent VTE and clinically relevant bleeding (major or non-major) were documented. The study was approved by institutional review boards and informed consent was obtained.

Results: Of the 5223 patients, 1414 (27%) were managed entirely as outpatients (724 in the edoxaban and 690 in the warfarin groups). The initial presentation was symptomatic deep-vein thrombosis (DVT) in 1183 of these patients (84%) and pulmonary embolism in 231 patients (16%). Of the DVT patients, recurrent VTE occurred in 18 (3.0%) given edoxaban and in 21 (3.6%) given warfarin (risk difference [edoxaban-warfarin] -0.61, 95% CI -2.65 to 1.42). Clinically relevant bleeding occurred in 46 patients given edoxaban (7.7%) and in 48 patients (8.3%) given warfarin (risk difference -0.59, 95% CI -3.68 to 2.49). Major bleeding occurred in 8 patients given edoxaban (1.3%) and in 8 patients (1.4%) given warfarin (risk difference -0.04, 95% CI -1.36 to 1.27).

Conclusions: Most patients chosen for outpatient management had symptomatic DVT at presentation. For outpatient treatment of DVT, a regimen of heparin followed by edoxaban had similar efficacy and safety to a regimen of heparin and warfarin.

PB 454 | Reliability and Validity of a Point of Care Test from Urine Samples of Patients on Therapy with Apixaban, Rivaroxaban and Dabigatran

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Background: The determination of the absence or presence of direct oral anticoagulants (DOACs) may be required in emergency situation of patients. They are excreted between 30% and 80% into urine. A point of care test (POCT) was developed to determine the absence or presence in urine samples of patients.

Aims: We aimed to analyze the reliability and validity of the POCT from urine samples patients on treatment with apixaban (A), rivaroxaban (R) and dabigatran (D).

Methods: Urine samples were obtained from patients (n=29 per group) treated with A (5mg bid), R (20mg od), D (110mg or 150mg bid) and patients without anticoagulant therapy (controls, C). Teststrips with pads containing the reagents (dry chemistry) for determination of A, R, and D were incubated with patient`s urine for 10 min. Then colours on the pads were white (A, R) and rose (D) in the presence (positive) and yellow (A, R) or ochre (D) in the absence (negative) of DOAC. The colours were objectified using the CMYK colour scale. Two observers identified independently the colours the pads visually by naked eye in comparison to the colour of the CMYK scale. Teststrips were photographed in a lightbox. Colours were analysed by ImageJ software program (grey scale). The concentration of DOACs was measured by liquid chromatography tandem mass-spectrometry (LC-MS/MS).

Results: The reliability (kappa index) and validity of the POCT were 100% (95% confidence intervals 74 to 100%) for A, R, and D. Ranges of results of ImageJ did not overlap for negative or positive results of colours on pads for A, R, and D using POCT. Validity was calculated using concentrations of DOACs (LC-MS/MS), which were below 10 ng/ml in control samples, 202 to 6.667ng/ml (A), 169 to 9.579 ng/ml (R) and 1.057 to 15.996ng/ml (D).

Conclusions: The present POCT from urine samples of patients offers a rapid, reliable and valid detection of DOACs in medical emergency situations. Additional pharmacological investigations and a study in a real-world setting are performed.

PB 456 | XALIA-LEA, a Non-interventional Study Comparing Rivaroxaban with Standard Anticoagulation for Initial and Long-term Therapy in Deep Vein Thrombosis

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Background: XALIA-LEA is a complementary study to XALIA. Regional enrolment differed; the later start date enabled the inclusion of patients with isolated pulmonary embolism (PE) in XALIA-LEA after approval.

Aims: To provide rivaroxaban safety and effectiveness information from routine practice in an unselected venous thromboembolism (VTE) population from Latin America, Eastern Europe, Middle East, Africa and Asia.

Methods: Patients aged ≥18 years, with objectively confirmed acute deep vein thrombosis and/or PE, with intended anticoagulant treatment for ≥3 months were eligible. Patients received rivaroxaban or standard anticoagulation (heparin/fondaparinux alone or overlapping with/followed by a vitamin K antagonist [VKA]). Therapy type, dose and duration were at the physician's discretion. VTE and major bleeding Cox regressions were adjusted for cancer and stratified by index VTE type. The all-cause mortality Cox regression was stratified by cancer and index VTE type.

Results: XALIA-LEA enrolled 1987 patients between Jun 2014 and Oct 2015; 8 patients did not receive anticoagulant therapy and 7 received other non-VKA oral anticoagulants, and were excluded from the primary analysis. Early switchers (n=285) given >2-14 days heparin/fondaparinux or 1-14 days VKA before starting rivaroxaban were included in a separate sensitivity analysis. The primary analysis comprised 1285 rivaroxaban- and 402 standard anticoagulation-treated patients (Table 1). Annualized rates of: major bleeding 2.7% (n=19) and 8.2% (n=15) (HR=0.35; 95% CI 0.18-0.69); recurrent VTE 2.6% (n=18) and 8.3% (n=15) (HR=0.33; 95% CI 0.17-0.67); and all-cause mortality 4.2% (n=29) and 15.8% (n=29) (HR=0.27; 95% CI 0.16-0.45), respectively.

Conclusions: XALIA-LEA provides information on VTE treatment in regions not studied in XALIA. Baseline characteristics between groups were more similar than in XALIA (including age and cancer rates). Safety and effectiveness outcomes with rivaroxaban were consistent with XALIA.

TABLE 1 Baseline demographic and clinical characteristics

Characteristic N (%) unless otherwise stated	Rivaroxaban (N=1285)	Standard anticoagulation (N=402)
Age (years), mean (SD)	59.6 (17.1)	58.0 (18.0)
Male sex	623 (48.5)	180 (44.8)
Asia Pacific	720 (56.0)	167 (41.5)
Eastern Europe, Middle East and Africa	473 (36.8)	196 (48.8)
Latin America	92 (7.2)	39 (9.7)
First available Creatinine Clearance <50 ml/min	126 (9.8)	61 (15.2)
DVT only (remaining patients had PE with or without DVT)	882 (68.6)	238 (59.2)
Cancer at baseline	216 (16.8)	69 (17.2)
Previous VTE	150 (11.7)	55 (13.7)

PB 457 | Rivaroxaban for Initial Therapy in Patients with Pulmonary Embolism. A Real-life Study

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Background: The efficacy and safety of initial therapy with rivaroxaban in patients with acute pulmonary embolism (PE) has not been consistently studied yet in real-life studies.

Aims: To compare the outcomes during rivaroxaban therapy in PE patients, according to starting rivaroxaban in less than 48 hours, from day 3 to day 7, or beyond the eight day after PE diagnosis.

Methods: We used the RIETE (Registro Informatizado Enfermedad Trombo Embólica) database to compare the rate of VTE recurrences,major bleeding and death during therapy with rivaroxaban.

Results: From January 2013 to September 2016, 1518 PE patients received rivaroxaban initially: 591 (39%) starting within the first 48 hours, 402 (26%) from day 3 to day 7 and 525 (35%) beyond the 8th day. Patients starting rivaroxaban within the first 48 hours were younger and were less likely to have renal insufficiency than those starting later. Otherwise, there were few differences in other clinical characteristics or risk factors for PE among subgroups. During rivaroxaban therapy (mean, 192 days), the rate of major bleeding was: 2.20 (95%CI: 0.80-4.78), 2.31 (95%CI: 0.74-5.39) and 0.65 (0.07-2.35) major bleeds per 100 patient-years, respectively. One patient that started beyond the 8th day had a recurrent PE and no patient died of PE or bleeding.

Conclusions: The use of rivaroxaban in patients starting < 48 hours after PE carried similar rates of PE recurrences or major bleeding than in those starting the drug later.

TABLE 1 Outcomes according to rivaroxaban starting

	Rivaroxaban starting < 48 hours		Rivaroxaban starting 3-7 days		Rivaroxaban starting ≥ 8 days	
	N	Events per 100 patient-years	N	Events per 100 patient-years	N	Events per 100 patient-years
Patients, N		591		402		525
Duration of therapy (days)		167±120		194±153		211±209
DVT recurrences	1	0.37 (0.005-2.04)	2	0.92 (0.10-3.34)	0	-
PE recurrences	0	-	0	-	1	0.33 (0.004-1.81)
Major bleeding	6	2.20 (0.80-4.78)	5	2.31 (0.74-5.39)	2	0.65 (0.07-2.35)
Overall death	4	1.46 (0.39-3.75)	2	0.92 (0.10-3.34)	7	2.28 (0.91-4.69)
Fatal PE	0	-	0	-	0	-
Fatal bleeding	0	-	0	-	0	-

PB 458 | Impact of Age, Comorbidity and Polypharmacy on the Efficacy and Safety of Edoxaban for the Treatment of Venous Thromboembolism: An Analysis of the Randomized, Double-blind Hokusai-VTE Study

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Background: Many patients with venous thromboembolism (VTE) have multiple comorbidities and take many concomitant medications, which represents a higher risk for both recurrent VTE and bleeding, particularly in the elderly. The lack of comparative data of direct oral anticoagulants (DOACs) versus warfarin in these complex patients may result in physician preference to utilize monitored warfarin therapy.

Aims: To determine the effects of advanced age, comorbidities, and polypharmacy on the efficacy and safety of edoxaban and warfarin.

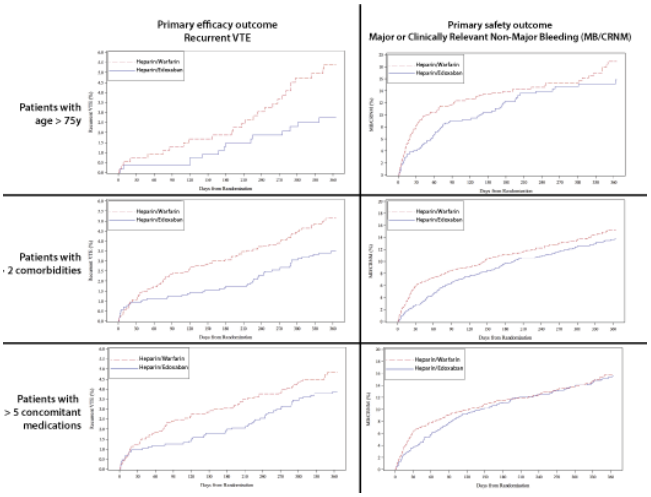

FIGURE 1 Primary efficacy and safety outcome in subgroups of frail patients

TABLE 1 Outcomes by categories of frailty

	Recurrent VTE			Major or clinically relevant non-major bleeding		
	edoxaban, n/N, rate (%)	warfarin, n/N, rate (%)	HR (95% CI)	edoxaban, n/N, rate (%)	warfarin, n/N, rate (%)	HR (95% CI)
<80 years	123/3866 (3.2%)	131/3857 (3.4%)	0.94 (0.73-1.20)	311/3866 (8.0%)	377/3857 (9.8%)	0.81 (0.70-0.94)
≥80 years	7/252 (2.8%)	15/265 (5.7%)	0.51 (0.21-1.24)	38/252 (15.1%)	46/265 (17.4%)	0.87 (0.56-1.33)
no comorbidities	11/571 (1.9%)	9/590 (1.5%)	1,23 (0.51-2.97)	44/571 (7.7%)	38/590 (6.4%)	1,23 (0.80-1.89)
1-2 comorbidities	64/1853 (3.5%)	52/1778 (2.9%)	1,19 (0.82-1.71)	119/1853 (6.4%)	167/1778 (9.4%)	0,67 (0.53-0.85)
>2 comorbidities	55/1694 (3.3%)	85/1754 (4.9%)	0,66 (0.47-0.93)	186/1694 (11.0%)	218/1754 (12.4%)	0,86 (0.71-1.05)
≤2 concomitant medications	43/1256 (3.4%)	32/1168 (2.7%)	1,25 (0.79-1.97)	73/1256 (5.8%)	82/1168 (7.0%)	0,82 (0.60-1.13)
3-5 concomitant medications	32/1328 (2.4%)	41/1357 (3.0%)	0,79 (0.50-1.26)	87/1328 (6.6%)	138/1357 (10.2%)	0,62 (0.47-0.81)
>5 concomitant medications	55/1534 (3.6%)	73/1597 (4.6%)	0,78 (0.56-1.11)	189/1534 (12.3%)	203/1597 (12.7%)	0,96 (0.79-1.18)

Methods: Using data from the Hokusai-VTE study, we report rates of recurrent VTE and of clinically relevant bleeding by age category (< 65, 65-75, and >75; < 80 vs ≥80 years), by comorbidities (0, 1-2, >2) and by concomitant medications (< 3, 3-5, >5). We present hazard ratios (HR) and corresponding 95% confidence intervals (CI) for edoxaban versus warfarin. Kaplan-Meier methodology was used to construct time-to-event curves. Pre- and post-dose levels of edoxaban were measured at the month 3 visit by mass spectrometry. For warfarin-treated patients, the time in therapeutic range was calculated. The study was approved by institutional review boards; informed consent was obtained.

Results: Recurrent VTE increased with advanced age, with multiple comorbidities, and with polypharmacy in warfarin-treated patients. Edoxaban was more effective in patients age ≥75 years and in patients with multiple comorbidities (*Table 1*, *Figure 1*). In the 517 patients above 80 years, recurrent VTE rates were 2.8% for edoxaban and 5.7% for warfarin (HR 0.51, CI 0.21-1.24). Bleeding increased with age, comorbidity, and polypharmacy regardless of treatment, but the relative safety of edoxaban versus well-managed warfarin was maintained. Age, comorbidity and polypharmacy did not impact on edoxaban pre- and post-dose levels.

Conclusions: Our data should reassure physicians that edoxaban is convenient, more effective and at least as safe as warfarin in these high-risk patients.

PB 459 | Prothrombin Complex Concentrate for Reversal of XA Inhibitors - Real Life Data

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Background: The recommendation for reversal of Xa inhibitors with Prothrombin Complex Concentrate (PCC) is based on limited experience in healthy volunteers and real life data is lacking.

Aims: Outcome of patients who received PCC for reversal of XA inhibitors.

Methods: A retrospective study of patients who received 4 factors PCC between 4.2015 -1.2017 at a tertiary academic hospital. Decision for reversal was made by a coagulation expert.

Results: Forty/70 patients (mean age 78±10 years, 72% male, 90% with atrial fibrillation) received PCC at a mean dose of 30+11 u/kg for reversal of apixaban (n=25), rivaroxaban (n=13) or enoxaparin (n=2). Indication was major bleeding in 28(70%), mostly intracranial hemorrhage (ICH) (19/28, 68%). Seventeen patients (42%) including 5 with bleeding, required reversal for an urgent surgery/procedure; 7 abdominal, 3 neurosurgical, 2 orthopedic and 1 thorax surgery, 3 cholecystomy tube insertion and 1 vaginal procedure. Surgeons reported good hemostasis for all patients, blood products were given in 3/17 surgeries (2 orthopedic and 1 gynecology). Six of 17(35%) patients died 1-46 days after PCC, none due to bleeding. Among 19 patients with ICH, 6 were discharged home, 10 to rehabilitation/nursing homes, among them 4 unconscious or necessitated mechanical ventilation, 3 died 2-29 days after PCC (17.6%). During this period 17 patients treated with PCC for warfarin ICH, 5 discharged home(NS) and 9 (53%) died within 30 days (p=0.07). Drug levels obtained before and after PCC in 5 patients and were reduced in approximately 34-50% except for one patient with high levels and no reduction. 30 days thrombotic complications occurred in one patient (2.5%).

Conclusions: Good hemostasis in surgery obtained after PCC in patients under XA inhibitors despite incomplete reversal of drug levels. Thirty-day mortality from ICH tended to be lower in patients under XA inhibitors who received PCC as compared to warfarin treatment. Our findings support the use of PCC in these patients.

PB 460 | Clinical Characteristics and Treatment of Patients with Cancer-associated Venous Thromboembolism: Results from the GARFIELD-VTE Registry

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Background: Patients with active cancer are susceptible to venous thromboembolism (VTE). Although guidelines recommend low-molecular-weight heparin (LMWH) for cancer-associated thrombosis (CAT), direct oral anticoagulants (DOACs) and other agents also are prescribed.

Aims: To compare the clinical characteristics of VTE patients with and without active cancer and to determine the initial anticoagulant treatment patterns for CAT using data from the GARFIELD-VTE prospective global registry.

Methods: Baseline data in 701 patients with active cancer were compared with those in 7307 patients without active cancer who received initial anticoagulant treatment between 2014 and 2016.

Results: Patients with active cancer tended to be older than those without active cancer (median [IQR]: 65.7 yrs [56.2-73.6] and 59.4 yrs [45.1 -71.5], respectively), and more were of Asian ethnicity (31.7% and 14.6%, respectively). Common sites of underlying cancers in men, women and overall are shown in the Table below. The proportion of patients with deep-vein thrombosis (DVT) was similar in patients with and without cancer (59.3% and 61.3%, respectively) as was the proportion with pulmonary embolism ± DVT (40.7% and 38.7%); a slightly

higher proportion of cancer patients had upper limb DVT (12.2% and 8.1%, respectively). Patients with active cancer were more likely to receive LMWH monotherapy or another parenteral anticoagulant as initial therapy than those without cancer (64.0% and 13.1%, respectively), and were less likely to receive a DOAC with or without a heparin lead-in (22.8% and 53.2%, respectively).

Conclusions: These data show differences in the clinical characteristics and treatment patterns of VTE in patients with and without active cancer. In keeping with guidelines, the majority of cancer patients are treated with LMWH monotherapy. However, DOACs are prescribed in almost 25% of patients.

TABLE 1 The most common sites of cancer in VTE patients

Men (n=219)		Women (n=232)		Overall (n=451)	
Lung	21.9%	Gynecological	22.0%	Lung	17.3%
Colorectal	15.5%	Breast	18.1%	Colorectal	12.4%
Prostate	11.4%	Lung	12.9%	Gynecological	11.3%
Urological	9.1%	Colorectal	9.5%	Breast	9.3%
Lymphoma	8.2%	Lymphoma	7.8%	Lymphoma	8.0%

PB 461 | Rivaroxaban for Scheduled Work-up of Patients with Suspected Deep Venous Thrombosis: A Prospective Interventional Outcome Study - The Ri-Schedule study

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Background: Scheduled work-up of deep vein thrombosis (DVT) can reduce the need for emergency diagnostic imaging and avert prolonged waiting of patients in the emergency room (ER). Current guidelines suggest using low molecular weight heparin (LMWH) if the diagnostic work-up is expected to be delayed. The Ri-Schedule study aims to investigate the safety of rivaroxaban in the pre-diagnosis phase of DVT and the feasibility of scheduled work-up according to a predefined criteria.

Aims: Our aim is to report the study design and preliminary results on the feasibility of scheduled work-up after including 50% of the estimated sample size.

Methods: Ri-Schedule is a prospective outcome study (NCT02486445). The primary endpoint is safety of rivaroxaban (15 mg every 12 hours, max 2 tablets) defined as the composite endpoint of serious bleeding and/or death related to bleeding encountered within 48 hours after the last tablet was ingested if DVT was excluded or until the initiation of anticoagulant if a DVT was diagnosed. The feasibility is defined as the proportion of patients who can be managed according to the criteria shown in the table. Patients are recruited from the ER of Østfold Hospital, Norway. Work-up of suspected DVT includes Wells score, D-dimer and compression ultrasonography. The study was approved by the Regional Ethics Committee and consent is acquired from all patients.

Results: Out of 1195 screened patients, 836 have been recruited in 22 months. Of these 328 (39%) fulfilled the predefined criteria and received rivaroxaban to allow for scheduled work-up; 333 (40%) did not meet criteria 1-10 (table) while 175 (21%) had already received LMWH and were therefore not given rivaroxaban. DVT was diagnosed in 136 (16%) patients. No death has been encountered so far in the study due to scheduled work up.

Conclusions: Scheduled work-up was allowed in 40% of the patients according to the study criteria. This figure is likely to be higher if the final study results support the implementation of these criteria in the future.

TABLE 1 Selection criteria for scheduled work-up of DVT

1	Diagnostic work-up is expected to exceed 2 hours
2	Absence of active cancer
3	No suspicion of PE
4	No suspicion of active or recent bleeding
5	No signs of threatened circulation or intractable pain in the lower limb
6	Physician considers it safe to discharge the patient
7	Absence of comorbidity that require hospital admission and GFR >45 ml/min
8	Absence of contraindication to rivaroxaban
9	Absence of logistic factors that may hinder scheduled work-up
10	Patient do not oppose discharge before diagnosis is completed

PB 462 | Real-world Adherence with Direct Oral Anticoagulants in Adults with Atrial Fibrillation

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Background: Adherence to anticoagulation (AC) therapy is critical as it directly impacts clinical outcomes and healthcare utilization.

Aims: To evaluate patterns of medication adherence and persistence in a real-world setting in atrial fibrillation (AF) patients treated with direct oral anticoagulants (DOACs).

Methods: AF patients newly initiating a DOAC with a minimum of 6 months of continuous health plan enrollment pre and post-index date (date of first DOAC) were identified from the Truven Health MarketScan® Commercial and Medicare Supplemental databases (2009-2013). DOAC adherence (proportion of days covered (PDC)), persistence and predictors of adherence were assessed at 6 and 12 month post-index.

Results: Of 57,048 AF patients included, 51.8% (N=29,523) were AC naïve and 48.2% (N=27,525) were non-AC naïve (age: 67.0±12.7 vs. 71.1±11.3 years, p< 0.001; male: 18,282 (61.9%) vs. 16,787 (61.0%), p=0.0216, respectively). A majority of patients received dabigatran as their index DOAC (N=42,129; 73.8%). The mean PDC in AC naïve and non-AC naïve patients at 6 and 12 months was 72.2% vs. 83.2% (p< 0.001) and 63.7% vs. 79.8% (p< 0.001), respectively. Persistence with

DOAC therapy in AC naïve and non-AC naïve patients at 6 and 12 months ranged from 59.2% and 76.0% (p< .0001) to 31.7% and 50.8% (p< .0001), respectively. Predictors of higher DOAC adherence were older age and higher number of concomitant medications; and of lower adherence were higher number of comorbidities and AC naïve user status.

Conclusions: While medication adherence and persistence declined over time, both were lower (at 6 and 12 months post-index) in AC naïve compared to non-AC naïve patients. Our findings can help target future strategies or interventions on patient education and AC management in those naïve to DOAC therapy. Future investigation should examine potential reasons for differences in DOAC adherence and persistence between non-AC vs. AC naïve patients, and its implication on patient outcomes.

PB 463 | Oxygen Saturation to Improve Risk Stratification in Hemodynamically Stable Patients with Acute Pulmonary Embolism

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Background: In patients with acute pulmonary embolism (PE), risk stratification for short-term death is crucial to drive clinical management. The European Society of Cardiology (ESC) proposed a risk stratification model based on clinical risk scores (PESI or sPESI), right ventricle dysfunction (RVD) and elevated serum troponin (2014 ESC model).

Aims: To assess whether the accuracy of the 2014 ESC model to predict in-hospital death in hemodynamically stable patients with acute PE can be improved by the inclusion of patient oxygen saturation.

Methods: Consecutive hemodynamically stable patients with symptomatic, confirmed PE included in prospective cohorts were merged in a collaborative database and included in the study if information on sPESI score, oxygen saturation in air at admission, RVD (at echocardiography or CT angiography) and serum troponin were available. Patients' risk was classified as low (sPESI zero), intermediate-low (sPESI>0, RVD or increased troponin) and intermediate-high (RVD and elevated troponin). The primary study outcome was in-hospital death occurring within 30 days.

Results: Of 863 patients, 237 (27.5%) were categorized as low-risk, 468 (54.2%) as intermediate-low risk and 158 (18.3%) as intermediate-high risk according to the 2014 ESC model. Death occurred in 69 patients (8.0%), and in particular in 1.7% of low, 10.3% of intermediate-low (HR 5.88, 95% CI 2.11-16.34) and 10.8% of intermediate-high risk (HR 6.37, 95% CI 2.12-19.06) patients. Oxygen saturation in air lower than 85% at admission was an independent predictor of death (HR 3.36, 95% CI 2.00-5.63) across the 2014 ESC risk categories. c-statistics showed an improvement of the discriminatory power by including oxygen saturation in air lower than 85% to the 2014 ESC model (0.73; 95% CI 0.67 - 0.79 vs. 0.69, 95% CI 0.63 - 0.75).

Conclusions: A model adding a simple and routine test as oxygen saturation to the 2014 ESC model has an incremental prognostic value for risk stratification in hemodynamically stable patients with acute PE.

PB 464 | Direct Anticoagulants and Vitamin K-antagonists Exert Differential Effects on Markers of Subclinical Cardiovascular Disease: Results from the MyoVasc Study

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Background: Experimental data indicate differential effects of novel oral anticoagulants (NOAC) and vitamin K-antagonists (VKA) on the development and progression of (subclinical) cardiovascular disease (CVD).

Aims: To compare the effects of NOACs compared to VKAs on intermediate CVD phenotypes.

Methods: In the prospective, single-center cohort study MyoVasc, 2,000 individuals aged 35 to 84 years with documented cardiac dysfunction were enrolled between 2013 and 2016. In a highly standardized clinical visit, parameters of cardiovascular structure and function were assessed and concentrations of humoral biomarkers were measured.

Results: Overall, the sample comprised 229 NOAC users and 404 VKA users. Multivariable analysis adjusted for age, sex, traditional cardiovascular risk factors and comorbidities revealed an independent relationship between NOAC vs. VKA intake and better diastolic function (E/E´: β -0.23 [-0.36/-0.10]; p< 0.001), less left ventricular mass (β -5.4 g/m2.7 [-11/-0.25]; p=0.041), lower log-NT-pro-BNP (β -0.38 pg/ml [-0.67/-0.096]; p=0.0094) and higher levels of ApoA-1 (β +0.12 g/L [0.042/0.20]; p=0.0029). In propensity score weighted analyses, the most pronounced differences between NOAC and VKA intake were observed for E/E´ (Δ-2.36), stiffness index (Δ-0.5 m/s), ApoA-1 (Δ+0.06 g/l), ejection fraction (Δ-1.7%), left ventricular mass (Δ-1.98 g/m2.7), NT-pro-BNP (Δ-139 pg/ml) and CRP (Δ-0.4 mg/l;

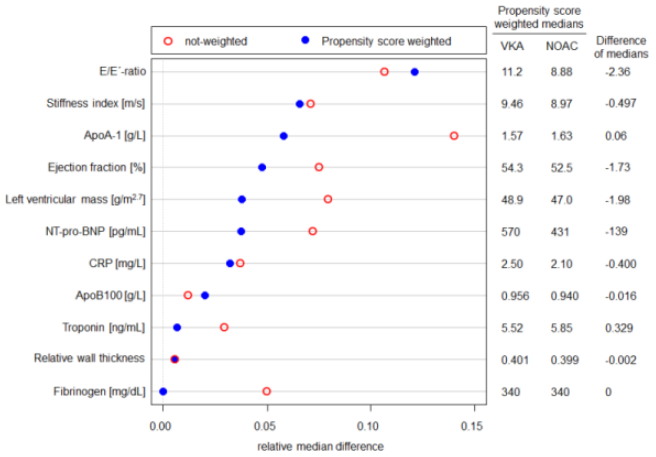

FIGURE 1 Differences in surrogate markers of cardiovascular disease and inflammatory biomarkers in VKA users and NOAC users

Figure). Sensitivity analyses in the homogeneous subsample of individuals with atrial fibrillation (CHA_2DS_2-VASc score ≥1, exposure to anticoagulation therapy ≥3 months) and in a subsample excluding individuals with symptomatic heart failure confirmed the consistency of the results.

Conclusions: The present analysis demonstrates differential relations between biomarkers of cardiovascular structure and function and lipid metabolism in individuals with VKA and NOAC therapy. The potentially beneficial effect of NOACs needs to be further elucidated and may be relevant for the management of anticoagulation therapy.

PB 465 | Lifelong Apixaban Treatment Is Cost-effective for Idiopathic Venous Thromboembolism

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Background: Dutch guidelines advise lifelong anticoagulant treatment with direct oral anticoagulants (DOACs) or vitamin K antagonists (VKAs) for Dutch patients with idiopathic venous thromboembolism (VTE) who do not have high bleeding risk.

Aims: The aim of this study was to analyze the economic effects of lifelong treatment of apixaban in the Netherlands, based on updated and adapted previous modelling exercises for use of apixaban in acute VTE-patients.

Methods: We performed a cost-effectiveness analysis (CEA) simulating a population of 1,000 VTE patients. Two different treatment strategies were tested: lifelong apixaban treatment vs. no treatment after the first 6 months (base case analysis). In scenario analysis, the initial treatment period of 6 months with LMWH/VKA was also included. The primary outcome of the model is the incremental

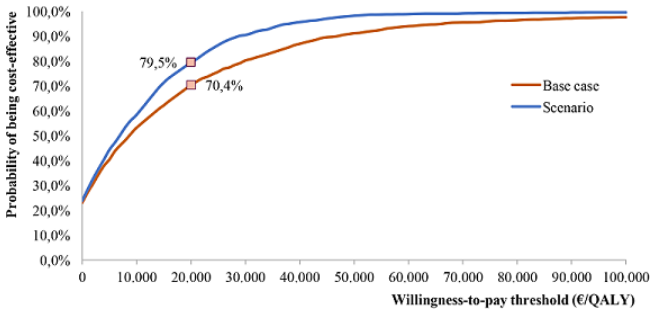

FIGURE 1 Cost-effectiveness acceptability curve: base-case and scenario analysis

cost-effectiveness ratio (ICER) in costs (€) per quality adjusted life-year (QALY), with one QALY defined as one year in perfect health. To account for any influence of the uncertainties in the model a probabilistic sensitivity analysis (PSA) was conducted, in which the ICER was recalculated 2,000 times while varying all input parameters over their range. These results were summarized in a cost-effectiveness acceptability curve (CEAC). The treatment was considered cost-effective with an ICER less than €20,000/QALY, which is the most commonly used willingness-to-pay (WTP) threshold for preventive drugs in the Netherlands and other European countries.

Results:

The model showed a significant reduction in recurrent-VTE and no increase in major bleeding events for lifelong treatment. Deterministic results showed ICERs of €9,830/QALY and €8,231/QALY in the base case and scenario analysis, respectively. The probability of being cost-effective at a WTP threshold of €20,000/QALY of 70.4% and 79.5% respectively resulted, see CEAC in figure 1.

Conclusions: Lifelong treatment with apixaban is effective and cost-effective in Dutch VTE patients with idiopathic VTE.

PB 466 | Factors Associated with the Anticoagulant Regimen and Duration of Hospitalization in Patients with Acute Pulmonary Embolism: FOCUS, a Prospective Multicenter Cohort Study

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Background: The ongoing multicenter ´Follow-up after acute pulmonary embolism´ (FOCUS) observational study has set out to prospectively follow, over a 2-year period at predetermined intervals with standardized follow-up (FUP) protocols, the clinical and hemodynamic course of 1,000 unselected patients with confirmed acute symptomatic pulmonary embolism (PE).

Aims: To examine the association between the patients´ baseline characteristics and 1) the type of anticoagulant drug prescribed as well as 2) the length of hospitalization.

Methods: Baseline data of the patients enrolled as of December 2016 were analyzed. Multivariable stepwise logistic models were fit with clinically relevant covariates to study their association with 1) prescription of vitamin K antagonists (VKA) or ‚single oral drug therapy' with a non-vitamin K-dependent oral anticoagulant (NOAC), and 2) length of hospitalization < 48 hours. FOCUS was approved by ethics committee and patient informed consent obtained.

Results: Data of 575 patients who completed FUP visit on discharge were analyzed. Of these, 78 (13.6%) were discharged on VKA, 305 (53.0%) on rivaroxaban, 101 (17.6%) on apixaban, and 91 (15.8%) on parenteral agents (*Table 1*).

The absence of right ventricular dysfunction markers (adjusted Odds Ratio [aOR] 2.1 [95% Confidence Interval 1.2-3.7]), a negative history of renal dysfunction (aOR 4.8 [2.3-10.0]), and simplified Pulmonary Embolism Severity Index indicating low clinical risk (aOR 3.8 [2.0-7.1]) were associated with NOAC prescription. Patients on NOAC were more often discharged within 48 hours from admission (*Table 2*).

Conclusions: Overall, the majority of acute PE patients enrolled in FOCUS were discharged on rivaroxaban or apixaban. Although principally approved for all stable PE patients, NOAC were mainly prescribed in patients with low clinical risk and no right ventricular dysfunction. NOAC use was associated with shorter length of hospitalization compared to VKA.

TABLE 2 Factors associated with a hospitalization of 48 hours or shorter in patients receiving either NOAC or vitamin K antagonists

	Crude Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio (95% Confidence Interval)
NOAC use	5.3 (1.6-17.2)	4.0 (1.1-13.2)
Age >=75 years	0.4 (0.2-0.8)	-
Male sex	0.9 (0.5-1.4)	-
Active cancer	0.3 (0.1-2.0)	-
Right ventricular dysfunction detected at echocardiography or computer tomography scan, or positive cardiac biomarkers	0.04 (0.01-0.3)	0.04 (0.01-0.3)
Personal history of renal dysfunction	0.6 (0.2-1.8)	-
Diabetes mellitus	0.2 (0.05-0.9)	0.2 (0.1-1.0)
Chronic heart failure	0.2 (0.02-1.3)	-
Body mass index >40 or weight >120 kg	0.5 (0.2-1.4)	-

TABLE 1 Baseline characteristics of the patients included in FOCUS

	Vitamin K antagonist (n=78)	Rivaroxaban (n=395)	Apixaban (n=101)	Parenteral agent (n=91)
Age (years), mean (Standard Deviation)	64.9 (16.0)	58.5 (14.7)	66.3 (14.7)	64.5 (13.6)
Male sex, n (%)	47 (60.3)	160 (52.2)	48 (47.5)	51 (56.0)
Body mass index >40 or Body weight >120 kg, n (%)	10 (12.8)	27 (8.9)	10 (9.9)	6 (6.6)
Active cancer, n (%)	7 (9.0)	13 (4.3)	2 (2.0)	32 (35.2)
Estimated glomerular filtration rate <30 mL/min, n (%)	10 (12.8)	6 (2.0)	3 (3.0)	1 (1.1)
sPESI = 0, n (%)	13 (16.7)	165 (54.1)	37 (36.6)	30 (33.0)

PB 467 | Real-life Use of Non-vitamin K Antagonist Oral Anticoagulants in Patients with Cancer Associated Venous Thromboembolism: Data from a Prospective Cohort

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Background: Cancer associated venous thromboembolism (CAT) has an increased risk of recurrence. The use of novel oral anticoagulants (NOACs) is controversial in patients with CAT.

Aims: The aim of this study is to assess mortality, recurrent VTE and bleeding complications in patients with and without CAT receiving NOACs.

Methods: Consecutive patients with acute objectively confirmed venous thromboembolism (VTE) receiving NOACs within 1 month from the diagnosis are included in an ongoing prospective cohort study started in September 2013.

Results: 678 patients were included (mean age 66 years) of whom 132 with CAT (19.7%). 54 patients with CAT (41%) were on CHT or RT and 29 (22%) had a metastatic disease. Site of cancer were genitourinary, breast, gastrointestinal, lung and haematological in 25, 24, 22, 17 and 6 %, respectively. ECOG score at NOAC prescription was 0, 1, 2, 3 and 4 in 35, 23, 6, 18 and 18% of CAT patients, respectively. Index PE occurred in 38.6% and 40.7% of patients with CAT and without (p=0.65). 186 out of 273 patients with PE (68.1%) also presented a DVT. DVT only was observed in 61.4% and 59.4% of patients with CAT and without (p=0.68).

435 patients had at least 3 months of follow-up (mean 10.1 months). 250 continued treatment (57.4%), 139 suspended (32%) and 46 died (10.6%): 36 were cancer related deaths (78.3%). Non cancer related death occurred in 2 patients with CAT and in 8 patients without (2.4% vs 2.3%, p=0.92). No fatal VTE recurrences occurred. One patient with cerebral metastases died from intracranial bleeding. Overall VTE recurrences and clinically relevant bleedings occurred in 9 vs 19 patients with and without CAT, respectively (10.9% vs 5.4%, p=0.03).

Conclusions: In our real-life study, one-fifth of patients with VTE prescribed with NOACs have cancer. A trend toward higher rates of both recurrence and bleeding was observed in CAT compared to non-CAT patients, with similar rates of non cancer related mortality.

PB 468 | The YEARS Algorithm for Suspected Pulmonary Embolism Leads to Much Shorter Diagnostic Turnaround Time than Conventional Algorithms

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Background: Recently, the safety of the YEARS algorithm (fig 1), designed to simplify the diagnostic work-up of PE, was demonstrated (YEARS study, 2016 ESC abstract 5727). We hypothesize that by design, YEARS is associated with a shorter diagnostic turnaround time at the emergency ward due to simultaneous assessment of pre-test probability and D-dimer level.

Aims: To investigate whether implementation of the YEARS diagnostic algorithm is associated with a shorter diagnostic turnaround time compared to the conventional algorithm.

Methods: We selected consecutive outpatients with suspected PE included in the YEARS study (n=308) and ADJUST-PE study (Righini M et al, JAMA 2014; n=233) Different time points of the diagnostic process were extracted from the electronic patients chart system (Table 1). Patients who were initially evaluated for a different acute condition, i.e. myocardial infarction, were excluded from this analysis.

TABLE 1

		Conventional algorithm		Years algorithm		
Turnaround time between:	Patient cohort	Number of patients	Mean time minutes (SD)	Number of patients	Mean time minutes (SD)	Mean absolute difference (95%CI),p value
Start of diagnostic algorithm and order for CT scan	All patients referred for CT scan	162	133(69)	219	82(44)	51 (40-60), p<0,001
Start of diagnostic algorithm and initial dose of anticoagulant	All patients with proven PE	57	261(105)	119	215(87)	46 (17-76), p=0,002
Start of diagnostic algorithm and discharge from emergency ward	All patients managed without CT	68	189(106)	89	135(69)	54 (27-82), p<0,001
Start of diagnostic algorithm and discharge from emergency ward	All patients	233	271(114)	308	215(109)	56 (36-77), p<0,001

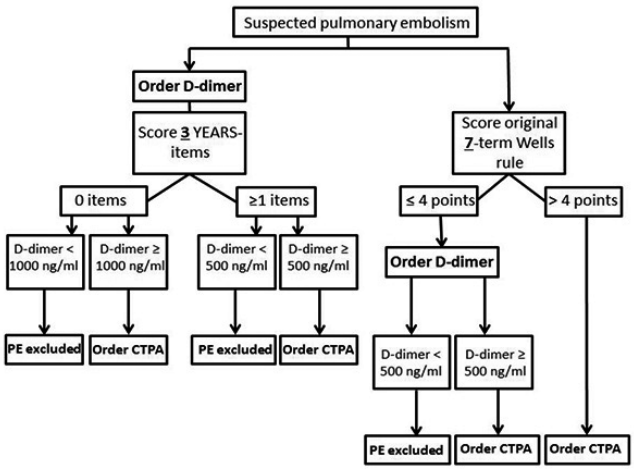

FIGURE 1 The YEARS algorithm versus conventional algorithm

Results: All predefined diagnostic turnaround times were significantly shorter after implementation of YEARS. Specifically, the time between start of the diagnostic algorithm and order for CT-scan decreased from 133 to 82 minutes (min) and patients were discharged earlier from the emergency ward: 189 versus 135 min for patients managed without CT and 271 versus 215 min for the complete study population. Importantly, patients diagnosed with PE by CTPA received the first dose of anticoagulants 46 min (95%CI 17-76) faster than those managed according the conventional algorithm.

Conclusions: YEARS was shown to be associated with a significantly shorter diagnostic turnaround time compared to the conventional diagnostic algorithm, leading to faster treatment initiation in case of confirmed PE and more efficient use of the emergency ward resources.

PB 469 | The Assessment of Apixaban Reversal in a Porcine Trauma Mmodel by Thrombelastography (TEG)

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Background: Approved point of care tests for direct oral anticoagulants (DOAC) are currently unavailable. For the thrombelastography (TEG) device, a DOAC cartridge with a specific FXa-inhibitor-channel has been developed (AFXA).

Aims: To assess the efficacy of TEG as point of care device to measure the concentration of the FXa-inhibitor apixaban in a preclinical model, following andexanet reversal.

Methods: Given ethical approval, 15 pigs received oral apixaban (3 d, qd). Standardized trauma consisted of blunt liver injury and bilateral femur fractures. 15 min after trauma, animals randomly received 1) Ringer's solution (control, n=5), 2) andexanet bolus (1000 mg, n=5), or 3) andexanet bolus+infusion (1000 mg + 600 mg/h for 2 h, n=5).

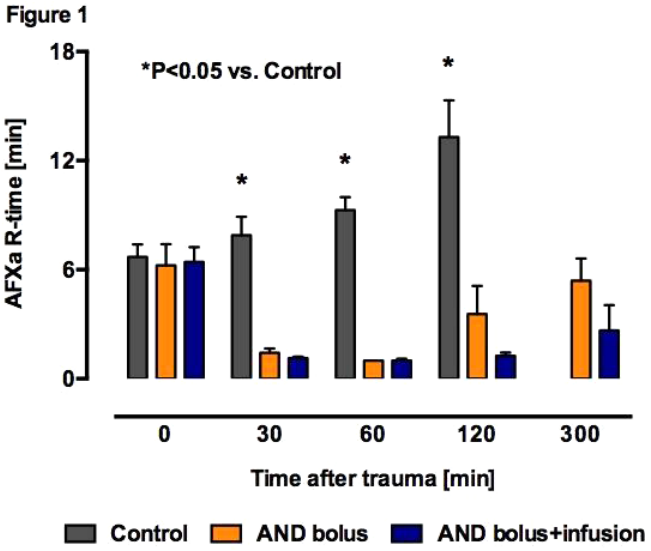

FIGURE 1 TEG6s AFXa R-time [min

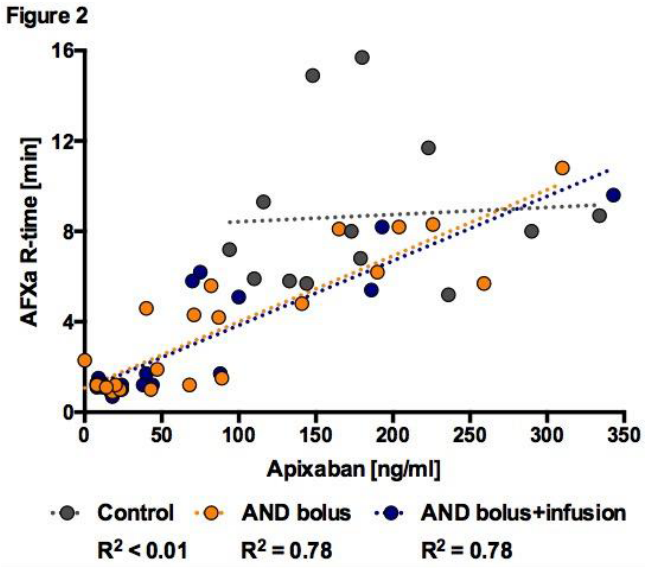

FIGURE 2 Correlation between AFXa R-time and Apixaban concentration

Animals were observed for 300 min or until death. Apixaban levels (anti-FXa assay), TEG and blood loss (BL) were recorded. ANOVA (mean±SEM) and Pearson's r were used.

Results: Apixaban levels prior to trauma infliction were comparable (183±26 ng/mL, n=15). Andexanet bolus or bolus+infusion resulted in about 70% reduction in total BL as compared to control animals (control: 3913±235 mL). Due to clearance of andexanet (1), apixaban levels returned faster after bolus compared to bolus+infusion (Fig.1). The increase of apixaban concentrations was detected by TEG (120 and 300 min post trauma). The difference in concentrations of apixaban strongly correlated with the AFXA R-time, both before trauma and after andexanet dosing (bolus and bolus+infusion: R^2=0.78; Fig.2). However, in control animals, no correlation between AFXA R-time

and apixaban levels after trauma was measured due to continuous blood loss (R^2< 0.01).

Conclusions: The present data show that new generation of TEG cartridges might be effective as point of care test to assess the concentration of apixaban. However, consumptive coagulopathy with massive blood loss may affect this correlation.

Reference: 1. Ghadimi K et al. Expert Rev Hematol 2016; 9:115-22.

PB 471 | Plasma Fibrin Clot Properties in the G20210A Prothrombin Mutation Carriers Following Venous Thromboembolism: The Effect of Rivaroxaban

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Background: G20210A prothrombin mutation is associated with two- to three-fold increase in the VTE risk, but its prothrombotic mechanism is still unclear.

Aims: We investigated whether the G20210A prothrombin mutation modifies plasma fibrin clot properties in patients after venous thromboembolism (VTE) and how rivaroxaban treatment affects these alterations.

Methods: We studied 34 prothrombin mutation heterozygous carriers and sex- and age-matched 34 non-carriers, all at least 3 months since the first VTE episode, before and during treatment with rivaroxaban. Clot permeability (K_s) and clot lysis time (CLT) with or without elimination of thrombin activatable fibrinolysis inhibitor (TAFI) were assessed at baseline, 2-6 hours after and 20-25 hours after intake of rivaroxaban (20 mg/d).

Results: At baseline, the prothrombin mutation group formed denser clots (K_s -12%) and had impaired fibrinolysis (CLT +14%, and CLT-TAFI +13%) compared with the no mutation group (all p< 0.05) and were similar to those observed in 15 healthy unrelated prothrombin mutation carriers. The G20210A prothrombin mutation was the independent predictor for K_s and CLT before rivaroxaban intake. At 2-6 hours after rivaroxaban intake, clot properties improved both in G20210A carriers and non-carriers (K_s +38% and +37%; CLT -25% and -25%; CLT-TAFI -20% and -24%, respectively, all p< 0.001), but those parameters were worse in the prothrombin mutation group (K_s -12.8%, CLT +17%, CLT-TAFI +13%, all p< 0.001). Rivaroxaban concentration correlated with fibrin clot properties. After 20-25 hours since rivaroxaban intake most clot properties returned to baseline.

Conclusions: The G20210A prothrombin mutation affects alterations to clot density and lysability. Rivaroxaban treatment, though improves fibrin clot properties, cannot abolish more prothrombotic fibrin clot phenotype observed in prothrombin mutation carriers following VTE.

PB 472 | A Cross-Sectional, Multinational, Multidisciplinary Survey of Physicians Surrounding the Management of Bleeding Complications in the Setting of Direct Oral Anticoagulant Use

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Background: Management of life-threatening bleeding episodes in patients receiving direct oral anticoagulants (DOACs) is a significant clinical concern.

Aims: We aimed to assess clinicians' knowledge regarding the evaluation and management of DOAC-associated bleeding.

Methods: We conducted a multinational cross-sectional survey of physicians from a variety of disciplines. The 32-question survey was electronically administered (SurveyMonkey®) and distributed via email through several medical associations in Canada and the United States.

Results: A total of 518 physicians have completed the survey to date, with a completion rate of 91%. Most respondents were from either Canada (72%) or the US (15%) and were staff physicians (78%). The survey was completed by thrombosis specialists (14%), hematologists (13%), emergency physicians (32%), anesthesiologists (18%), general practitioners (4%), neurologists (3%), general surgeons (3%) and other specialties (13%). A majority of physicians (58%) stated that they did not feel confident in managing serious DOAC-associated bleeding complications. Most clinicians surveyed did not identify the correct laboratory investigation to detect or quantify individual DOACs in bleeding patients. Only 29% of physicians would use thrombin time to identify dabigatran, whereas 42% and 41% would use calibrated anti-Xa levels to identify rivaroxaban and apixaban, respectively. Similarly, only 37% of surveyed clinicians would use dilute thrombin time to quantify plasma concentrations of dabigatran. Table 1 indicates laboratory testing availability according to survey respondents. Participants' responses regarding the use of pro-hemostatic agents are illustrated in Figure 1.

TABLE 1 DOAC Laboratory Testing Availability

DOAC Laboratory Test	% Total Respondents Reporting Availability
Dilute Thrombin Time	17
Ecarin Clotting Time	7
Heparin Calibrated Anti-Xa Levels	41
DOAC Calibrated Anti-Xa Levels	15
Serum DOAC Levels	5
Do Not Know	34
None of the Above	24

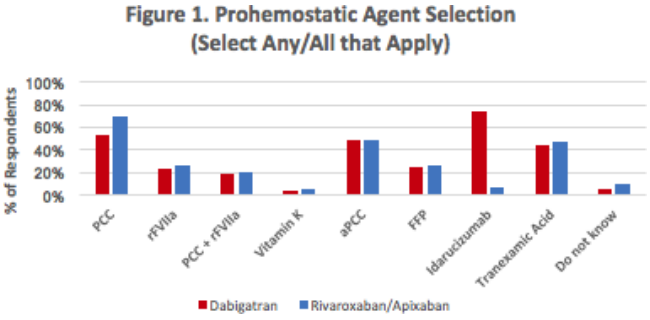

FIGURE 1 Prohemostatic Agent Selection

Conclusions: There appears to be important knowledge gaps regarding the assessment and management of DOAC-associated life-threatening bleeding in the majority of surveyed clinicians. Targeted educational initiatives regarding the management of DOAC-associated bleeding are warranted to enhance patient safety.

PB 473 | Apixaban in Mechanical Circulatory Support - Evaluation in a Mock Circulatory Loop with Human Blood

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Background: In light of decreased intracranial haemorrhage with apixaban compared to warfarin in atrial fibrillation trials, we conducted an ex vivo study of thrombus formation in an ASTM 1841 hemocompatibility testing loop incorporating a continuous flow left ventricular assist device (HeartWare HVAD.

Aims: The aim of this study was to determine the effect of low and high dose apixiban on intra-pump thrombus formation.

Methods: Blood from volunteers were used in four experimental conditions: un-anticoagulated blood (control); blood from patients on warfarin; in vitro low dose apixaban (equivalent 2.5mg BID); and in vitro high dose apixaban (equivalent 5mg BID). Afterload, preload and loop temperature were controlled to physiological targets. The primary outcome was time to formation of intra-pump thrombosis based on 20% rise in power characteristics. A secondary outcome was changes in haemostasis over one hour of stable pump use as measured by microparticle activity (PPL). platelet aggregation (multiplate), rotational thromboelastometry and von Willebrand factor levels (CBA/Ag).

Results: A total of 10 runs were completed. Overall time to intra-pump thrombosis formation was prolonged in the anticoagulated runs when compared to control (83.1vs 70.6 mins). Low dose apixaban was comparable to warfarin (80.5mins vs 80.3), however high dose apixaban showed no evidence of thrombosis after 90mins. Baseline total clotting times were prolonged in the anticoagulation groups (control 91.2s, warfarin 120s, low dose apixaban 164.4s, high dose apixaban > 180s). The reduction in clotting times over one hour pump running was similar in the control, warfarin and low dose apixaban groups (44.3s vs 37.8s vs 45.8s).

Conclusions: In an in vitro setting, low dose apixaban provides similar anticoagulant property as warfarin in terms of prolongation of thrombosis formation. Higher dose apixaban may have lower pump thrombosis rates. Further studies in patient groups are needed before change in clinical practice.

PB 474 | Management of Major Bleeding and Outcomes in Patients Treated with DOACs: Results from the START SSC Event Register

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Background: Management of major bleeding in patients treated with direct oral anticoagulants (DOAC) is still not well established.

Aims: START SSC Events, branch of the START register, aims to describe the management and outcomes of bleeding or recurrent thrombotic events during DOAC treatment in routine clinical practice. We present the results of the management of bleeding patients.

Methods: Adult patients with major bleeding (based on ISTH definition) during DOAC treatment for atrial fibrillation (AF) or venous thromboembolism (VTE) were enrolled. Baseline characteristics, laboratory results, site of bleeding, management strategies and outcomes at hospital discharge and after 6 months were recorded on a web based-form.

Results: Between January 2015 and December 2016, 173 patients on DOACs (25% on apixaban, 24% on dabigatran 50% on rivaroxaban, and 1% on edoxaban) were enrolled, 117 had major bleeding events. AF was the indication for treatment in 84%, 62% were males, median age was 79 years, median creatinine clearance was 59.5 mL/min. The site of bleeding was cerebral in 53 patients (15 fatal during hospitalization), gastrointestinal in 42 (2 fatal), and in other sites 22 (1 fatal). Bleeding occurred within the first 90 days of DOACs treatment in 45% of patients. Haemoglobin, PT, and aPTT were available in nearly 90% of cases while specific DOACs measurements in only 20%. Therapeutic strategies included surgery or invasive procedures in 17% of patients; red blood cells transfusion in 25%, prothrombin

complex concentrates in 23%, antifibrinolytic drugs in 7.6%, fresh frozen plasma in 2.1%; 3 patients on dabigatran were treated with idarucizumab. At 6-month follow-up, there were 16% deaths, 64% complete resolutions, and residual disability in 32% of patients with intracranial haemorrhage.

Conclusions: Our data confirm a high heterogeneity in the management of bleeding complications in patients treated with DOACs. Only rarely specific measurements of DOACs activity drove management strategies.

PB 475 | High Recanalization Rate in Patients with Proximal-vein Thrombosis Treated with the New Direct Oral Anticoagulants

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Background: In patients with proximal deep venous thrombosis (DVT) of the lower extremities, the persistence of residual vein thrombosis (RVT) has been reported to increase the risk of subsequent complications. With the use of standard anticoagulant therapy (LMWH followed by vitamin K antagonists), the rate of RVT has consistently been reported to occur in approximately 50% of patients after 3 and 6 months.

Aims: In an Italian registry, we sought to investigate the rate of RVT in 352 consecutive outpatients with proximal DVT who were treated with the new oral anticoagulants (NOA). We compared this rate with a historical cohort of 1094 patients who had been treated with standard anticoagulation.

Methods: 282 patients had been treated with Rivaroxaban, 28 with Dabigatran and 42 with Apixaban. In each patient the common femoral vein at the groin and the popliteal vein at the popliteal fossa were scanned in transverse plane at 3 and/or 6 months after the acute event. Veins were assessed during compression, and RVT was defined as the persistence of thrombotic material resulting in a diameter of at least 4 mm.

Results: RVT was detected in 143 patients treated with NOA (41.2%) after three months and in 58 patients (21.1%) after six month; the corresponding in patients treated with standard anticoagulation was 52.3% and 54.5%, respectively. After adjusting for the baseline characteristics, the odds ratio of RVT in patients treated with the NOA as compare to those treated with conventional anticoagulation was 0.63 (95% CI 0.48 - 0.81) after three months and 0.17 (95% CI 0.11 -0.26) after six months.

Conclusions: Our study results show for the first time that in patients with proximal DVT treated with the NOA the persistence of ultrasound detectable RVT occurs less frequently than in patients treated with standard anticoagulation. Whether this observation has implications for the development of subsequent complications remains to be demonstrated.

PB 476 | Clot Waveform Analysis of Activated Partial Thromboplastin Time Measurement Curves Reveals How Direct Oral Anticoagulants Impact In Vitro Coagulation

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Background: Clot waveform analysis (CWA) has been reported to extend the interpretation of activated partial thromboplastin time (APTT) measurement curves. The parameters are obtained from successive derivatives of the clotting reaction curve, dissecting the coagulation cascade pathway. The first, second, and third derivatives represent the thrombin activity for fibrin generation, the activated factor X (Xa) activity for thrombin generation, and the IXa activity for Xa generation, respectively.

Aims: To assess the in vitro effects of direct oral anticoagulants (DOACs), CWA was applied to the APTT assay of plasma samples spiked with each drug.

Methods: Pooled plasma samples from healthy volunteers were spiked with one of three Xa inhibitors, rivaroxaban, apixaban, and edoxaban, or a direct thrombin inhibitor (DTI), dabigatran. APTT was assayed using the Actin regent (SIEMENS) and the automated blood coagulation analyzer, CS-5100 (Sysmex). In addition to the four DOACs, another DTI, argatroban was subjected to analogous assays. For CWA, the first and second derivatives were automatically gained using the CS-5100 program. The third derivative was calculated using data from the CS-5100 analysis.

Results: All the maximum positive values in the successive derivatives were decreased dependently on the concentrations of each drug to a varying degree (Fig 1, 2). The patterns of decrease seemed to depend on whether the drug is a DTI or a Xa inhibitor. Moreover, the negative values in the second and third derivatives appeared putatively due to consumption of thrombin and factor Xa, respectively, to form complexes with plasma serine protease inhibitors. The maximum negative values decreased dependently on the concentrations of each drug

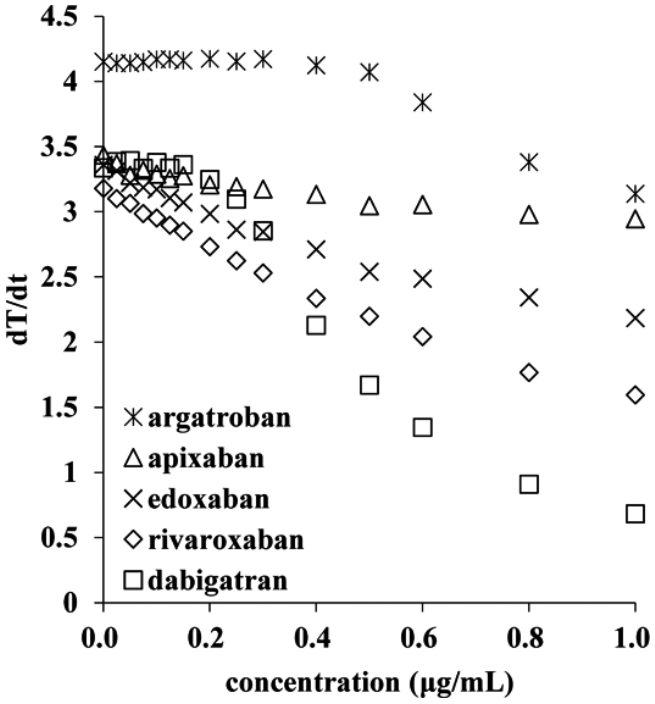

FIGURE 1 Maximum positive values of the first derivative of the clotting reaction curves were decreased dependently on the concentrations of each drug

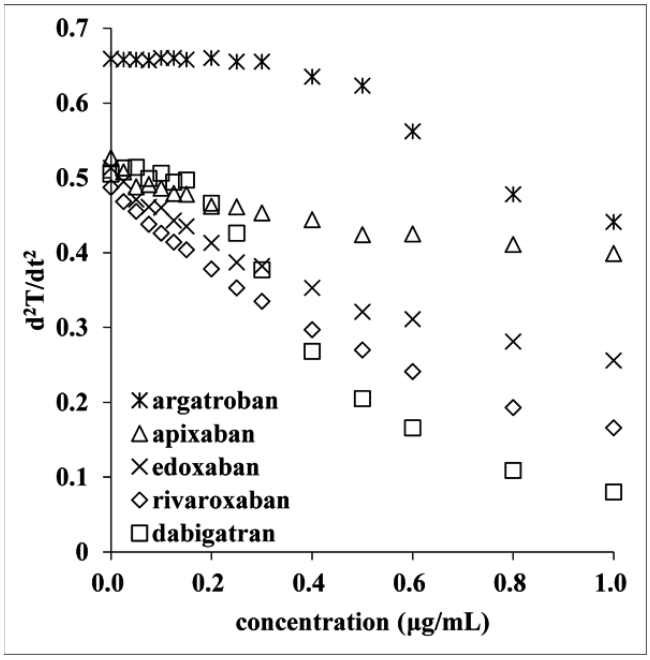

FIGURE 2 Maximum positive values of the second derivative of the clotting reaction curves were decreased dependently

were likely consistent with the suppressed generation of thrombin and factor Xa.

Conclusions: CWA revealed that the effects of DOACs involve not only direct inhibition of generation of thrombin or fibrin but also prevention of thrombin positive feedback.

PB 477 | Concentration Correlation of Direct Oral Anticoagulants as Measured by the TEG® 6s Oral Anticoagulant Assay

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Background: No fast, reliable point of care assay is currently available for measuring the anticoagulant effect of DOACs (Direct Oral Anticoagulants). Although DOACs do not require routine monitoring there can be situations where measurements may be necessary (e.g. perioperative management, patients with renal failure, thromboembolic or bleeding events, suspected overdose or lack of adherence).

Aims: This *In-vitro* study aimed to characterize the Oral Anticoagulant Assay (OAC; Haemonetics Corp, MA) as a new method to assess four currently available DOACs (dabigatran, rivaroxaban, apixaban and edoxaban).

Methods: After obtaining informed consent venous blood (in 3.2% citrate) from healthy volunteers (n≥ 4 for each DOAC) was analyzed with the fully automated OAC cartridge with an Ecarin based DTI channel and an HFXa based AFXA channel. Blood was spiked with increasing concentrations of a particular DOAC to achieve target levels. Concentrations were chosen to represent sub-therapeutic, therapeutic and in some cases supra-therapeutic concentrations of each DOAC according to published literature. Pearson's r was used to analyze correlation.

Results: A direct linear relationship was observed between the elongation of TEG®6s R-time and the spiked DOAC concentrations. DTI-R time was strongly correlated with dabigatran with Pearson coefficient of r=0.97 (0-300 ng/ml). A strong correlation was demonstrated between AFXA channel R-time and both rivaroxaban and apixaban (r=0.92; 0-300 ng/ml). Edoxaban showed a relatively weak correlation (r=0.52; 0-500 ng/ml). In edoxaban group response was highly donor dependent and interestingly in one donor no response was observed even at supra-therapeutic level indicating possible resistance.

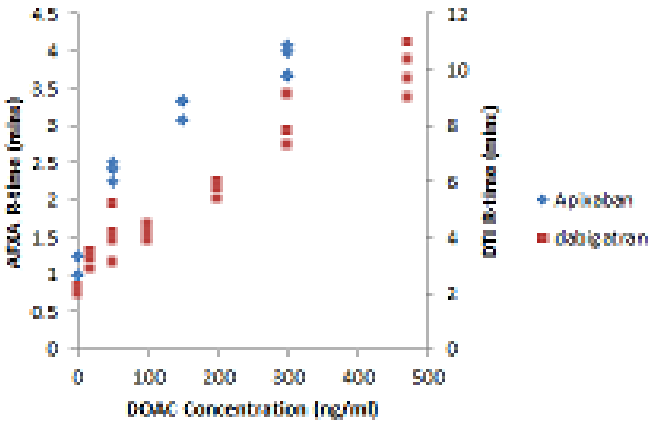

FIGURE 1 In- Vitro Apixaban and dabigatran Concnetration Correlation in blood from healthy volunteers (n=4)

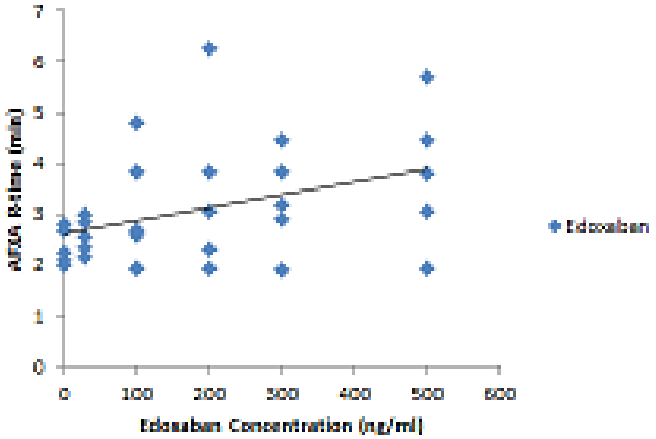

FIGURE 2 In-Vitro Edoxaban Concentration Correlation in blood from healthy volunteers (n=5)

Conclusions: A strong linear concentration response was observed for TEG® R time in this in-vitro study for all DOACs except edoxaban. As efficacy and safety of DOACs are correlated with drug concentrations in certain critical situations the TEG® 6s OAC Assay can be a valuable tool in such situations.

PB 478 | Self-poisoning with Direct Oral Anticoagulants: A Series of Five Cases Admitted to the Intensive Care Unit

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Background: There are limited reports of self-poisoning with direct oral anticoagulants (DOAC). Additionally specific monitoring of DOAC concentrations is rarely reported in the published series.

Aims: To describe the clinical features and management of DOAC-self-poisoned patients referred to intensive care unit (ICU).

Methods: We prospectively included all DOAC-self-poisoned patients referred to the intensive care unit of Lariboisière - F. Widal Hospital during a two-year period. Collected data included medical history, all ingested toxicants, clinical features, laboratory parameters, patients' management and outcome. DOAC anti-Xa or anti-IIa activities were measured using dedicated calibrators (Liquid Anti-Xa, Stago; Hemoclot-DTI, Hyphen).

Results: Five DOAC-self-poisoned patients with multi-drug ingestions including rivaroxaban (patients 1, 2, 3), apixaban (patient 4) and dabigatran (patient 5) were admitted to the ICU during 2 years (Figure 1). All patients except one were depressive or already committed suicide attempt. Clinical presentation was attributed to the co-ingested

Patient	1	2	3	4	5
Age / Gender	21 / Female	82 / Male	49 / Male	62 / Male	48 / Male
Body-weight	107 kg	70 kg	150 kg	112 kg	140 kg
Medical history	Depression and bipolar disorder	Previous suicide attempt / mixed anxiety-depressive disorder	None	Depressive disorder	Previous suicide attempt
Ingested DOAC	rivaroxaban	rivaroxaban	rivaroxaban	apixaban	dabigatran
Ingested dose	420 mg	210 mg	280 mg	230 mg	3000 to 4500 mg
Other co-ingested drugs	9 g valpromide 16 g paracetamol 80 mg propranolol	60 mg perindopril arginine/amlodipine 12.5 mg bisoprolol	6 g amiodarone 1.2 g propranolol	100 mg amlodipine 750 mg hydrochlorothiazide 520 mg bisoprolol 4 g paracetamol 400 mg atorvastatine 600 mg clomipramine 20 g potassium	5 pen insulin aspart > 2 sticker tramadol > 2 sticker perindopril arginine/amlodipine
Renal Function (Cockcroft formula)	192 mL/min	56 mL/min	192 mL/min	125 mL/min	305 mL/min
Clinical presentation	Bradycardia	Hypovolemic shock	Wrist bilateral phlebotomies and hypovolemic shock	Hypovolemic shock	None

FIGURE 1 Clinical and laboratory data in five DOAC self-poisoned patients.

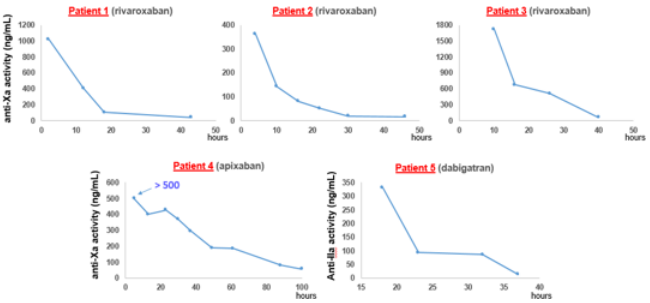

FIGURE 2 Monitoring of anti-Xa (rivaroxaban or apixaban) or anti-IIa (dabigatran) activities in the five self-poisoned patients. X-axis indicates the

drugs. None of the patients developed bleeding on admission and in the following days. Plasma DOAC concentrations on admission were up to 1800 ng/mL for rivaroxaban. Patient 3 admitted with wrist bilateral phlebotomies required stiches. Neither hemostatic treatment nor anti-Xa antidote was used. Activated charcoal was administered only in patient 2. In all these 5 patients, the renal function remained normal. Specific anti-Xa or anti-IIa activities were monitored until becoming undetectable (Figure 2).

Conclusions: Despite elevated serum DOAC concentrations, none of the 5 DOAC-poisoned patients presented bleedings. In these patients, normal renal function and absence of drug-drug interactions altering DOAC pharmacokinetics allowed the complete elimination of the toxicant between in less than 4 days.

PB 479 | New Insights in Interindividual Variability of Pharmacodynamic (PD) Response to Rivaroxaban through Thrombin Generation in the Presence of an Active Protein C Pathway: DRIVING Study Results in Healthy Volunteers

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Background: DRIVING was a prospective study on interindividual variability to R exposure among 60 healthy volunteers (Gouin *et al*, *J Thromb Haemost* 2016).

Aims: The present study aims at assessing the PK-PD relationship of R, with thrombin generation (TG) as the PD parameter.

Methods: Blood was sampled at baseline and after a single 40-mg R dose over a 24h-period (11 time-points). TG in platelet poor plasma was studied with the fully automated system ST Genesia (Stago). It differs from the calibrated automated thrombogram system by a calibration performed in plasma with a known amount of human thrombin in buffered solution and AMC fluorophore. Three experimental conditions were used: intermediate (STG-DrugScreen) or low (STG-ThromboScreen, TS) tissue factor concentration, the latter with and without thrombomodulin (+TM/−TM, Stago).

Results: Endogenous thrombin potential (ETP), peak height (PH) and time to peak (TTP) are shown at baseline, Tmax and T24 along with R plasma concentrations (Figure 1).

Using TS, adding TM markedly reduced ETP and PH, especially at Tmax, without return to baseline at T24. The PK-PD relationship was modeled by an exponential, with a decay rate (k) presenting a large between-subject variability, both for ETP (k = 2.8×10^{-3} [−TM] *vs* 13.4×10^{-3} [+TM] (ng/mL)$^{1-}$; CV: 37 vs %25) and PH (k = 6.8×10^{-3} [−TM] *vs* 15.2×10^{-3} [+TM] (ng/mL)$^{1-}$; CV: 15 vs %24)(Figure 2).

Conclusions: Phenotyping TG with ST-Genesia allows a reliable assessment of the PD response after R intake. In addition to PK variability, a substantial between-subject variability of TG parameters was evidenced in presence of R. In subjects receiving R, the involvement of the inhibitory dynamic system of activated protein C, formed in presence of TM by thrombin still generated, is important to take into account, in contrast to what occurs in subjects under vitamin K antagonist therapy, whose proteins C and S are altered.

	T0	T_{max}	T_{max}						
Rivaroxaban concentration (ng/mL)	0 [0 ; 0]	186.1 [143.3-216.2]	31.1 [22.6;37.1]						
	DrugScreen			*ThromboScreen - TM*			*ThromboScreen + TM*		
	T_0	T_{max}	T_{24}	T_0	T_{max}	T_{24}	T_0	T_{max}	T_{24}
ETP (nM·min)	1272 [1194;1436]	1130 [1042;1251]	1212 [1104;1352]	1064.0 [982;1248]	649.5 [538.2;829.9]	987.6 [896.8;1110]	551.3 [426.2;740.7]	39.9 [17.7;79.8]	251.1 [177.4;319.4]
PH (nM)	396.7 [368.9;423.2]	122.7 [111.8;170.9]	279.5 [251.5;306]	214 [171.6;262.2]	47.9 [34.5;65.1]	110.9 [96.96;134]	144.9 [102.3;189.8]	7.7 [3.21;14.4]	51.5 [34.7;66.4]
TTP (min)	2.08 [1.96;2.23]	6.37 [5.36;7.45]	3.08 [2.77;3.47]	4.37 [4.04;4.66]	11.8 [10.55;13.04]	7.40 [6.74;8.08]	3.80 [3.66;4.11]	10.9 [9.60;13.8]	5.87 [5.36;6.48]

FIGURE 1 Rivaroxaban concentrations and TGT parameters using STG-Drugscreen and STG-Thromboscreen (-TM / +TM) (median, Q1, Q3)

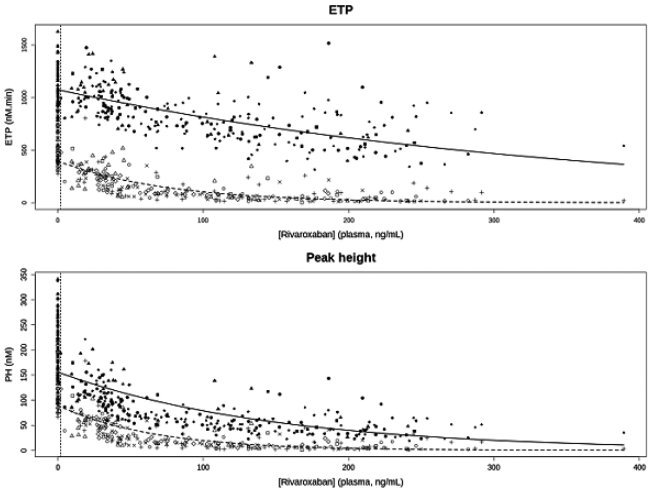

FIGURE 2 Relationship between ETP / PH (STG-Thromboscreen) and rivaroxaban plasma concentrations. Closed symbols [-TM]; open symbols [+TM

PB 480 | DTI/DXI Interferences with Global Coagulation Tests in Daily Care Emergency Admissions - Results of the Prospective Dresden NOAC Registry (NCT01588119)

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Background: Emergency management of orally anticoagulated patients is challenging, also because type of anticoaguolant may be unknown and direct thrombin inhibitors (DTI) and direct FXa inhibitors (DXI) may or may not affect global coagulation tests such as prothrombin time (PT), international normalized ratio (INR) or activated thromboplastin time (aPTT), depending on the assay and the time between last drug intake and blood sampling.

Aims: To evaluate impact of the interferences of DTI/DXI with global coagulation tests.

Methods: DTI/DXI patients with emergency hospital admissions were identified in the prospective, non-interventional *Dresden NOAC Registry*. Impact of drug class and time between last intake and blood sampling on the admission laboratory testing (proportion of normal vs. abnormal PT, INR, aPTT) was evaluated.

Results: 237 emergency admissions (46 DTI cases; 191 DXI cases) into 36 different hospitals were identified. DOAC specific testing was performed in 9 DTI (8 thrombin time, 1 ecarin clotting time) and in 12 DXI cases (chromogenic aXa assays).

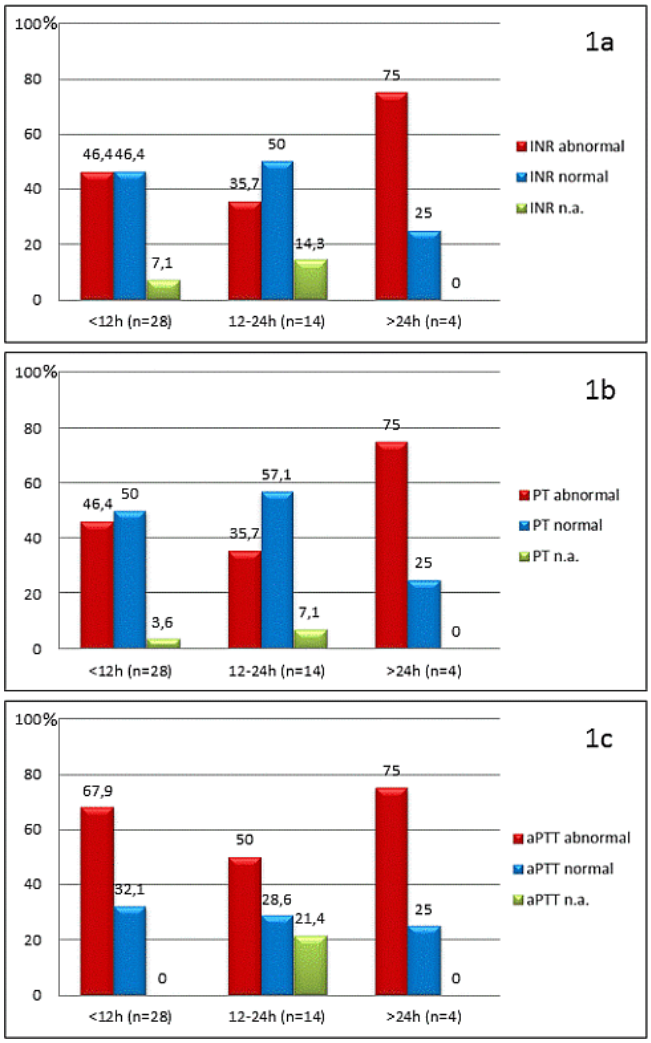

FIGURE 1 Effects of DTI intake on INR (1a), PT (1b), and aPTT (1c); n.a.=not available

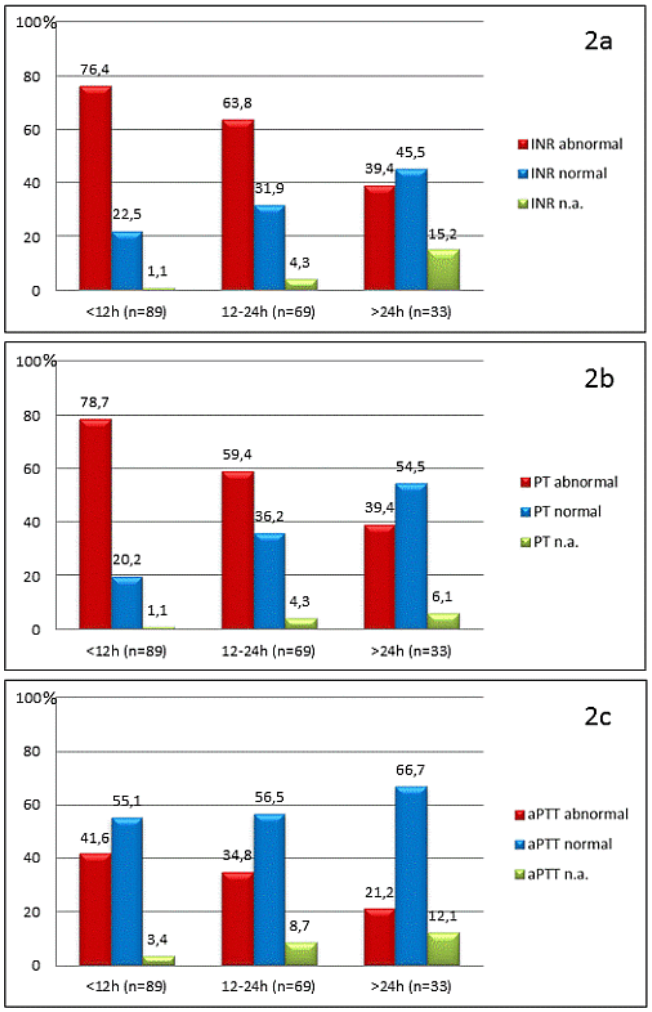

FIGURE 2 Effects of DXI intake on INR (2a), PT (2b), and aPTT (2c); n.a.=not available

Within 12 hours after last intake, only 46% of DTI recipients had abnormal INR or PT values but 67.9% had abnormal aPTT. These numbers decreased to 35.7% (PT and INR) and 50.0% (aPTT), if DTI was taken within 12-24h before blood sampling (figure 1 a-c).

In contrast, DXI recipients demonstrated abnormal INR or PT values in 76.4 and 78.6%, respectively, if last intake was < 12h before blood sampling, which decreased to 63.8 (INR) and 59.4% (PT) if DXI was taken within 12-24h and to 39.4%, if DXI before blood sampling (figure 2 a-c).

APTT was abnormal in 41.6% (< 12h); 34.8% (12-24h) and 21.2% (< 24h), respectively.

Conclusions: Within 12 hours after last intake, approximately 70-80% of DTI and DXI recipients demonstrate abnormal coagulation test results across a spectrum of assays, which may be helpful to identify unknown presence of DTI/DXI at high plasma concentrations. However, >12h after last intake, global coagulation tests are of little predictive value.

PB 481 | No Additional Diagnostic Value of Chest X-ray Prior to the YEARS Algorithm in the Diagnostic Management of Suspected Pulmonary Embolism

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Background: The YEARS algorithm was designed to simplify the diagnostic process of suspected pulmonary embolism (PE) and to reduce the number of required CTPA-scans (*Figure 1*). A recent outcome study confirmed the safety and efficacy of YEARS (2016 ESC abstract 5727), with PE ruled out in 48% of patients without need for CTPA. Chest X-ray (CXR) is an important test in the initial evaluation of

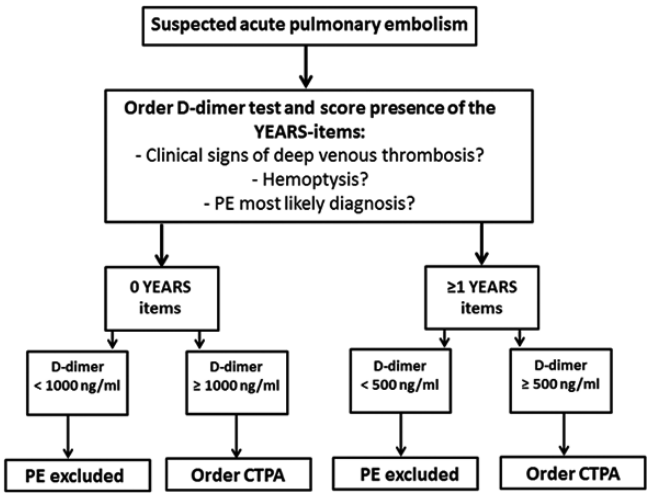

FIGURE 1 The YEARS algorithm

*Example: Assuming that the pre-test probability of PE is 28% in a certain patient with suspected PE and an indication for CTPA according to YEARS, the post-test probability of PE in case of a normal CXR result would be 28%*1.07 = 30%. The post-test probability of PE in this patient with any abnormality on CXR would be 28%*0.89 = 25%.*

	All patients (n=1473)		*Patients in whom CTPA was indicated according to the YEARS algorithm (n=763)*	
Results CXR	*Positive LR (95%CI)*	*Negative LR (95%CI)*	*Positive LR (95%CI)*	*Negative LR (95%CI)*
Normal CXR	0.86 (0.78-0.96)	1.39 (1.13-1.69)	1.07 (0.95-1.21)	0.89 (0.73-1.10)
Pleural effusion	1.14 (0.66-1.99)	0.99 (0.95-1.03)	0.58 (0.33-1.01)	1.05 (1.01-1.10)
Consolidation	1.84 (1.38-2.44)	0.88 (0.82-0.95)	1.35 (0.99-1.84)	0.93 (0.86-1.01)
Malignancy/mass	0.93 (0.40-2.17)	1.00 (0.98-1.03)	0.51 (0.22-1.22)	1.03 (1.00-1.06)
Congestive heart failure	0.98 (0.45-2.15)	1.00 (0.97-1.03)	0.67 (0.29-1.50)	1.02 (0.99-1.05)
Pneumothorax	0.00	1.00 (0.99-1.01)	n.a.	n.a.
(Rib)fracture	0.00	1.00 (0.99-1.03)	0.00	1.00 (0.99-1.01)
Atelectasis	0.49 (0.06-3.75)	1.00 (0.99-1.02)	0.37 (0.05-2.96)	1.01 (0.99-1.02)

n.a. = not applicable

FIGURE 2 Overview of LRs and CXR results in two groups; all patients and patients in whom CTPA was indicated according to the YEARS algorithm

suspected acute cardiopulmonary disease and is cheaper and associated with less radiation exposure than CTPA.

Aims: To investigate whether CXR provides incremental diagnostic value to YEARS in patients with suspected PE, i.e. whether the post-test odds of PE after certain CXR findings would allow to change the decision of the algorithm to perform CTPA or not.

Methods: This post hoc analysis was assessed in 1473 consecutive patients with suspected PE who were managed according to YEARS and were also subjected to CXR as part of standard policy. The likelihood ratio's (LR) of 7 main CXR findings for a final diagnosis of PE were calculated.

Results: 214 patients were diagnosed with PE at baseline (15%), of which 137 had a normal CXR (64%). PE was ruled out in 1259 patients, with 7 thrombotic events during 3-month follow-up (0.5%), of whom 1069 had a normal CXR (72%), for an Odds Ratio of 1.60 (95%CI 1.18-2.18) compared to patients with PE. For the overall population, only the finding of a rib fracture or pneumothorax, present in 6 patients (0.4%), significantly changed (lowered) the post-test probability of PE (*Figure 2*). For patients with an indication for CTPA (PE prevalence 28%), only the CXR finding of a rib fracture, present in 2 patients (0.3%), lowered the post-test probability to such an extent that CTPA would no longer be required to rule out PE.

Conclusions: In our cohort of patients with suspected PE, CXR was more frequently abnormal in patients who were diagnosed with PE than in those in whom PE was ruled out. The incremental diagnostic value of CXR to the YEARS algorithm to rule out was limited.

PB 482 | Rivaroxaban Levels in Patients Plasmas Are Comparable by Measuring through Two Different Calibrators in Two Different Systems

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Background: Rivaroxaban (Riva) is used for thromboembolic prevention in patients with Atrial Fibrillation (AF) and in venous thromboembolism (VTE) treatment, without laboratory monitoring, but in particular situations measured plasma Riva levels is needed.

Aims:

A) To verify the analytical performance of HemosIL liquid anti Xa calibrated by Riva specific Calibrators in an ACL TOP coagulometer.

B) To compare the results with those obtained by STA-Liquid Anti Xa calibrated with specific RIVA calibrators in a TCoag Destiny Plus coagulometer,

C) to compare results obtained by calibrating each system with the other branch calibrator.

Methods: 53 samples drawn at any time (through or peak) from consecutive patients taking Riva because of AF or VTE were processed in three different centers. Riva was measured by HemosIL Liquid anti Xa in a ACLTOP 300 and STA liquid Anti Xa in a TCoag Destiny Plus

TABLE 1 Rivaroxaban methods comparison by using two different test systems and calibrators

Comparison of reagent-calibrator combinations	HemosIL-HemosIL vs STA-STA	HemosIL-HemosIL vs HemosIL-STA	STA-STA vs STA HemosIL
n	53	42	52
Slope (95%CI)	1.053 (0.961 to 1.142)	1,024 (0,953 to 1,096)	1,062 (0,985 to 1,139)
Y intercept (95%CI)	-3,87 (-19,94 to 12,19)	11,7 (-2,0 to 25,5)	-16,5 (-30,7 to -2,2)
R (Corr Coef)	0.953	0.976	0.967
BIAS %	2.85	10	-6.9

coagulometer. HemosIL Rivaroxaban and STA Rivaroxaban Calibrators and controls were used. Data Analysis: Precision and trueness were evaluated by EP15A3, linearity by EP6 and methods comparison by EP 9 CLSI protocols. Methods comparison statistics was performed by Deming regression and Bland Altman plots in EP evaluator software.

Results: Riva HemosIL showed CVr 2.18 and 1.32%, CVi 3.06 and 3.27% and BIAS 10.5 and 13.3% giving TE of 16.0 and 19.8% for Low and High Riva controls, respectively. Linearity was verified from 8.2 to 525 ng/mL Deming regression equations and BIAS % calculated by methods comparison are included in table.

Conclusions: The analytical performance of HemosIL anti Xa calibrated by specific calibrators is acceptable, with precision and trueness similar to that reported for low molecular weight heparin. The best method comparison results were obtained when each reagent is used in their specific system with their specific calibrators. However, both tests are robust and calibration procedure with both calibrators can be used showing comparable results.

PB 483 | Multicenter Observational Study to Evaluate TEG®6s Oral Anticoagulant (OAC) Assay in Apixaban-treated Patients

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Background: Detection of anticoagulant effects of NOACs and NOAC classification are important in situations such as trauma, stroke or emergent surgery where a major intervention may increase survival. Currently, no standardized point-of-care test is available to evaluate the anticoagulant effects of apixaban.

Aims: The aim of the study was to evaluate the anticoagulant effect of apixaban-treated patients and compare with results obtained from healthy volunteers by using the point-of-care TEG®6s Oral Anticoagulant (OAC) assay.

Methods: Healthy volunteers (n=27) and patients on apixaban (n=50) were enrolled across 5 sites. Protocol was approved by site IRBs and informed consent was obtained prior to blood draw. To ensure that a range of drug concentrations were captured, blood samples were obtained at non-trough (< 9hrs) and trough (9-12hrs) levels as indicated from last dose (2.5 or 5.0 mg BID). The viscoelastic OAC assay utilizes human Factor Xa and Ecarin as reagents for AFXA and DTI assays, respectively. Data derived from patients on apixaban were compared against the reference range (based on a separate healthy cohort; n=111) to determine sensitivity and specificity for detection.

Results: The majority of patients were prescribed apixaban for atrial fibrillation. Patients had significantly longer Reaction Time (R) by AFXA assay ($p < 0.001$) but not DTI assay ($p=0.55$) as compared to healthy volunteers. AFXA-R time >1.9 min indicated detection of anticoagulation effect with 96% sensitivity and 100% specificity (Fig A). A DTI R-time cut-off < 2.3 min classified an anti-Xa with 98% sensitivity

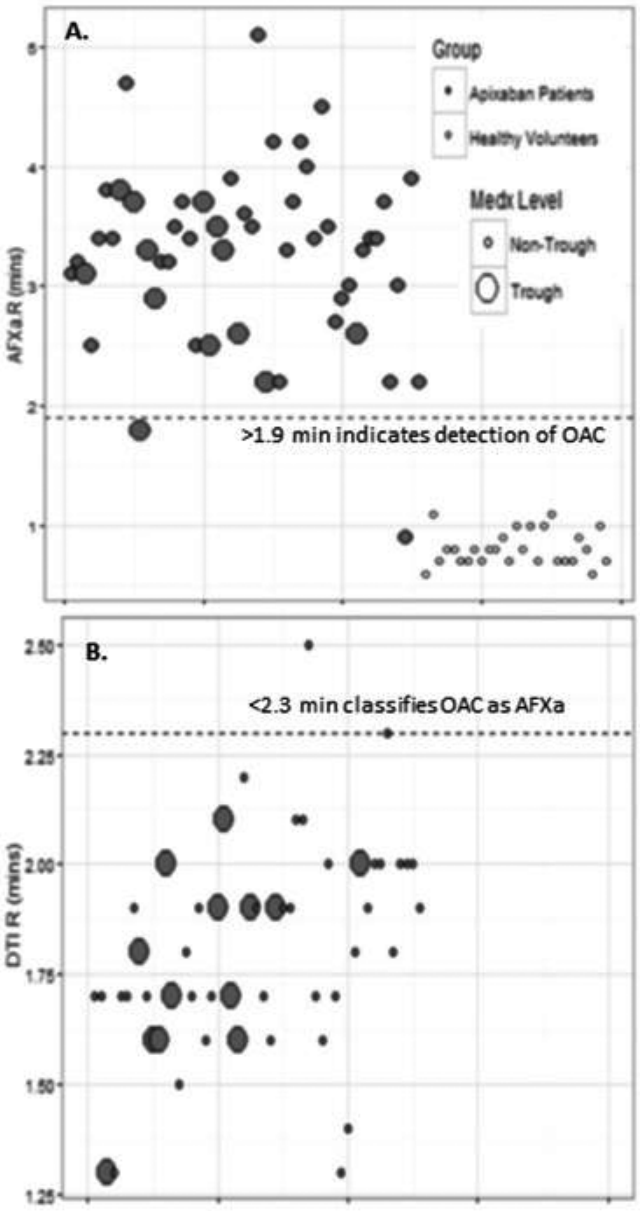

FIGURE 1

(Fig B). There was a large interindividual variability in anticoagulant effect that was not attributable to dose or trough measurements.

Conclusions: The automated TEG®6s device with its OAC assay may be an effective tool to rapidly identify anticoagulant effects in apixiban-treated patients and thus, facilitate care in emergent situations.

PB 484 | Management Strategies and Long-term Outcomes in Patients with Upper Extremity Deep Vein Thrombosis: Findings from the XALIA Study

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Background: Upper extremity deep vein thrombosis (UEDVT) accounts for approximately 5-10% of venous thromboembolism (VTE) events. Evidence on the treatment and clinical history of UEDVT is either indirect or from small observational studies. No study has assessed the efficacy and safety of the non-vitamin K antagonist oral anticoagulants in patients with UEDVT.

Aims: To report the results of a subgroup analysis of patients with UEDVT from the XALIA study.

Methods: XALIA was a prospective, non-interventional study of rivaroxaban in the treatment of acute VTE. Patients aged ≥18 years scheduled to receive ≥3 months of anticoagulation with rivaroxaban or standard of care (SOC) were eligible. Baseline characteristics, management strategies and unadjusted incidence rates of recurrent VTE, major bleeding and all-cause mortality for patients with UEDVT were compared between treatment groups.

Results: Overall, 164 patients with UEDVT were enrolled; 61 (37.2%) received rivaroxaban and 103 (62.8%) received SOC. Mean age was 48.8 years in the rivaroxaban group and 53.9 years in the SOC group; 3 (4.9%) and 5 (4.9%) had creatinine clearance < 50 ml/min, 4 (6.6%) and 48 (46.6%) had cancer; and UEDVT was provoked by a transient risk factor in 16 (26.2%) and 54 (52.4%), respectively. Twelve (19.7%) patients in the rivaroxaban group and 51 (49.5%) in the SOC group were hospitalized (mean duration 4.9 and 9.1 days, respectively). Median treatment duration was 132 and 161 days, respectively. The annualized symptomatic recurrent VTE incidence was 0% and 6.95% (95% CI 1.89-17.8), major bleeding rates were 0% and 3.51% (0.42-12.67) and all-cause mortality was 0% and 15.4% (7.03-29.2), respectively.

Conclusions: In XALIA, most patients with UEDVT received SOC, and nearly half of the SOC group had cancer. Rivaroxaban-treated patients most frequently had unprovoked UEDVT and only a minority were hospitalized. In these patients, rivaroxaban appears to be a safe and effective treatment strategy.

PB 485 | Measurement of Apixaban and Rivaroxaban Levels in Real Life Clinical Practice: An Instrument to Improve Anticoagulation Management?

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Background: Rivaroxaban (R) and apixaban (A) are oral anticoagulants that function as direct anti-factor Xa inhibitors (DFXaI). Although routine monitoring is not required, knowledge of drug levels may be required in bleeding, thromboembolic events, procedures, extreme body weight, suspected non-compliance or overdose. Anti-Xa chromogenic assays have become recently available to determine plasma levels of DFXaI.

Aims: We analysed all DXaI plasma level requests received in our lab in 12 months. We recorded the clinical details and anticoagulation outcome for every patient (pt).

Methods: We utilised chromogenic anti-factor Xa assays (Technoclone®) to measure A and R levels. The expected mean plasma levels for A 2.5 mg bd were 21 (trough) and 62
(peak) ng/ml while for 5mg bd were 50 and 125ng/ml respectively (Frost, 2013). For R 20 mg od the expected peak levels were 223 (160-360) while the mean trough levels were 22 (4-96) ng/ml (Mueck, 2008). Pts were advised to take their tabs as usual.

Results: A total of 97 samples from 78 pts (M:44/F:34), median age:66 (32-98) years were recorded.
41 pts suffered from atrial fibrillation and 37 had DVT/PE. 29 pts were on A 2.5mg, 41 on A 5mg and
28 on R 20mg. The main reasons to request drug levels were: extreme body weight:17, gastric/bowel//pancreas pathology: 12, carbamazepine use: 11, chronic kidney disease(CKD): 10, clinician reassurance: 10, history of bleeding:9, recurrent DVT/pt compliance:6. Median drug levels for R were 47 (1,07-437,4) ng/ml, for A 2.5mg 93.74 (2.82-254.6) and for A 5mg they were 116.9 (5.39-442.68) ng/ml. Anticoagulation was changed in 8/78 pts (10%); it was stopped in 1 A pt with CKD, A was reduced in
3 pts; 1 on amiodarone, 1 with pancreatic cancer, 1 on carbamazepine. A was changed to other anticoagulant in 4 pts; 1 with new thrombosis, 1 with bleeding and 2 with drug interactions.

Conclusions: DFXaI are safe even in complex clinical scenarios like carbamazepine co-use or bowel pathologies. Lab testing would favor implementation of DOACs but its role remains to be defined.

PB 486 | Comparison of Bleeding and Thromboembolic Event Rates in Atrial Fibrillation Patients with Good and Poor Warfarin Control before and after Switching to Direct Oral Anticoagulants

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Background: It is unclear which non-valvular atrial fibrillation (NVAF) patients would most clearly benefit from switching from warfarin to a direct oral anticoagulant (DOAC). There have been no randomized controlled trials to examine this common scenario, and providers have differing opinions.

TABLE 1 Event rates before and after switching to DOAC

	Good control before switch	Good control after switch	p-value	Poor control before switch	Poor control after switch	p-value
# total patients	119	119		71	71	
# patient years	275.1	213		116	82	
Median months of treatment per patient	22.8	18		13	12	
Mean/median TTR (%)	72/71	N/A		47/50	N/A	
Stroke/TIA (# per 100 pt-yr)	1.5	0.5	0.31	2.6	0	/
Bleeding requiring ED visit or hospitalization (# per 100 pt-yr)	9.8	3.3	0.006	7.8	2.4	0.08
Stroke/TIA or bleeding requiring ED visit or hospitalization (# per 100 pt-yr)	11.3	3.8	0.003	10.3	2.4	0.04

Aims: To examine outcomes in NVAF patients with good and poor INR control before and after switching from warfarin to a DOAC.

Methods: Since 2009, abstractors at 6 anticoagulation services have entered patient data into the Blue Cross Blue Shield of Michigan funded Michigan Anticoagulation Quality Improvement Initiative (MAQI^2) registry. NVAF patients with at least 6 months of warfarin treatment before switching to a DOAC were stratified into two groups according to INR control based on time in therapeutic range (TTR): good control (TTR ≥ 60%) and poor control (TTR < 60%). The composite rate of stroke/TIA or bleeding requiring ED visits or hospitalizations was calculated for both groups before and after switching to a DOAC. Comparisons were made using Poisson regression and Chi-square.

Results: A total of 190 patients were identified with 119 (63%) having good INR control and 71 (37%) having poor INR control. After switching to a DOAC, the composite event rate in patients with good control was reduced by 7.5 per 100 pt-yr (11.3 to 3.8, p=.003) while the composite event rate in patients with poor control was reduced by 7.9 per 100 pt-yr (10.3 to 2.4, p=.04) (Table 1). This represents similar relative composite event rate reductions (66% vs. 77%, p=0.21) in the good and poor INR control groups, respectively (Table 2).

TABLE 2 Relative percent event rate reduction after switching to DOAC

	Good control	Poor control	p-value
Stroke/TIA	67%	100%	0.01
Bleeding requiring ED visit or hospitalization	66%	69%	0.77
Stroke/TIA or bleeding requiring ED visit or hospitalization	66%	77%	0.21

Conclusions: NVAF patients with good and poor INR control from our cohort benefitted equally after switching from warfarin to a DOAC. Both groups experienced a significant reduction in rate of our composite outcome. Our results suggest that patients with both good and poor INR control may be appropriate candidates to switch to a DOAC.

PB 487 | Measurement of Direct Oral Anticoagulant Drug Levels Following Bariatric Surgery

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Background: Absorption of the direct oral anticoagulants (DOACs) occurs to a variable extent in the stomach and small intestine. While bariatric surgery may result in decreased drug absorption, there is limited data regarding its impact on the absorption of DOACs.

Aims: We evaluated peak plasma drug concentrations in a case series of patients receiving rivaroxaban or apixaban for atrial fibrillation or venous thromboembolism, following bariatric surgery.

Methods: Our institution is a regional bariatrics centre. All bariatric patients receiving DOACs are assessed post-operatively, and peak plasma DOAC concentrations routinely are evaluated 2 to 4 hours post-dose with validated assays. We performed a retrospective review of DOAC levels in bariatric patients receiving therapeutic dose DOACs at our centre between January 2015 and January 2017.

Results: 10 patients were identified who resumed a DOAC after bariatric surgery (7 rivaroxaban, 3 apixaban). Peak drug concentrations were measured at median (range) 7.0 days (0.0-29.0) after anticoagulant resumption. Mean body weight (SD) was 140.1 kg (23.2), mean body mass index was 47.9 kg/m^2 (5.5), and mean eGFR was 88.7 ml/min/1.73 m^2 (13.2). Among patients receiving rivaroxaban, mean peak concentration was 299.3 ng/ml (55.4; reference range 189-419). Among patients receiving apixaban, mean peak concentration was 132.7 ng/ml (26.4; 91-321). 6 patients demonstrated levels consistent with prior published ranges, while 3 were below published ranges. No thrombotic or bleeding events occurred during hospitalization.

Conclusions: This case series provides preliminary data suggesting that standard therapeutic doses of DOACs in morbidly obese patients after bariatric surgery may achieve peak anticoagulant levels within or close to prior published ranges. The pharmacokinetic properties

Sex	Surgery	Weight (kg)	eGFR	Anticoagulant	Peak (ng/ml)
F	SG	146.7	84.0	Riva. 20 mg OD	273
M	GB	141.0	78.0	Riva. 20 mg OD	363
F	SG	148.6	101.0	Riva. 20 mg OD	262
M	SG	194.0	102.0	Riva. 20 mg OD	117*
F	SG	118.0	74.0	Riva. 20 mg OD	892^
M	Other	153.0	92.0	Riva. 15 mg BID	172*
F	SG	125.0	65.0	Riva. 20 mg OD	147*
M	SG	117.0	94.0	Apix. 5 mg BID	163
M	GB	138.0	106.0	Apix. 5 mg BID	120
F	SG	120.0	91.0	Apix. 5 mg BID	115

FIGURE 1 Patient Characteristics and Peak Direct Oral Anticoagulant Levels After Bariatric Surgery

of DOACs and clinical significance of these findings requires further evaluation in prospective studies in bariatrics and morbid obesity.

PB 488 | Use of a 4-factor Prothrombin Complex Concentrate for Management of Direct Xa Inhibitor-induced Major Bleeding

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Background: While direct Xa inhibitors provide safety advantages over warfarin, concern still exists over the lack of a specific antidote for management of major bleeding events. Four factor prothrombin complex concentrate (4PCC) has demonstrated the ability to reverse coagulation parameters in healthy subjects, but efficacy and safety data are lacking in patients with bleeding events.

Aims: Assess the efficacy and safety of 4PCC in management of direct Xa inhibitor-induced major bleeding in real world practice.

Methods: This retrospective study included all patients who received 4PCC at our hospital for a major bleeding event on a direct Xa inhibitor from 11/14 to 10/16. Patients receiving other anticoagulants were excluded from the analysis. Patient demographics, medication use, laboratory values, and outcomes were collected from patient charts. All descriptive data are presented as mean ± standard deviation.

Results: We identified 23 patients who received 4PCC for management of direct Xa inhibitor-induced major bleeding, 65% of which were traumatic and 65% of which were cranial. Patients were 78% female, age 75±10 years, and weight 84.6±21.9 kg. Indications for a direct Xa inhibitor included atrial fibrillation (n=13) and venous thromboembolism (n=10). Patients were balanced between receiving apixaban (n=12) and rivaroxaban (n=11), with a HASBLED Score of 1.9±0.8. Nine patients were also on aspirin. The 4PCC dose was 3342±1474 U at 38.6±12.5 U/kg. 4PCC stopped bleeding in 15 patients, while 4 required surgery to stop bleeding. 4PCC stopped bleeding within 26.6±37.8 hours. Five patients also received PRBC and 4 received FFP and/or platelets. Five patients died during hospitalization (3 during acute bleeding event), and two thrombotic events occurred within the subsequent 30 days.

Conclusions: While 4PCC was not 100% successful, it did provide a rapid and safe strategy for management of a direct Xa inhibitor-induced major bleeding. More experience in non-cranial bleeding will be needed at our institution.

PB 489 | Is Rivaroxaban Associated with Headache and Dizziness? A Retrospective Study

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Background: Direct oral anticoagulants are effective in the prevention of stroke in atrial fibrillation and treatment of venous thromboembolism (VTE). Clinicians at different centers have made observations of headaches and dizziness being associated with rivaroxaban, which disappear upon switching to a different anticoagulant. However, these observations have not been reported in the literature.

Aims: To evaluate the incidence of headache and dizziness associated with rivaroxaban compared to a control group receiving apixaban.

Methods: A retrospective chart review was performed. We hypothesized that the incidence of headache and/or dizziness would be significantly higher in patients receiving rivaroxaban.

Results: A total of 491 patients were included in the rivaroxaban group and 397 in the apixaban group. The groups were similar with regards to gender but not for age (median age of 69 versus 78; $P < 0.001$). The most common indication for anticoagulation in the rivaroxaban group was treatment of VTE compared to management of atrial fibrillation in the apixaban group. The median duration of anticoagulation therapy was significantly longer for the apixaban group compared with the rivaroxaban group (19 months versus 11 months; $P < 0.001$). Number of patients who had headache was not different between the two groups, 3 (0.6%) versus 1 (0.25%). Two of the 3 patients on rivaroxaban and the patient on apixaban had resolution of headache after switching to a different anticoagulant. Number of patients who had dizziness was also not different, 4 (0.8%) versus 3 (0.75%). Two of the 4 patients on rivaroxaban and one patient on apixaban had resolution of dizziness after switching to a different anticoagulant.

Conclusions: Data from our study suggests that the incidence of headache and dizziness in patients receiving rivaroxaban was very low and not different from a control group of patients on apixaban. Switching

to a different anticoagulant resolved either symptom in most of the cases.

PB 490 | Adherence to Rivaroxaban, Dabigatran, and Apixaban for Stroke Prevention for Newly Diagnosed and Treatment Naïve Atrial Fibrillation Patients: 2013-2014

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Background: Few studies have assessed adherence to non-vitamin K, oral anticoagulants (DOACs), especially using contemporary data now that multiple DOACs are available.

Aims: This study compared adherence and treatment patterns among DOACs for stroke prevention in NVAF.

Methods: Incident, treatment-naïve NVAF patients were identified during 2013-2014 from a large claims database. Patients were included who initiated rivaroxaban, dabigatran, or apixaban within 30 days after diagnosis. Adherence to the index medication and adherence to any oral anticoagulant was assessed using the proportion of days covered (PDC) at 3, 6, and 9 months. The number of switches and gaps in therapy were also evaluated. Analyses were stratified by stroke risk scores and a logistic regression model was used to control for factors that may predict high adherence.

Results: Dabigatran had lower adherence (PDC=0.76, 0.64, 0.57) compared to rivaroxaban (PDC=0.83, 0.73, 0.66; p< 0.001) and apixaban (PDC=0.82, 0.72, 0.66; p< 0.001) at 3, 6, and 9 months of follow and twice the number of switches to either other anticoagulants or antiplatelet therapy. Adherence was higher overall as stroke risk increased and showed dabigatran had consistently lower adherence compared to the other DOACs. Adjusted analyses showed that increasing age and comorbid hypertension and diabetes were associated with higher adherence.

Conclusions: In this real-world analysis of adherence to DOACs, rivaroxaban and apixaban had favorable profiles compared to dabigatran and rivaroxaban appeared to have higher overall adherence among the DOACs. Clinicians and managed care organizations should consider the implications of lower adherence on clinical outcomes and quality assessment.

PB 491 | Determination of Rivaroxaban, Apixaban, Edoxaban and Dabigatran by Ultra-performance Liquid Chromatography-tandem Mass-Spectrometry and Chromogenic Assays from Urine Samples of Patients

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Background: The direct oral anticoagulants (DOAC) apixaban (A), edoxaban (E), rivaroxaban (R), and dabigatran (D) are excreted into the urine between 30% and 80%. Ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) methods the reference procedure to quantify DOACs in plasma samples.

Aims: To develop an UPLC-MS/MS method for DOACs in urine and evaluate the analytical performance compared to chromogenic substrate (CS) assays from urine samples of patients treated with A, R and D.

Methods: Urine samples were analysed with ACQUITY H-Class UPLC System, Xevo TQ-S Mass spectrometer, ACQUITY UPLC CSH C18 Column (Waters) and Risperidon D4 as internal standard. Samples were eluted with gradients of ammoniumformiate pH 3.5 and methanol. Artificial urine was spiked up to 10,00ng/ml of DOACs to evaluate of the method. The method was validated using urine samples from patients (n=29 per group) treated with A (5mg bid), R (20mg od), D (110mg or 150mg bid) and controls. Modified Coamatic (Haemochrom) and S2238 CS assays were adopted to determine DOACs activity from patient's urine samples. Results of UPLC-MS/MS and CS assays were compared by statistical analysis.

Results: Linearity of he UPLC-MS/MS method was from 0 to 10,000ng/ml for A, E, R, and D, LLOD between 0.1 and 2.0ng/ml, LLOQ 0.4 and 3.6ng/ml, intra- and inter-assay coefficient of variation of 2% to 11% (n=8 determinations). Background noise of clinical samples of A, R and D corresponded to mean±sd: 5.3±6.7ng/ml, 0.4±0.2ng/ml, 1.2±0.2ng/ml (UPLC-MS/MS), 3.6±3.5ng/ml, 3.6±3.5ng/ml, and 1.2±1.2ng/ml (CS assays). The results from urine samples of DOAC patients and statistical analysis of the comparison of methods are given in the table.

Conclusions: UPLC-MS/MS is more precise than CS assays for determination of DOACs in urine. CS assay indicates the presence of active

TABLE 1 Concentrations of DOACs from patient's urine determined by UPLC-MS/MS and chromogenic substrate assays and statistical comparison of methods

	UPLC-MS/MS method . . . ng/ml . . . . . mean+/-sd	chromogenic substrate assay . . . . ng/ml . . . . . mean+/-sd	t-test . . . . . p value	Pearson coefficient of correlation . . . r value	Intraclass coefficient of correlation . . . r value	Maloney Rastogi test . . p value
Apixaban	1849+/-1420	1533+/-1624	0.4344	0.6956	0.6821	0.3387
Rivaroxaban	2829+/-1909	4148+/-1947	0.0117	0.7257	0.5925	0.8828
Dabigatran	5650+/-3698	5674+/-4532	0.9821	0.7868	0.7767	0.0901

metabolite/s of R in urine. UPLC-MS/MS may serve as gold standard method for development of a point of care test from urine samples of patients to determine DOACs in emergency medicine.

PB 492 | Length of Hospital Stays Following Acute Pulmonary Embolism in England: Observational Cohort Study Using the CPRD-HES Databases

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Background: The development of low molecular weight heparin (LMWH) and more recently the DOACs has revolutionised the anticoagulation management of patients with DVT, such that outpatient management in the UK is accepted as the standard of care; this is not the case for PE. The newly updated 2017 British Thoracic Society (BTS) Guidelines for the management of PE recommend that patients with PE should receive a DOAC (Grade A recommendation) and those with a PESI score I/II, sPESI 0 or meeting the Hestia criteria should be considered for outpatient management (Grade B recommendation).

Aims: The aim of this study is to describe hospital admission patterns following the diagnosis of acute VTE in England.

Methods: We performed a retrospective cohort study of all VTE (DVT and PE) events identified in the UK Clinical Practice Research Database linked to Hospital Episode Statistics (CPRS-HES) from April 1st 2008 to March 31st 2012. VTE occurrence was defined by a VTE read code or ICD-10 code and matched to hospitalisation, measured in number of days.

Results: 11,353 VTE events were identified, 5114 (45%) PE and 6239 (55%) DVT. Of the 5114 PE episodes identified, 3021 (59%) resulted in hospitalisation. The median duration of hospitalisation for PE patients was 6 days, with an inter-quartile range of 3-10 days (Q1-Q3), compared to a median hospital stay for DVT patients < 1 day.

Conclusions: PE patients spend almost a week in hospital, whereas DVT patients are in general discharged the same day. The increasing availability, experience and confidence in the use of DOACs as well as the development of ambulatory PE pathways in line with the new UK BTS Guidelines, utilising risk assessments such as PESI and Hestia criteria, is likely to result in a paradigm shift in the management of PE patients in the UK with more patients being managed in the outpatient setting.

PB 493 | Early Uptake of Edoxaban: A Danish Nationwide Cohort Study

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Background: Edoxaban is the fourth direct oral anticoagulant (DOAC) to enter the market. In Europe, edoxaban is licensed for use in atrial fibrillation as well as treatment and prevention of pulmonary embolism and deep vein thrombosis. The place for edoxaban in a field with four highly similar drugs is not clear.

Aims: To describe the early uptake of edoxaban in the first six months after market entry in Denmark.

Methods: Using nationwide health registries, we identified new users of edoxaban (n=170) from June 6 (day of marketing) through November 2016. For comparison, we also identified new users of dabigatran (n=1,319), rivaroxaban (n=8,755), and apixaban (n=6,932). Users were compared according to the indication of use and described with regard to previous anticoagulant experience, comorbidity and comedication.

Results: The rate of edoxaban initiation increased to 1.2 per 100 000 person months in November, compared to 3.4, 33.9 and 26.2 for dabigatran, rivaroxaban, and apixaban, respectively.

Atrial fibrillation was the most common registered indication for edoxaban use (57%) as well as the other DOACs (38-51%). Overall, users of edoxaban were comparable to users of the other DOACs with regard to age, gender distribution and comorbidities. However, 85% of edoxaban users had previously received anticoagulant treatment

TABLE 1 Baseline characteristics of new users of direct oral anticoagulants (DOAC) in Denmark between June and November 2016

	Edoxaban (n=170)	Dabigatran (n=1,319)	Rivaroxaban (n=8,755)	Apixaban (n=6,932)
Age, median (IQR)	73 (69-81)	73 (68-81)	72 (63-80)	76 (68-83)
Male gender	92 (54%)	773 (59%)	4,804 (55%)	3,737 (54%)
Switching from warfarin	38 (22%)	235 (18%)	1,166 (13%)	1,009 (15%)
Switching from other DOAC	71 (42%)	281 (21%)	728 (8%)	907 (13%)
Indication: atrial fibrillation	97 (57%)	672 (51%)	3,398 (39%)	3,548 (51%)
Previous myocardial infarction	23 (14%)	79 (6%)	616 (7%)	604 (9%)
Previous bleeding event	25 (15%)	166 (13%)	909 (10%)	1,012 (15%)
Previous ischaemic stroke / transient ischemic attack	12 (7%)	149 (11%)	745 (9%)	1,002 (15%)
Use of low-dose aspirin	24 (14%)	270 (21%)	1,685 (19%)	1,632 (24%)

compared to 34-48% for users of the other DOACs. Further, edoxaban users generally had lower use of other medication.

Conclusions: While the use of edoxaban is still limited compared to other DOACs, its use is increasing. Edoxaban is primarily used in atrial fibrillation, and edoxaban users are often switchers from another oral anticoagulant. Continued monitoring of DOACs, including effectiveness and safety, is considered essential to the safe and rational use of these drugs. However, selective prescribing may be a challenge when comparing DOACs.

PB 494 | Fibrin Monomer Complex and Dilute Prothrombin Time as a Marker for Thrombotic Tendency in Anti-Xa DOAC Therapy

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Background: Direct oral anticoagulant targeted to factor Xa(Anti-Xa DOACs) such as Rivaroxaban, Apixaban and Edoxaban, a direct thrombin inhibitor, have been administered for prevention of cerebral embolism in patients with non-valvular atrial fibrillation (NVAF), and the monitoring is not required in general clinical setting. However, some evaluation criteria are needed to prevent major bleeding and/or other severe events.

Aims: In this study, we compared the anticoagulant effect of anti-Xa DOACs in patients with NVAF by dilute prothrombin time (dPT), fibrin monomer complex (FMC), D-dimer and general clotting assay.

Methods: Citrated plasma samples were obtained from Japanese patients (Pt) with NVAF receiving Rivaroxaban (n=593), Apixaban (n=471) and Edoxaban (n=310) therapy. Depending on clinical setting, they were classified into 4 groups: no particular event (N, n=1325), minor bleeding (B, n=36), major bleeding (n=0) and thrombus (T, n=13). Sample characteristics were classified according to blood collection time after ingestion: peak group is within 4 hours after ingestion, and others were trough group. Laboratory tests such as dPT (indicated by Ratio of Inhibited Thrombin Generation, RITG), FMC and D-dimer was determined by Sysmex CS series. Anti-Xa DOACs were determined with anti Xa Assay (Hyphen Biomed).

Results: In trough group, a significant difference was confirmed in PT-INR between N (1.21) and T (1.16), in RITG between N (15.7) and T (1.3) and between N (15.7) and B (34.2), and in FMC between N (3.1 ug/mL) and T (10.3 ug/mL), but no significant difference was confirmed in other items. In peak group, the significance was lower than trough group.

Conclusions: This study suggested that PT-INR, RITG and FMC were useful tool for understanding anticoagulant effect during oral anti Xa DOACs treatment. Especially, FMC measuring at trough might be a marker to judge thrombotic tendency, and also RITG measuring at trough might judge bleeding and thrombotic tendency.

PB 495 | Adherence to Rivaroxaban for the Management of Acute Venous Thromboembolism (VTE) - Early Results from the ‚Follow-up in Rivaroxaban Patients in Setting of Thromboembolism' (FIRST) Registry

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Background: Medication non- adherence is a global public health concern with 30-50% of medicines not taken as recommended. Those prescribing anticoagulants must consider these concerns with the

TABLE 1 Adherence screening questionnaire results from the FIRST Registry

Adherence Screening Questionnaire	1 month n= 95/104	End of treatment n= 102/104	p Value
Morisky Score - Mean (/4) (Only patients who answered all 4 questions included)	3.78	3.76	NS
	1 month questionnaire	End of treatment questionnaire	
Adherence Screening Questionnaire (Questions regarding patient beliefs taken from 16-item screening tool)	Responded no	Responded no	p Value
Do you feel that you need to take your rivaroxaban regularly? n (%)	1 (1)	7 (7)	≤ 0.05
When you feel better do you sometimes stop taking your rivaroxaban? n (%)	104 (99)	100 (95)	≤ 0.05
Do you only take your rivaroxaban when you feel you need to? n (%)	101 (96)	103 (98)	NS
Sometimes if you feel worse when you take your rivaroxaban, do you stop taking it? n (%)	104 (99)	104 (99)	NS
Sometimes do you stop taking your rivaroxaban so your body can take a break from its effects? n (%)	102 (97)	102 (97)	NS
Are you careless at times about taking your rivaroxaban? n (%)	92 (88)	98 (93)	NS

increased use of the direct oral anticoagulants, which require less clinician patient interface.

Aims: To evaluate patients adherence to rivaroxaban at the start and at the end of treatment for the management of acute VTE, and explore what factors cause patients concern about their treatment with rivaroxaban.

Methods: FIRST is a non-interventional, investigator led, multicentre registry investigating the incidence of long-term complications of VTE treated with rivaroxaban without bridging heparin in routine practice. Following written informed consent patients were administered an adherence screening questionnaire (16 items), including the 4-item Morisky Medication Adherence Scale, 1 month into rivaroxaban treatment and at the end of treatment.

Results: To date, 14 UK hospitals have recruited 600 patients. Results from the first 298 patients recruited and who have completed their treatment are presented. Reassuringly, results suggest that adherence to rivaroxaban is very high during the first month and does not decrease with time (Table 1). Whilst a quarter of patients reported being worried or concerned about their treatment, 29% at 1 month and 32% at the end of treatment, this did not impact on overall adherence. Most were worried about side-effects (25%) and the long-term effects rivaroxaban may have, with the latter increasing with time (11% versus 18% NS).

Conclusions: Adherence to rivaroxaban is high during the acute management of VTE, although we did find that some patient's beliefs about taking rivaroxaban, did change between one month and the end of treatment. Further results from FIRST will provide valuable information about these beliefs and their relationship with adherence, particularly when rivaroxaban is prescribed long-term during the secondary prevention phase.

PB 496 | Level of Fatigue after Initiation of Rivaroxaban for Treatment of Venous Thromboembolism

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Background: Rivaroxaban, a direct oral Factor Xa inhibitor, was the first direct oral anticoagulant approved for treatment of venous thromboembolism (VTE). Clinical trials showed that rivaroxaban was non-inferior to conventional anticoagulation for VTE with regard to efficacy and safety. However, increased fatigue symptoms have been reported by some patients after the initiation of rivaroxaban in routine use, but data on this potential side-effect is currently non-existing.

Aims: The aim of this study was to assess fatigue levels before, during and after treatment with rivaroxaban.

Methods: Consecutive patients were recruited from the thrombosis clinic of Østfold Hospital, Norway after a diagnosis of VTE. Fatigue was assessed with the Fatigue Questionnaire (FQ), consisting of 11 questions with scores from 0 to 3 (Higher scores=higher level of fatigue). The FQ consists of 2 subdimensions (physical and mental fatigue), as well as a total sum score (total fatigue). Fatigue measurement was performed at baseline, after 3 weeks of treatment and 1 month after the discontinuation of rivaroxaban or at 6 months if treatment was continued beyond 6 months. Data were analyzed using descriptive analyses and the Mann-Whitney test. Ethics committee approved the study and informed consent was acquired from patients.

Results: Median age of 55 patients (35 males) was 57 years; 33 had DVT, 17 pulmonary embolism and 5 had both). Fatigue data from all timepoints were available in 30 patients. Table 1 summarizes the mean dimensional and total fatigue scores during the study period. There was no statistically significant change in physical, mental or total fatigue scores between baseline, 3 weeks and end of treatment or at 6 months.

TABLE 1 Mean (standard deviation) fatigue score for patients receiving rivaroxaban at baseline, 3 weeks and after the end of treatment period

Fatigue score	Baseline	3 weeks	4-6 months
Total fatigue	15.27 (4.21)	14.84 (3.24)	15.20 (4.61)
Physical fatigue	10.89 (3.49)	10.24 (2.71)	10.30 (3.47)
Mental fatigue	4.38 (1.39)	4.60 (1.29)	4.90 (1.61)

Conclusions: In this observational study we found no indication of increased fatigue at 3 weeks as compared to baseline in patients receiving rivaroxaban for VTE. We are, however, limited by small sample size, and further studies are needed to assess this.

PB 497 | The Combination of Rivaroxaban and Dabigatran Is Superior to Either Agent Alone in Suppressing Mechanical Heart Valve-induced Thrombin Generation

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Background: Although direct oral anticoagulants (DOACs) are at least as effective as warfarin for stroke prevention in atrial fibrillation and treatment of venous thromboembolism, dabigatran is less effective than warfarin in patients with mechanical heart valves (MHVs). The utility of rivaroxaban in this setting is unknown.

Aims: Compare the capacities of rivaroxaban, dabigatran, or their combination with warfarin to suppress thrombin generation (TG) induced by components of MHVs.

Methods: TG was quantified in the absence or presence of valve leaflets or sewing ring segments (SRS). Studies were done in recalcified

human plasma containing added rivaroxaban and/or dabigatran at concentrations achievable with clinically available dosing regimens, or in plasma from patients on warfarin with varying international normalized ratio (INR) values.

Results: Mean endogenous thrombin potential (ETP) increased by 17%, 80%, and 52% (from a background of 4401 ± 192 nM·min) in the presence of leaflets, Dacron SRS, and Teflon SRS, respectively. At INR values above 2, warfarin reduced ETP below background levels. By contrast, concentrations of rivaroxaban and dabigatran required to reduce ETP to a similar extent as warfarin at an INR of 2 were 86 and 260 ng/mL, respectively; concentrations higher than those measured at trough with the currently approved dosing regimens. Whereas 50 ng/mL rivaroxaban or 100 ng/mL dabigatran on their own had little effect on leaflet induced ETP, when combined, ETP was significantly suppressed.

Conclusions: MHVs induce TG in concentrations that overwhelm dabigatran. Like dabigatran, rivaroxaban is less effective than warfarin in preventing TG in the presence of MHVs. These results suggest rivaroxaban is unlikely to be effective for patients with a MHV. Although rivaroxaban and dabigatran suppress TG induced by MHVs to a greater extent when combined than they do as monotherapy, the safety of such combinations is unknown.

PB 498 | Oral Rivaroxaban for the Treatment of Antiphospholipid Syndrome (APS)

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Background: Rivaroxaban has been proposed as an effective and safety strategy for venous thromboembolism patients that could prevent symptomatic or fatal recurrence of VTE and prevent the major bleeding. But whether it is useful in patients with antiphospholipid syndrome (APS) is uncertain.

Aims: -

Methods: The historical cohort study in Fu Wai Hospital-The National Center of Cardiovascular Disease included 20 antiphospholipid syndrome patients between March 17, 2014, and June 26, 2016. All of them were treated with Rivaroxaban (15 mg twice daily for 3 weeks, followed by 20 mg once daily). We analysed the baseline characteristics and record recurrence of thrombotic events, Chronic thromboembolic pulmonary hypertension (CTEPH), bleeding event, and all-cause death. The thrombotic events and CTEPH diagnosis were confirmed by available clinical, laboratory, and imaging findings.

Results: Of 20 APS patients, 7 (35%) male and 13 female with 42 years old (15-84) as the median of age and the eldest was 84 years old. For the first thromboembolic event, 11(55%) were pulmonary embolism (PE), 8(40%) were deep venous thrombosis, 1(5%) were coronary heart disease. The median treatment time of Rivaroxaban is 14.5 months. During the follow-up, recurrence of thrombotic events was recorded in 4 patients (20%, 3 patients recur PE at the 6, 7 and 21 month respectively during the treatment of Rivaroxaban, 1 patient occur left atrial thrombus at the 22 month). 2 (10%) patients were confirmed as CTEPH during the long term follow up. 1(5%) patient occur major bleeding (hemoptysis), 5(25%) patients occurred non-major bleeding. No death occurred.

Conclusions: Patients with antiphospholipid syndrome (APS) is likely to happened recurrence thrombotic events, in our study, with the treatment of Rivaroxaban the thrombotic events recurrence rate was 20%, with only 1(5%) major bleeding happened. A fixed-dose regimen of rivaroxaban alone for the treatment of antiphospholipid syndrome (APS) had a potentially improved benefit-risk profile.

PB 499 | Improving Appropriate Use of NOACs: Success of Online CME

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Background: Anticoagulant therapy is often not appropriately initiated in patients with venous thromboembolism (VTE), resulting in poor patient outcomes.

Aims: To determine if an online continuing medical education (CME) intervention could improve knowledge and competence of cardiologists and hematologists/oncologists (hem/oncs) regarding appropriate use of non-vitamin K oral anticoagulants (NOACs).

Methods: An online CME activity was developed as a 15-minute video-based discussion on appropriate initiation of NOAC therapy in the setting of VTE. The effects of education were assessed using a linked pre-/post-assessment study design. For all questions combined, the McNemar's chi-square test was used to assess differences from pre- to post-assessment. P values are shown as a measure of significance; P values < .05 are statistically significant. Cramer's V was used to calculate the effect size (> 0.3 are large, 0.16-0.3 are medium, and < 0.16 are small).

Results: Comparisons of individually linked pre-assessment question responses to the respective post-assessment responses demonstrated statistically significant improvements for cardiologists (N = 31; P < .05; V=0.243) and hem/oncs (N=48; P< .05; V=0.186). An average of 42% of cardiologists and 47% of hem/oncs selected the best response on pre-assessment, which rose to an average of 67% on post-assessment for both specialty groups. Statistically significant relative improvements were observed (all P< .05):

A 74% improvement among hem/oncs (31% vs 54%) and a 72% improvement among cardiologists (32% vs 55%) in knowledge of strategies to improve adherence to NOAC therapy

A 55% improvement (42% vs 65%) among hem/oncs (42% vs 65%) in recognition of dosing regimens for the different NOACs

A 103% improvement for cardiologists (32% vs 65%) in selection of effective strategies to improve adherence to NOAC therapy

Conclusions: The significant improvements observed in this intervention demonstrate the benefits of using effective online CME to educate appropriate target audiences.

PB 500 | Influence of Anthropometric, Hematological and Biochemical Parameters on Plasma Levels of Direct Oral Anticoagulants

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Background: Direct oral anticoagulants (DOACs) were developed to overcome the limitations of the previous standard therapeutic approach with vitamin K antagonists. However, the influence of anthropometric biochemical and hematological parameters on levels of DOACs was investigated only in part.

Aims: The aim of this study concerns the influence of anthropometric, biochemical and hematological parameters on the plasma level of different DOACs.

Methods: Globally, 141 patients (M:F=60:81; median age= 76.5, IQR: 69-82) were studied: 21 on dabigatran (DBT), 66 on rivaroxaban (RVX) and 54 on apixaban (APX) therapy for AF and VTE. The dose and the precise time elapsed after dosing were recorded in all cases. PT, APTT, fibrinogen, blood count, hematocrit (Htc), AST, ALT and creatinine with BMI were also measured in all patients. Plasma level of DBT, RVX and APX was measured using a liquid anti-Xa assays on a STA Compact Max® instrument (Diagnostica Stago). Statistical analysis was performed with SPSS (v. 21) software.

Results: DBT concentration did not show any significant correlation with the above hematological, biochemical and anthropometric factors in univariate analysis. RVX showed an inverse correlation with the time elapsed after dosing (ρ= -0.751, p< 0.001), which remained also in multivariable regression analysis (p< 0.001 and p=0.028, respectively). Finally, APX level was inversely associated in bivariate non-parametric analysis only with Htc and Hb levels (ρ= -0.643, p= 0.018 and ρ= -0.637, p= 0.019, respectively). In multivariable analysis only Hb was inversely correlated with APX concentration
(ρ= -0.476, p=0.029).

Conclusions: These results showed that: a) DBT, RVX and APX can be safely prescribed in adult patients of any BMI ranging from 23 to 38; b) APX shows a strong negative correlation with Hb and Htc level, so that APX concentration increases by about 20 ng/ml as a function of a Hb decrease of about 1gr/dl and vice versa. Hence, Hb level should not be ignored when APX is prescribed.

PB 501 | Non Vitamin-k Oral Anticoagulants (NOAC) Dose Reduction and Adverse Outcomes - A Single Center Experience

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Background: NOACs have become the mainstay of therapy for patients with atrial fibrillation (AF) and venous thromboembolism (VTE). However, recommended doses are frequently reduced due to safety concerns leading potentially to reduced effectiveness and safety.

Aims: We aimed to test the effect of NOAC dose administered on effectiveness and safety in a cohort of consecutive patients admitted to our center due to recurrent thrombosis or major bleeding events.

Methods: Consecutive patients treated with either dabigatran, rivaroxaban or apixaban for atrial fibrillation or venous thromboembolism presenting to the ER with major bleeding or thrombotic episode (arterial or venous) between 01/01/2016 - 31/12/2016 formulated the study group. For each patient detailed medical and drug history, drug compliance, base line blood tests and NOAC blood level were carefully assessed. Adverse thrombotic and bleeding episodes were then related to NOAC doses. Dose reduction was considered adequate if 2/3 criteria (age>75, weight < 55 kg, CCT < 50 ml/m) were present.

Results: Thirty nine patients (23 M) with a mean age of 74 ± 14 were admitted during 2016 to various hospital wards because of thrombotic or bleeding events. Of the patients admitted 20 received apixaban (7 with reduced dose), 16 patients received rivaroxaban (5 reduced dose) and 3 patients received dabigatran (1 reduced dose). All 13 dose reductions were considered unjustified. In patients receiving adequate NOAC dose 15/26 (58%) and 11/26 (42%) had a thrombosis or bleeding episode, respectively, while most of the patients with reduced dose (11/13, 85%) had a thrombotic episode and 2/13 (15%) a bleeding episode (P=0.09).

Conclusions: In patients with AF or VTE treated with NOACs unjustified dose reductions was associated with increased rate of thrombosis. Dose reduction should be carefully assessed in these patients.

PB 502 | Reversal of Rivaroxaban Using Prothromplex Total, a 4-Factor Prothrombin Complex

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Background: The absence of approved specific antidote for reversal of rivaroxaban means that life-threatening bleeding events or reversal for emergency procedures are currently managed with 4-factor prothrombin complex (4F-PCC) in our hospital. A number of commercial preparations exist with variations in contents and limited data on individual efficacy.

Aims: To describe and review the efficacy and safety of a 4-factor prothrombin complex (4F-PCC) (Prothromplex Total, Shire) in reversing rivaroxaban in a series of patients.

Methods: Requests for 4F-PCC to reverse rivaroxaban were screened by haematologists and used as triggers to identify subjects over a 2-year period. Progress and outcomes data were then prospectively collected for analysis.

TABLE 1 Patient Characteristics

Patient	#1	#2	#3	#4	#5	#6	#7	#8
Age/sex	81/Female	81/Male	82/Female	65/Male	76/Female	80/Male	66/Female	64/Female
Creatinine clearance (ml/min)	54	65	36	NA	26	53	105	33
Rivaroxaban dose (mg/day)	15	20	15	20	15	15	20	15
Indication for anticoagulation	Atrial fibrillation	Atrial fibrillation	Atrial fibrillation	Atrial fibrillation	Atrial fibrillation	Atrial flutter	Deep vein thrombosis	Atrial flutter
Indication for reversal	Right frontal intra-parenchymal haematoma	Sub-dural haematoma	Sub-arachnoid haematoma	Haemopericardium, post-coronary angiogram	Urgent percutaneous nephrostomy	Basal ganglia haematoma	Tracheostomy Bleed	Massive Hemoptysis
Dose of 4F-PCC (U/kg)	40	47	46	49	30	45	40	66
Prothrombin Time (PT) (seconds) before 4F-PCC	11.4	14.3	19.2	NA	11.7	15.2	17.6	16.9
PT after 4F-PCC	10.5	10.5	12.2	NA	NA	NA	11.9	17.1
Bleeding post infusion	No	Yes (4 days later)	No	No	No	No	No	Yes

Results: Eight patients were given Prothromplex to reverse life-threatening bleed or for urgent procedure. Seven patients were on rivaroxaban for atrial fibrillation/flutter with one for deep vein thrombosis. The indications for reversal were intracranial haemorrhage (ICH)(n=4), haemopericardium post coronary angiogram(n=1), massive hemoptysis(n=1), tracheostomy bleed (n=1) and urgent percutaneous nephrostomy(n=1). Six patients received a single dose of Prothromplex 50 iu/kg rounded to the nearest vial (range 40-49 iu/kg). The patients undergoing percutaneous nephrostomy and massive hemoptysis received 30 iu/kg and 65 iu/kg respectively. There were two mortalities from septic shock (the patient with hemoptysis) and ICH. The first death was unrelated to bleeding while the second was contributed by delayed therapy. Prothromplex effectively controlled bleeding in 6 of 8 patients. There were no thrombotic events.

Conclusions: This data supports the utility of Prothromplex in the management of the majority of patients on rivaroxaban who suffer life-threatening bleeding episodes or require urgent interventional procedures. 50 iu/kg appears efficacious and safe although higher optimal dosing has not been ascertained.

PB 503 | Similar Rivaroxaban Plasma Concentrations Induce Different Anticoagulant Effects in Individual Obese Patients

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Background: Rivaroxaban (RVX), a direct inhibitor of factor Xa, has been developed to bypass the traditional anticoagulant limitations. RVX has predictable pharmacokinetics and pharmacodynamics, thus, there is no need for laboratory monitoring. However the "one dose fits all" concept based on randomized clinical trials might not be always adequate for individual patients in clinical practice.

Aims: To asses thrombin generation (TG) and spatial clot growth (SCG) at similar plasma concentration of RVX in:

1. different obese patients before bariatric surgery.
2. individual patients before and after surgery.

Methods: Citrate blood was collected during 24 hours from obese patients receiving a single dose of RVX (10 mg) before bariatric surgery

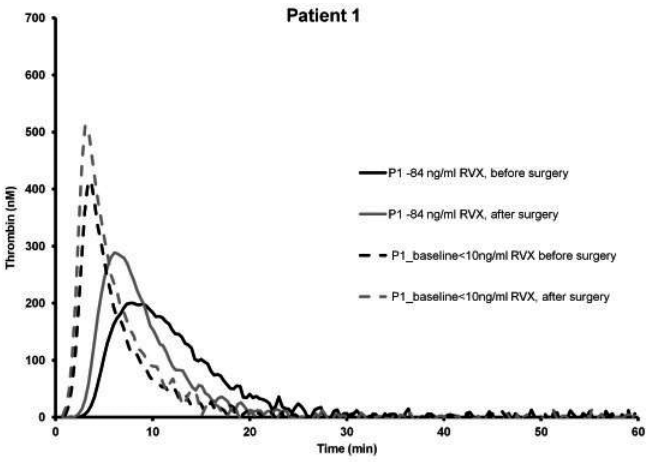

FIGURE 1 Thrombin generation profiles of Patient 1 before and after bariatric surgery

and on the third postoperative day. RVX plasma concentration was quantified with an anti-Xa chromogenic assay. RVX anticoagulant effect was assessed *ex-vivo* by monitoring tissue factor induced TG in platelet-poor-plasma (PPP) with calibrated automated thrombogram (CAT). TG and SCG were recorded with Thrombodynamics analyzer.

Results: Despite similar RVX-concentrations, patient TG and SCG profiles significantly differed. Large inter- and intra-individual variations were observed among patients (Figure 1).

At similar RVX concentrations, patients had higher thrombotic profiles after surgery. Despite RVX, spontaneous clots appeared in patient's plasma.

Conclusions: Obese patients show, in response to similar concentrations of RVX, inter-individual and intra-individual differences in TG inhibition. These differences reflect the variable anticoagulant effect in each individual. High prothrombotic tendency in individual obese patients is suggested by Thrombodynamics assay. The lower TG inhibition and increased SCG observed for the same patient after surgery might indicate that the preoperative RVX dose is less effective in providing a good prophylactic anticoagulation.

PB 504 | Reversal of Dabigatran in Emergency Situations

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Background: In 2015, the Reverse AD study, a phase III trial that evaluated the efficacy and safety of Idarucizumab as a specific agent for the reversal of Dabigatran, were published. There is still little data on the use of Idarucizumab in real life.

Aims:

Methods: We retrospectively reviewed the patients who received Idarucizumab for the reversal of Dabigatran at our center to date.

Results: Case 1: Male of 81 years old, traumatic brain injury on August 3, 2016. HTA. Sigma adenocarcinoma pT3N0 (year 2012) Hepatic recurrence in (2015). Atrial flutter CHA2DS2Vasc 3, anticoagulated with Dabigatran 110mg c / 12h, last dose of Pradaxa at 14 h before. Weight 66.8 Kg. Normal neurological examination. Tests: EGF 80.9 ml / min, Coagulation: TP 11.8 sec, TTPa 29.7 sec. Ethanol in plasma 267 mg / dl. Anti-IIa (performed on the following day using admission sample) 8.87 ng / ml (28-155ng / ml) Cranial CT scan: Haemorrhage of 5 mm in right semioval center. An urgent reversal is performed with Idarucizumab 5 g and observation 36 h with good evolution.

Case 2: Male 65 years old, Hypertension. Atrial fibrillation and non-obstructive hypertrophic cardiomyopathy (MYBPC3 mutation Y749 C). Anticoagulated with Pradaxa 150 mg / 12 hours. Work accident: section of the deep flexor tendon of the 5th finger, of the superficial flexor tendon of the 5th finger and of the ulnar collateral nerve of the 5th finger. Requires urgent surgery. Last intake of dabigatran (21:00 hours of the previous day, 14 hours before). Weight 74kg. Test: Coagulation: TP 15.1 sec, TTPa 43.70 sec (R 1.46) Anti-IIa: 91.87 ng / ml (39.8-74.3 ng / ml) FGE: 59 mL / min. Idarucizumab was administered prior to surgery that was performed without incident and restart Pradaxa at 24 h.

Conclusions: Few cases of use of Idarucizumab in real life have been published. In our experience the treatment has been proven effective and safe. The anti-IIa measurement is not available in most centers. The use of Idarucizumab can be oriented with data from the coagulation study.

PB 505 | Can we Successfully Reverse a "Massive Overdose" of Dabigatran?

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Background: Dabigatran (Dab) is used in clinical practice for stroke prevention in atrial fibrillation. Specific antidotes for these new drugs are still under development. Idarucizumab is already approved by FDA in clinical practice as Dab inhibitor. Are the recommended doses enough to reverse the anticoagulant effect and clotting assays?

Aims: Present a clinical case where the recommended doses for Idarucizumab weren't enough for reversing the anticoagulant effect, and question if the results of RELY- ABLE and RE-VERSE AD trials cover overdoses of Dabigatran such as > 1000 ng/mL.

Methods: Clinical Case

Results: A 76 y/o female with clinical history of Pott's disease, hypertension, diverticulosis, iron deficiency *anemia*, atrial fibrillation (AF) medicated with Dab (110mg), was admitted at emergency care with vomits, nausea, melena, diffuse abdominal pain and no other relevant symptoms. Hgb-12.1g/dL, Leu-19x10^9/L (Neu-17x10^9), Plt-307x10^9/L, RPC-0.8mg/dL, aPTT>120", INR-8, eGFR-CKD-EPI=28 and normal liver function. Later she developed hypotension with renal function deterioration and active digestive bleeding, evolving to hypovolemic shock. Clotting assays revealed an aPTT and Thrombin Time > 120" and a diluted Thrombin Time (dTT) of 3374ng/mL. Clinical deterioration provoked her death on the 4th day. Although Idarucizumab administration, Dab levels were persistently high (1219ng/mL). The autopsy revealed that hypovolemic shock by a massive gastro-intestinal bleeding was the cause of death.

During hospitalization, besides Dab's withdraw, the therapy given was 4U PCC, 2 FFP and a pool of platelets, 5g Fbg, 2U rFVIIa and 2x5g Idarucizumab.

Conclusions: Two doses of Idarucizumab were not efficient on clinical outcome and did not reverse the clotting assays. Are we far from the ideal antidote? Is Idarucizumab able to reverse Dab's effect at a 1:1 stoichiometric ratio as known? Which Dab levels were assayed on trials? For ethical reasons managing massive overdose is limited on trials.

PB 506 | Routine Coagulation Tests for Assess Anticoagulant Activity of Direct Oral Anticoagulants

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Background: Direct oral anticoagulants (DOACs) do not require routine monitoring, however, in certain clinical situations assessment of anticoagulant activity may be important. Assays for quantification of DOACs are not available in most hospitals. It is important to evaluate locally if routine coagulation tests, prothrombin time (PT) or activated partial thromboplastin time (aPTT) can be used to assess DOAC anticoagulation.
Aims: Assess the sensitivity of PT and aPTT to different DOACs, correlating the results with quantitative assays: ecarin chromogenic assay (ECA) for dabigatran and specific anti-Xa for apixaban and rivaroxaban.
Methods: Blood samples were collected from 72 patients under dabigatran, 25 under apixaban and 39 under rivaroxaban, admitted to our hospital.
For each sample a quantitative assay (ECA or specific anti-Xa), PT and aPTT were performed.
Results for each qualitative assay were correlated with plasma drug levels and the ‚misprediction percentage' (MP), that represents how often the coagulation test values were in the normal reference range while the drug concentration was in or above the therapeutic range, was calculated.
Results: We found the strongest correlation and the lowest MP with Dabigatran (aPTT: R^2=0,615, MP=6,0%; PT: R^2=0,519, MP=23,9%).
At higher levels of Dabigatran (ECA>260ng/mL), all samples showed aPTT and PT values above reference ranges. Under 260ng/mL, PT values were very variable but aPTT remained above reference range as of 57 patients with ECA>50ng/mL, only 1 presented normal aPTT.
Correlation between routine coagulation tests and Apixaban were not as strong as with Dabigatran (aPTT: R^2=0,382, MP=36,4%; PT: R^2=0,470, MP=40,9%).
The weakest correlations and highest MPs were found in Rivaroxaban samples (aPTT: R^2=0,205, MP=71,9%; PT: R^2=0,375, MP=65,6%).
Conclusions: Routine coagulation tests not always show the same ability to quantify drug effect as described in literature. These assays are generally insufficient for measuring Xa inhibitors activity.

PB 507 | Direct Oral Anticoagulants in the Treatment of Deep Vein Thrombosis: The Experience of a Hemostasis Unit

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Background: Direct Oral Anticoagulants (DOACs) are novel therapeutic agents for DVT. According to their clinical trials they are superior to AVK anticoagulants concerning safety and bleeding risk.
Aims: The objective is to present our clinical experience in the treatment of DVT with DOACs, regarding effectiveness, side effects, bleeding risk and post-thrombotic syndrome.
Methods: For a period of 47 months (2012-2016), 102 (50 male, 52 female) patients with median age 47 years (17-78), with a VTE diagnosis and indication for *indefinitetreatment* were assessed. They were treated with DOACs (rivaroxaban, dabigatran, apixaban) for a median treatment duration of 17 months (7-47). The study included patients who were anticoagulant naive and patients who had previously used a coumadin anticoagulant. Effectiveness and side effects of the treatment were assessed. Additionally, major and minor bleeding and the course of the post-thrombotic syndrome were evaluated using the 2015 ISTH bleeding definition recommendations and the Villalta score respectively.
Results: One patient presented acute coronary syndrome (0,98%). No patient experienced a recurrent VTE episode, 6 patients had minor bleeding (5,88%) and 2 developed major bleeding without fatal outcome. One patient complained of pruritus (0,98%) and one of headaches (0,98%), symptoms which were attributed to DOACs. The treatment had no effect in the vast majority of the patients (97,1%) regarding the post thrombotic syndrome. 5 patients had an aggravation of the post thrombotic syndrome (4,9%), of whom 4 had their treatment discontinued.
Conclusions: DOACs are the drug of choice for the treatment of DVT. The clinical experience of the Hemostasis Unit is consistent with the results of the clinical trials regarding their effectiveness and safety. Nevertheless, long-term clinical experience is required in order to establish these conclusions.

PB 508 | Reversal of Dabigatran with Idarucizumab in Five Patients Admitted to the Emergency Department of Centro Hospitalar São João, Porto

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Background: Urgent reversal of direct oral anticoagulants (DOACs) is important when facing severe bleeding or emergency surgery. Idarucizumab, the specific reversal agent of dabigatran, is the only antidote for DOACs approved and available so far.
Aims: Presentation of 5 patients under dabigatran who needed urgent reversal with idarucizumab. Evaluation of anticoagulation parameters.
Methods: Five patients under dabigatran were admitted to emergency department. Three patients with severe bleeding events: 2 with intracraneal hemorrhages (ICH) after trauma, 1 with gastrointestinal bleeding (GIH) associated to angiodysplasia, anemia. Two patients needed urgent invasive procedures:
1 with sepsis needing an urological procedure, 1 with a severe compartment syndrome secondary to infection in the right arm. At admission all patients had prolonged activated partial thromboplastin time (aPTT) (>39 sec; N: 24,2-36,4 sec) and dabigatran concentrations within or above therapeutic range (>80 ng/ml), determined by ecarin chromogenic assay (ECA). Idarucizumab was administered: 2.5 g infusion, followed by 2.5 g 10 minutes later. ECA and aPTT were performed after the first and second vial.
Results: In blood samples collected 5 min after the first vial of idarucizumab, dabigatran concentration was not detectable (< 15 ng/mL

- detection limit of ECA) and aPTT was normal in all patients. Results were similar after the second vial. Patients with ICH completely recovered; dabigatran was restarted 2 weeks later. The patient with GIH did not need any further blood transfusion; anticoagulation was not restarted. Hemostasis was normal in the 2 patients submitted to invasive procedures; one patient died within 24 h due to her clinical condition.

Conclusions: Idarucizumab successfully reversed dabigatran effect in 5 patients. Our findings suggest the possibility of using a lower dose (2,5 g) of idarucizumab in selected patients (normal renal function and therapeutic concentrations of dabigatran) leading to a more cost-effective therapy.

PB 509 | Do All Patients Respond to Treatment with Direct Oral Anticoagulants?

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Background: Use of direct oral anticoagulants (DOACs) is increasing due to a wide therapeutic window, no requirements for laboratory monitoring and stable anticoagulation. Other advantages are little drug and dietary interaction, low bleeding rate and good compliance. However, variation of pharmacokinetic profiles, safety and efficacy still are important issues to be explored.

Aims: To present clinical cases, where a new thrombosis was diagnosed during the treatment with DOACs.

Methods: Review of medical records.

Results: Seven patients (4 women and 3 men, age 61-82 years, body mass index 25,3-39,9 kg/m^2) from our Patient Clinic of Department of Cardiology were included. Inclusion criteria: New thrombosis during treatment with DOACs and good compliance regarding intake of medicine. The patients were treated with standard dose of Rivaroxaban (5) and Apixaban (1). One patient initially was treated with Rivaroxaban and later with Apixaban. Their primary diagnosis was pulmonary embolism (PE) (5) or combination of deep vein thrombosis (DVT) and PE (2). Unprovoked thromboembolic event (TE) was seen in 2 patients and provoked in 5. Risk factors were: Surgery/immobilization, infection, atrial fibrillation and congenital thrombophilia. Thrombophilia testing was performed in 2 patients. Testing results were normal in one patient, whilst other patient had a combination of a heterozygous factor II and V gen mutation. All patients had normal liver function. 4 patients had normal (> 60 ml/min) estimated glomerular filtration rate (eGFR) , 2 patients had eGFR > 90 ml/min and one 58-60 ml/min. All patients were treated with DOAC as recommended at the time they were diagnosed with a new TE. No patients were treated with the drugs, which potentially could interact with DOACs.

Conclusions: Little is known about treatment failure of DOACs. Further clinical studies of different population, i.e obese, multimorbid patients and new possible mechanisms influencing action of DOACs could shed more light on DOAC's efficacy.

PB 510 | Measurement of Direct Factor Inhibitors Concentration in Practice Laboratory

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Background: Direct coagulation factors inhibitors or new oral anticoagulants are include in modern recommendations and widely use in prevention and treatment of thromboembolic events in fixed doses. But the problem of objective assessment of individual action by laboratory methods in clinical practice is not solved.

Aims: To validate the dabigatran (Dabi) and rivaroxaban (Riva) concentration measurement on the base of diluted thrombin time and ati-Xa activity in practice laboratory.

Methods: 50 patients with non valvular atrial fibrillation - 10 patients received dabigatran (68 [60; 74] years old, in dose 150 mg bid and 40 patients received rivarixaban 20 mg od, 72 [65; 76] years old. Quik test (PT) and aPTT screening tests (STA Compact, Stago reagents) as well as diluted thrombin time (Hemoclot Thrombin Inhibitors) and anti-Xa activity (Biophen DiXaI), both HYPHEN BioMed, using Sysmex CS-2000i automated analyzer were measured 3 times per day - final concentration (FC, before 1-st dose), peak concentration (PC, 4 hours after drug intake) and through concentration (TC, 8 hours after intake).

Results: Prolongation of aPTT in Dabi was max at PC and TC points (x1,3-1,7 control values) on the background of considerably different concentrations from 8,5 to 165,0 ng/ml (min in FC, max in PC points). In Riva group aPTT was unexpectedly prolonged in PC point (x1,4), Quick test in PC point - 53,6±21,7%. Riva concentration in FC, PC and TC - 67,9±50,6 ng/ml, 235,7±101,3 ng/ml and 182,6±108,1 ng/ml, respectively. There were not major hemorrhages, but 10% minor bleedings in Dabi and 15% - in Riva group without correlation with PC or TC, probably because of small number of observation.

Conclusions: Anti-Xa activity and Hemoclot Thrombin Inhibitors measurement by HYPHEN BioMed Kits is simple and repeatable laboratory assay and may be validated in routine laboratory for emergency situations in patients, receiving direct factors inhibitors.

PB 1000 | The Prevalence of Concomitant Deep Vein Thrombosis, Symptomatic or Asymptomatic, in the Patients with Symptomatic Pulmonary Embolism

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Background: The reported prevalence of concomitant deep vein thrombosis (DVT) in patients with pulmonary embolism (PE) is very

variable and the utility of routine venous compression ultrasound (CUS) is controversial.

Aims: To evaluate the prevalence of DVT in patients with symptomatic PE and to identify factors associated with the presence of DVT and with the symptomatic/asymptomatic character of DVT.

Methods: We conducted a single center retrospective study of prospectively collected data, including consecutive adult patients with objectively confirmed PE. The patients underwent clinical examination, complete CUS of both legs and were asked about medical history and risk factors for venous thromboembolism (VTE). For statistical analysis we used the t test and the chi-squared test.

Results: Of 428 patients with symptomatic PE (mean age 59±16.4 years; 52.3% men), DVT was found in 302 cases (70.6%), and proximal DVT in 202 (49.5%). Men had concomitant DVT significantly more often than women (79.5% versus 60.8%, P=0.02). DVT was asymptomatic in 173 patients (sensitivity of DVT symptoms 42.7%, specifity 93.7%). Patients with symptomatic DVT (compared to asymptomatic) were significantly younger (54.2 vs. 64.4 years, P< 0.0001) and had proximal location more frequently (78.3% vs. 64.2%, P=0.01). None of further considered factors (obesity, history of previous VTE, smoking, various risk factors) was associated with the presence/absence of symptomatic/asymptomatic DVT.

Conclusions: In patients with PE, the prevalence of concomitant DVT is quite high but clinical diagnosis is unreliable. In our group, the prevalence of concomitant DVT was significantly higher in men. Symptoms of DVT were more often present in younger patients or if thrombosis was proximal. Venous CUS may be helpful in PE diagnostic process, in risk stratification of PE or, potentially, for timely prevention of post-thrombotic syndrome. Criteria for proper ordering CUS in PE patients deserve further studies.

PB 1001 | Predicting Post-thrombotic Syndrome with Ultrasonographic Measurements after Proximal Deep Vein Thrombosis

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Background: Post-Thrombotic syndrome (PTS) is a common and potential severe complication of deep venous thrombosis (DVT). Elastic compression stocking therapy may prevent PTS if worn on a daily basis, but stockings are cumbersome to apply and uncomfortable to wear. Hence, identification of predictors of PTS may help physicians to target PTS prevention in those patients with a high risk of PTS.

Aims: To identify ultrasonographic (US) parameters assessed during or after treatment of DVT of the leg, that predict post thrombotic syndrome.

Methods: Systematic review and meta-analysis. Databases were searched for prospective studies including consecutive patients with

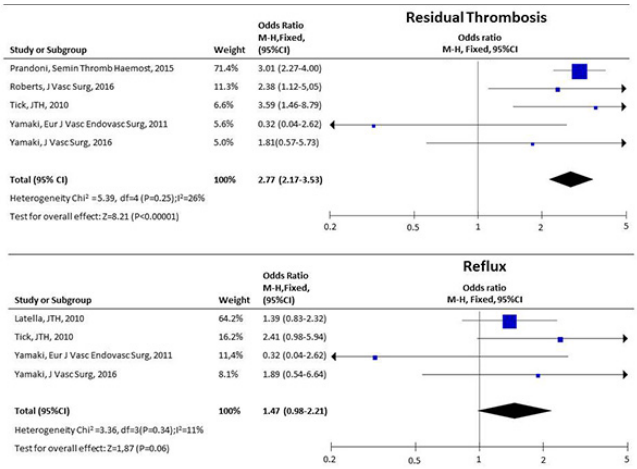

FIGURE 1 Forrest plot

DVT who received standardized treatment, had an ultrasonography during follow-up assessing findings consistent with vascular damage after DVT, and had a follow-up period of at least 6 months for the occurrence of PTS assessed by a standardized protocol.

Results: The literature search revealed 1156 studies of which 1068 were irrelevant after title and abstract screening by 2 independent reviewers. After full text screening of 88 studies, 6 relevant studies were included, with a total of 1550 analysed patients. All studies used different time points for US measurement with a range of 6 weeks to 6 months after DVT. The Villalta score or CEAP score were used for diagnosing PTS at 11 months to 6 years after DVT. Two US parameters proved to be predictive of PTS: reflux (retrograde flow >1 second after compression), for a pooled odds ratio (OR) of 1.5 (95%CI 0.98-2.2; figure 1); positive likelihood ratio (LR=+) of 1.7 (1.3-2.2); negative likelihood ratio (LR-) of 0.8 (0.7-0.9), and residual vein thrombosis, for a pooled OR of 2.8 (95%CI 2.2-3.5); LR+ of 1.8 (95%CI 1.6-2.1); LR- of 0.6 (0.5-0.7).

Conclusions: The US features reflux and residual thrombosis measured at least 6 weeks after DVT predict post-thrombotic syndrome and may be helpful in identifying patients with high risk that could benefit from compression therapy.

PB 1002 | Can Ultrasonographic and Laboratory Measurements One Year after Proximal Deep Vein Thrombosis Predict Post-thrombotic Syndrome?

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Background: Elastic compression stockings (ECS) are constricting, itchy and difficult to apply but may prevent post-thrombotic syndrome

TABLE 1.]

Ultrasound and laboratory features	Continue ECS group OR (95% CI), p value	Stop ECS group OR (95% CI), p value	Whole group OR (95% CI), p value
Reflux	1.1 (0.5-2.5), 0.8	1.1 (0.6-2.1), 0.9	1.1 (0.7-1.9), 0.7
Residual thrombosis	1.0 (0.5-2.2), 0.9	1.2 (0.6-2.1), 0.7	1.1 (0.7-1.8), 0.6
Thrombus score>1	1.1 (0.5-2.3), 0.8	1.1 (0.6-2.1), 0.8	1.2 (0.7-1.8), 0.5
D-dimer>0.5 mg/L	1.0 (0.3-3.3), 1.0	1.0 (0.4-2.6), 0.9	1.1 (0.5-2.2), 0.9
High sensitive CRP>3 mg/L	1.6 (0.6-3.8), 1.0	1.7 (0.8-3.8), 0.2	1.6 (0.9-2.7), 0.1
Fibrinogen>4 g/L	4.4 (1.1-18), 0.04	0.9 (0.2-3.4), 0.8	1.9 (0.7-4.8), 1.3

(PTS), a potential severe complication of deep venous thrombosis (DVT). The ability to predict PTS may help clinical decision making with regard to the optimal duration to wear ECS after DVT.

Aims: To assess the predictive value of ultrasonography and laboratory parameters for PTS development in the Octavia study, which was a randomized controlled trial that randomized patients who were compliant to ECS after DVT and had not developed PTS after 1 year, to continue or discontinue ECS treatment [G.C. Mol et al, BMJ 2016].

Methods: At baseline (12 months after acute DVT), ultrasonography was performed in all 518 study patients to assess reflux (retrograde flow > 1 second after compression), the presence of residual thrombosis and the thrombus score (amount of vein segments with a residual thrombus). D-dimer, fibrinogen and high-sensitive CRP levels were determined post-hoc in 275 patients.

Results: 51 of 256 patients randomized to the stop-ECS group and 34 of 262 patients in the continue-ECS group developed PTS during follow-up. Reflux, residual thrombosis, and the thrombus score did not predict PTS in univariate analysis, nor did any of the markers except for fibrinogen in the continue-ECS group, for an OR of 4.4 (95%CI 1.1-18) with a threshold of >4.0 g/L.

Conclusions: In our cohort, US features and laboratory tests were not predictive of PTS, making it unlikely that these parameters are helpful in making management decisions.

PB 1003 | Patient-reported Reasons for and Predictors of Non-compliance with Elastic Compression Stockings in the SOX Trial

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Background: Elastic compression stockings (ECS) are used to prevent and treat venous insufficiency. However, lack of compliance might be a limitation to achieving optimal effectiveness.

Aims: To describe patient-reported reasons for non-compliance with ECS and identify predictors of non-compliance in the SOX Trial population.

Methods: The multicenter randomized controlled SOX Trial compared active ECS (A-ECS) vs. placebo-ECS (P-ECS) to prevent post-thrombotic syndrome (PTS) after a first, symptomatic DVT (*Lancet* 2014). At each follow-up visit (1, 6, 12, 18 and 24 months), patients were asked how many days per week they wore study stockings and if not worn daily, to specify the reason. Compliance was defined as wearing ECS for 3 or more days per week. Potential predictors of ECS non-compliance were assessed in univariate and multivariate analyses.

Results: Of 776 study patients who had at least 1 follow-up visit, 61% were male, mean age was 55, mean BMI was 29. The reasons specified for non-daily use of study stockings at each visit fell into 3 main categories: 1) aversive aspects of ECS (uncomfortable, irritating, too hot, makes things worse, itchy, hard to put on, unaesthetic, don't help) in ~2/3 of patients, 2) patient behavior-related (too lazy, forgot, need a break) in ~1/3 of patients, and ‚other reason' in a minority of patients (see Figure). In multivariate analyses, predictors of ECS non-compliance were female sex (OR 1.4 [95% CI 1.1,1.9]) and smoker (OR 1.5 [1.05, 2.2]). Age, level of education, BMI, anatomical extent of DVT, Villalta score at baseline, and allocation to A-ECS vs. P-ECS did not predict non-compliance.

Conclusions: The most frequent reasons for non-daily use of ECS were aversive aspects of ECS. Females and smokers were significantly more likely to be non-compliant with ECS. Our results provide insight into why patients do not wear ECS, and suggest that improving the

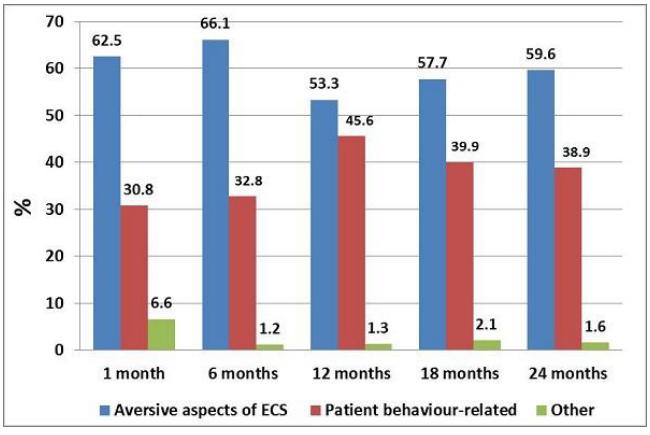

FIGURE 1 Reasons for non-daily use of ECS at each study visit

appeal and tolerability of ECS may have more impact than interventions to change patient behavior.

PB 1004 | External Validation of the Patient Reported Form of the Villalta Scale

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Background: Post-thrombotic syndrome (PTS) is a long-term complication of deep vein thrombosis (DVT). The Villalta scale is the recommended tool for diagnosing PTS, but requires a clinician's assessment of clinical signs in addition to patient self-assessment of symptoms. In a previous study, we developed and validated a self-administered tool for patient reporting of symptoms and clinical signs of the Villalta scale (PRV) (Utne et al Thromb Haemost, 115 (2), 361-7).

Aims: To perform an external validation of PTS diagnosed by the PRV scale.

Methods: The validity of PRV was assessed in 175 patients (57% male) diagnosed with DVT between 2010 and 2014. Median time from DVT to inclusion was 24 months (interquartile range (IQR) 23-27). Patients were requested to complete the PRV form before a scheduled visit. PTS diagnosed by the original Villalta scale during the visit served as the reference method. PTS was diagnosed if Villalta score was >4. Regional etics committee approved the study.

Results: PTS was diagnosed in 73 (42%) patients according to the original Villalta scale and in 81 (46%) by PRV. There was a very good agreement between the 2 measurements (kappa 0.82, 95% CI 0.73-0.90). The sensitivity of PRV to detect PTS was 95% and the specificity was 88%.

TABLE 1 Agreement between original Villalta and Patient Reported Villalta scale

	Original Villalta scale	Patient Reporte Villalta Scale	Kappa (CI 95%)
Post-thrombotic syndrome	73 (42)	81 (46)	0.82 (0.73-0.90)
No post-thrombotic syndrome	102 (58)	94 (54)	

Conclusions: This study confirms that patient Reported Villalta Scale is a valid and sensitive tool for diagnosing PTS. The tool can be particularly useful in clinical research, making studies on PTS less resource demanding by reducing the need for in-person clinic visits.

PB 1005 | High Accuracy and Reproducibility of CT Right-to-left Ventricular Diameter Measurement by Non-radiologists in Patients with Acute Pulmonary Embolism

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Background: Right ventricular (RV) dysfunction caused by acute pulmonary embolism (PE) is associated with poor short and long-term prognosis, i.e. higher risk of PE-related mortality and chronic thromboembolic pulmonary hypertension. RV dysfunction can be easily assessed by calculating the right-to-left ventricle diameter (RV/LV) ratio on standard computed tomography pulmonary angiography (CTPA) images (*Figure 1*). It is unknown whether dedicated training is required to adequately measure RV/LV ratio.

Aims: To assess the accuracy and reproducibility of CT RV/LV ratio measurement by non-radiologist clinicians without dedicated training and expertise in CT reading.

Methods: CTPA images of 100 patients with PE were reviewed by three independent non-radiologist clinicians and one experienced thoracic radiologist, after 1-time clear instructions by the latter. The maximum diameters were evaluated in the axial view by measuring the distance between the ventricular endocardium and the interventricular septum, perpendicular to the long axis of the heart. RV dysfunction was defined as a ratio of ≥1. Interobserver accuracy and

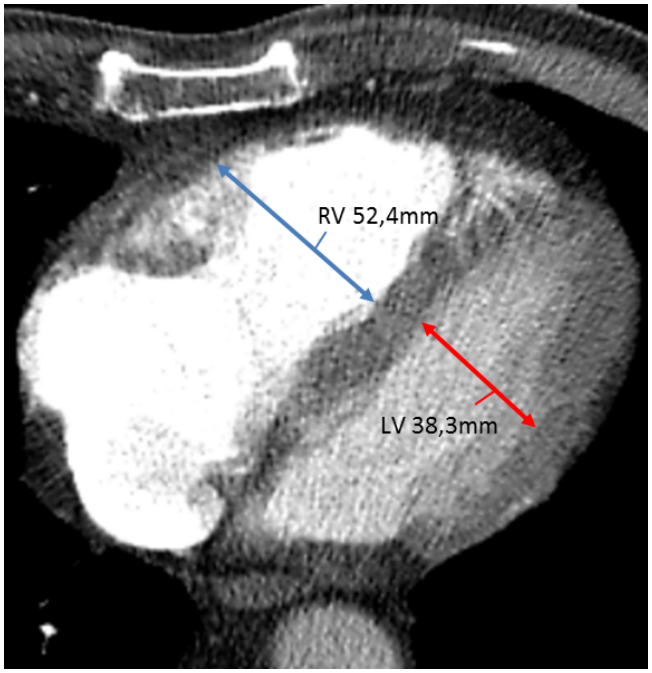

FIGURE 1 CTPA demonstrating the RV/LV ratio measurement in this patient was 1.4

reproducibility was determined using Kappa statistics, Bland-Altman analysis and Spearman´s rank correlation.

Results: The average interobserver difference in calculated RV/LV ratio's (±SD) between the three non-radiologist clinicians was very low: -0.01 (±0.11), 0.07 (±0.14) and 0.06 (±0.18) respectively, with an overall mean RV/LV ratio of 1.04. In line with this finding, Spearman´s rank correlation coefficient was 0.92, 0.88 and 0.85 indicating very good correlation (p< 0.01 for all). Furthermore, there was good agreement on the presence of a RV/LV ratio ≥1.0 compared to the ruling of the experienced radiologist, with kappa statistics ranging from 0.83-0.94.

Conclusions: After simple instruction, RV/LV ratio assessment on CTPA images by non-radiologist clinicians is accurate and reproducible, which may prove convenient in clinical practice.

PB 1006 | Nationwide Study on the Management and Outcomes of Patients with Superficial Vein Thrombosis under Real-life Conditions (INSIGHTS-SVT)

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Background: In Germany, representative and current data on the care situation and the outcomes of patients with acute superficial thrombosis (SVT) are missing.

Aims: The Investigating SIGnificant Health TrendS in the management of Superficial Vein Thrombosis study (INSIGHTS-SVT, registered at ClinTrials.gov NCT02699151) aims at closing this gap.

Methods: The study is observational, and physicians determine diagnostic procedures, treatment and the visit/contact schedule of their patients. Documentation about patient characteristics, diagnostics, comorbidities, and medical and non-medical treatment is collected at baseline, at approximately 10 days or approximately 45 days (depending on treatment), at 3 months and at 12 months. Patients are requested to fill in quality of life questionnaires (VEINES-QoI/Sym, EQ-5D-5L) at baseline and at 3 months, and report pain at every visit. The primary effectiveness outcome is the incidence of VTE at 3 months, the primary safety outcome is the combined incidence of major and clinically relevant bleeding events at 3 months. As quality measures, plausibility checks at data entry, queries based on statistical analyses, monitoring visits and adjudication procedures are applied.

Results: Results based on 709 patients are shown in the Table.

TABLE 1 Baseline characteristics (n= 709 patients)

Age, years	60.1 ± 14.7
Females, %	62.2
SVT in history, %	30.8
DVT/pulmonary embolism in history, %	15.1 / 2.8
Varicose veins, %	76.6
Known thrombophilia, %	5.4
Active cancer, %	2.3
SVT length of thrombus, cm	13.6
SVT distance form the saphenous femoral junction, cm	24.8
SVT in varicose veins, %	33.2

Conclusions: This large study provides a comprehensive picture on patients with SVT under clinical practice conditions in Germany.

PB 1007 | Post-Thrombotic Syndrome in Patients Treated with Rivaroxaban or Warfarin for Venous Thromboembolism

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Background: Post-thrombotic syndrome (PTS) is a common complication of deep vein thrombosis (DVT)±pulmonary embolism (PE).

Aims: To assess the relative hazard of PTS in venous thromboembolism (VTE) patients treated with rivaroxaban or warfarin in routine practice.

Methods: Using US MarketScan claims from 1/2012-6/2015, we identified adults with a primary diagnosis code for VTE during a hospitalization/emergency department visit (index event) and newly-started on rivaroxaban or warfarin. Patients lacking at least 6-months of continuous medical and prescription benefits prior to (baseline period) and at least 4-months after the index VTE or with a claim for an anticoagulant at baseline were excluded. PTS was defined as a code for ≥1 extremity venous study along with code(s) for both pain and swelling of the limb within 7-days of each other; lower extremity varicose veins; post-phlebitic syndrome; or other disorder of the circulatory system ≥3-months after the index event. Differences in baseline characteristics between rivaroxaban and warfarin users were adjusted for using inverse probability of treatment weights based on propensity scores. Cox regression was performed and reported as hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: In total, 10,463 rivaroxaban and 26,494 warfarin users were followed for a mean of 16±9 months. Duration of anticoagulation was similar between cohorts (mean=7-months). Rivaroxaban was associated with a significant 23% reduced hazard of PTS vs. warfarin (adjusted incidence rate difference=1.04 events/100 person-years). Reductions in PTS with rivaroxaban were consistent across subgroups (Figure 1) and when a ≥6-month post-index event gap was required before PTS diagnosis (HR=0.76, 95%CI=0.69, 0.85). (Figure 1) and when a ≥6-month post-index event gap was required before PTS diagnosis (HR=0.76, 95%CI=0.69, 0.85).

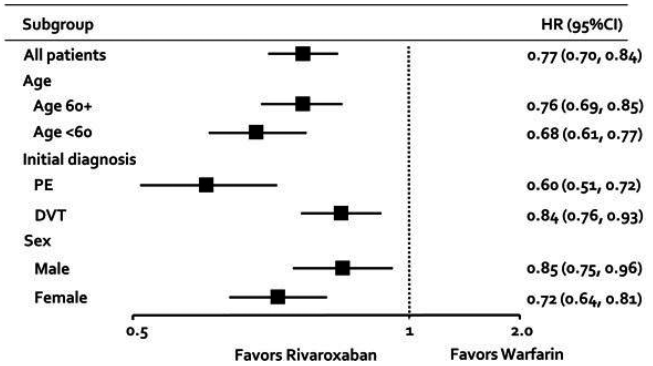

FIGURE 1 Development of Post-Thrombotic Syndrome in Patients Treated with Rivaroxaban or Warfarin for Venous Thromboembolism

Conclusions: We found rivaroxaban to be associated with less PTS vs. warfarin in VTE patients treated in routine clinical settings. Our results are consistent with those from the post-hoc PTS analysis of EINSTEIN-DVT trial.

PB 1009 | Possible Predictors of Long-term Dyspnea in Patients with Pulmonary Embolism - A Cross Sectional Study

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Background: In recent studies on long-term complications after pulmonary embolism (PE), up to 50% of patients still complain of dyspnea, have physical deconditioning, and/or impaired health-related quality of life (HRQoL). The etiology of these findings is still not fully established.
Aims: To evaluate the main differences between patients reporting dyspnea versus those without dyspnea, with regard to the severity of PE, cardiopulmonary comorbidities, employment status, BMI, smoking, exercise intolerance and HRQoL.
Methods: 203 patients diagnosed with PE at Ostfold hospital, Norway, were identified from hospital registries. Eligible patients were scheduled for a study visit, including a functional capacity test (6-minute walking test, 6MWT). HRQoL was assessed using both generic and disease-specific questionnaires (EQ-5D-3L and PE-mb-QoL). The severity of PE was assessed with both the proximal extension of the emboli (Fredrikstad score) and pulmonary embolism severity index (PESI) score.
Results: 96 (47%) patients of whom 52% were males reported dyspnea. Median time from diagnosis was 3.6 years (IQR 1.9-6.5). The severity of PE measured by the proximal extension of the clot (2.89 vs 2.98; p=0.57) and PESI score (70 vs 75; p=0.12) at index was not different between the groups. Patients with dyspnea had significantly more comorbidities (16% vs 4%, OR 4.8, 95%CI 1.5-14.9) and higher BMI (29.5 vs 27.7; p< 0.05). Unemployment was significantly higher in the dyspnea group as well (33% vs 17%; p< 0.05). Smoking history did however not differ between the groups (45% vs 42%, OR 1.1, 95%CI 0.6-1.9). As expected patients without dyspnea performed better on 6MWT (488m vs 413m; p< 0.005) and had better both generic and disease-specific HRQoL results (p< 0.005).
Conclusions: In our cohort of PE patients, persistent dyspnea was associated with poorer exercise capacity and HRQoL. Moreover, patients with persistent dyspnea were more frequently unemployed. We did not identify PE specific variables predictive of persistent dyspnea.

PB 1010 | Reliability Testing of the CAPTSureTM Pediatric Post-thrombotic Syndrome Tool

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Background: CAPTSure^{TM} is a new tool for diagnosis and severity rating of pediatric post-thrombotic syndrome, developed using an expert panel consensus method.
Aims: To test the inter- and intra-rater reliability of the clinician-reported (CR) and patient/proxy-reported (PR) elements of CAPTSure^{TM}, respectively.
Methods: Patients aged 0-18 yrs who sustained upper or lower extremity deep vein thrombosis (UE/LE DVT) were enrolled. All patients were assessed at least 6 months post-DVT by two raters to determine the reliability of the CR component of CAPTSure^{TM}. Patients/proxies completed an online version of CAPTSure^{TM} (symptoms questionnaire) at baseline and 2 weeks after. Ethics approval and participant consent were obtained.
Results: 47 of the planned 100 patients have been enrolled since November 2016. Median age at the time of study participation was 9 yrs (25th-75th percentile 4-13 yrs); 23 patients were males; 63% had sustained LE-DVT.
CR component: There was an 89% agreement for classification of collaterals; ulcers were not observed. The intraclass correlation coefficients (ICC) were 0.79 (95% CI 0.55-0.90), 0.49 (-0.10-0.76), and 0.86 (95% CI 0.65-0.95) for absolute thigh, calf, and arm circumference difference, respectively. The overall ICC for CR-CAPTSure^{TM} scores was 0.89 (95% CI 0.80-0.93).
PR component: Thus far, 24 participants completed their symptoms questionnaire a second time. The agreement for items of the PR component was as follows: skin redness and combined heaviness/tiredness/tightness 100%; heaviness 96%; paresthesia and endurance 92% and swollen limb 88%.
The overall agreement for pain was 89% (children 4-9 y.o: 88% agreement; children 10 y.o. and older: 91% agreement).
Conclusions: Preliminary results show good reliability of CR-CAPTSure^{TM} scores. Further testing is required in PR scores, since the low variability in the population thus far precluded estimation

of the ICC for PR-CAPTSure™ scores. Given our current recruitment rate, the study is expected to be completed in the next 4 months.

PB 1011 | Measuring Limb Edema in Children at Risk of Post Thrombotic Syndrome: Do I See what You Feel?

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Background: Limb edema is the most common finding of post-thrombotic syndrome (PTS). Pediatric tools for PTS diagnosis measure both patient/proxy-reported and clinician-assessed limb edema. How closely related these approaches are remains unknown.

Aims: To evaluate the correlation between patient- and proxy-reported and clinician-assessed limb edema, and the correlation between different techniques that estimate limb edema in children at risk of PTS.

Methods: Limb circumference difference, limb volume ratio, bioimpedance spectroscopy (BIS, which measures extracellular fluid content), durometry (which measures skin resistance to indentation) and self- or proxy-reported swelling were measured in 140 children at risk of PTS [n=70 with upper extremity (UE) deep vein thrombosis (DVT),

TABLE 1 Correlations between items assessing upper extremity limb edema in children with deep vein thrombosis

	Swelling patient-reported n=27	Swelling proxy-reported n=43	Circ. difference n=70	BIS ratio n=70	Limb volume ratio n=70	Durometry n=11
Swelling patient-reported n=27	-	-	0.11 (-0.02 - 0.26; p=0.57)	0.25 (0.17 - 0.50; p=0.19)	0.05 (-0.10 - 0.21; p=0.80)	-0.54 (-0.88 - -0.49; p=0.13)
Swelling proxy-reported n=43	-	-	0.36 (0.14 - 0.53; p=0.02)	0.21 (-0.03 - 0.46; p= 0.18)	0.42 (0.26 - 0.56; p=0.005)	-
Circ. difference n=70	0.11 (-0.02 - 0.26; p=0.57)	0.36 (0.14 - 0.53; p=0.02)	-	0.48 (0.27 - 0.64; p<0.001)	0.57 (0.38 - 0.71; p<0.001)	-0.004 (-0.77 - 0.67; p=0.99)
BIS ratio n=70	0.25 (0.17 - 0.50; p=0.19)	0.21 (-0.03 - 0.46; p= 0.18)	0.48 (0.27 - 0.64; p<0.001)	-	0.25 (0.01 - 0.45; p=0.04)	-0.27 (-0.74 - 0.81; p=0.42)
Limb volume ratio n=70	0.05 (-0.10 - 0.21; p=0.80)	0.42 (0.26 - 0.56; p=0.005)	0.57 (0.38 - 0.71; p<0.001)	0.25 (0.01 - 0.45; p=0.04)	-	-0.09 (-0.8 - 0.67; p=0.79)

TABLE 2 Correlations between items assessing lower extremity limb edema in children with deep vein thrombosis

	Swelling, patient-reported n=26	Swelling, proxy-reported n=44	Circ. difference thigh n=70	Circ. difference calf n=70	BIS ratio n=70	Limb volume ratio n=70	Durometry n=11
Swelling, patient-reported n=26	-	-	0.10 (-0.28 - 0.54; p=0.61)	-0.09 (-0.45 - 0.23; p=0.68)	0.42 (0.17 - 0.66; p=0.03)	0.01 (-0.33 - 0.41; p=0.94)	0.59 (0.22 - 0.87; p=0.09)
Swelling, proxy-reported n=44	-	-	0.49 (0.27 - 0.65; p<0.001)	0.05 (-0.16 - 0.26; p=0.74)	0.15 (-0.14 - 0.34; p=0.34)	0.40 (0.24 - 0.59; p=0.006)	-
Circ. difference thigh n=70	0.10 (-0.28 - 0.54; p=0.61)	0.49 (0.27 - 0.65; p=<0.001)	-	0.50 (0.30 - 0.66; p<0.001)	0.45 (0.24 - 0.62; p<0.001)	0.68 (0.53 - 0.79; p<0.001)	0.27 (-0.49 - 0.95; p=0.42)
Circ. difference calf n=70	-0.09 (-0.45 - 0.23; p=0.68)	0.05 (-0.16 - 0.26; p=0.74)	0.50 (0.30 - 0.66; p<0.001)	-	0.36 (0.14 - 0.55; p=0.002)	0.61 (0.44 - 0.74; p<0.001)	0.47 (-0.49 - 0.97; p=0.14)
BIS ratio n=70	0.42 (0.17 - 0.66; p=0.03)	0.15 (-0.14 - 0.34; p=0.34)	0.45 (0.24 - 0.62; p<0.001)	0.36 (0.14 - 0.55; p=0.002)	-	0.39 (0.17 - 0.57; p<0.001)	0.34 (-0.60 - 0.81; p=0.30)
Limb volume ratio n=70	0.01 (-0.33 - 0.41; p=0.94)	0.40 (0.24 - 0.59; p=0.006)	0.68 (0.53 - 0.79; p<0.001)	0.61 (0.44 - 0.74; p<0.001)	0.39 (0.17 - 0.57; p<0.001)	-	0.46 (-0.34 - 0.92; p=0.15)

n=70 with lower extremity (LE)-DVT]. Correlation between items was estimated using Pearson/Spearman correlation coefficient, as appropriate, for the UE and LE cohorts separately. Ethics approval/informed consents were obtained. This study was funded by PSI Foundation.

Results: Median age (25^{th}-75^{th} percentile) of recruited patients was 7 yrs (3-11) and 8.3 yrs (4-12) in the UE and LE-DVT group, respectively. In the UE-DVT group (Table 1), *proxy-reported* edema was weakly to moderately correlated with limb circumference difference (r=0.36, p=0.02) and with limb volume ratio (r=0.42, p=0.005), but not with BIS (r=0.21, p=0.18). No significant correlations for *patient-reported* edema and other items were found. In the LE-DVT group (Table 2), *proxy-reported* edema was moderately correlated with thigh circumference difference (r=0.49, p< 0.001) and limb volume ratio (r=0.40, p=0.006); *patient-reported* edema was correlated with BIS ratio (r=0.42, p=0.03). No significant correlations for durometry were found.

Conclusions: *Proxy-reported* edema best approximates the measurement of total limb size, whereas *patient-reported* edema measures a concept closer to limb fluid content. Edema as a sign and as a symptom seems to measure different aspects of PTS.

PB 1012 | Laboratory and Clinical Parameters at Diagnosis of Venous Thromboembolism (VTE) Are Not Associated to the Presence of Residual Vein Thrombosis (RVT)

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Background: RVT is a complication of VTE and can be detected by Doppler ultrasound (US). The grayscale median (GSM) method can provide quantitative analysis of the echogenicity of US images.

Aims: The purpose of this study was to investigate the relation between clinical parameters, the presence of RVT and the impact of RVT on post-thrombotic syndrome (PTS), which are until now a matter of debate.

Methods: This is a cross-sectional study with patients with lower limb VTE treated at the outpatient clinic of Hemocentro of Campinas, UNICAMP, between January 2015 and November 2016. Cancer, dyslipidemia, high blood pressure, diabetes, body mass index, D-dimer, FVIII, and C-reactive protein, and antiphospholipid antibodies were evaluated at the end of anticoagulation. No patient presented hereditary thrombophilia. PTS and US analysis were performed with a median of 60 months after VTE. RVT was defined as ultrasound incompressibility of at least 4 mm in the common femoral and/or popliteal vein. RVT echogenicity was determined by GSM. The classification of PTS was performed according to Villalta scale. A hundred and twenty-nine patients were included, with a median of 60 months after the acute episode.

Results: Fifty-seven patients (44.5%) showed RVT. Considering that 19 patients were excluded from this analysis due to lack of PTS data, 64 (58.18%) presented PTS: 46.87% mild, 10.9% moderate and 42.18% severe. Table 1 and 2 show clinical and laboratory parameters according to the presence or absence of RVT. There was no statistically significant difference when the presence of RVT was associated to all parameters analyzed. Furthermore, data did not shows any association between the presence of RVT and PTS, independently of the Villalta classification. The analysis of US images by GSM demonstrated no difference according to PTS classification.

TABLE 1 Clinical data of patients with and without residual vein thrombosis

Clinical data	Patients without thrombus (N=72)	Patients with thrombus (N=57)	P value
Genre: Male/ Female	30.10/69.90	39.30/60.70	0.27
Locals of occurrence: Left lower limb / Right lower limb	61.60/34.20	64.30/32.10	0.94
Characterization: Spontaneous/ Induced	35.60/64.40	33.90/60.70	0.97
PTS: No/ Mild/ Moderate/ Severe	26/18/4/14	20/12/3/13	0.94
HAS: Without/with	41/31	30/22	0.93
Diabetes: Without/ with	61/11	46/10	0.69
Dyslipidemia: Without/ with	55/17	43/13	0.95
Cancer: Without/ with	69/3	51/1	0.48
SAF: negative/ positive	60/13	49/4	0.09

TABLE 2 Laboratory data

TEST	Percentiles without RVT	Percentiles with RVT
Reactive C-Protein (P=0.84)	25:0.22 50:0.63 75:2.26	25:0.28 50:0.64 75:2.05
FVIII (P=0.09)	25:119.61 50:168.35 75:211.70	25:153.97 50:193.90 75:216.62
Total Cholesterol (P=0.32)	25:161.50 50:187.00 75:228.00	25:170.00 50:197.00 75:225.00
LDL Cholesterol (P=0.76)	25:95.00 50:109.00 75:144.00	25:93.50 50:115.50 75:134.50
Triglycerides (P=0.48)	25:86.00 50:113.00 75:181.00	25:85.00 50:127.00 75:187.75

Conclusions: Among our patients, laboratory and clinical parameters at diagnosis of VTE are not associated to the presence of RVT, and the presence of RVT is not correlated to PTS.

PB 1013 | An Innovative Approach to an Age-old Problem: Piloting a Smart Compression Garment Device

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Background: The efficacy of compression garments in the treatment and prevention of post-thrombotic syndrome in adults and children is poorly understood, due in part to the lack of an objective method to quantify compliance with wearing these garments. Lack of compliance has surfaced as a reason for contradictory results in clinical trials.

Aims: To develop and test a prototype of a device designed to objectively measure compliance to wearing compression garments.

Methods: In collaboration with the Bloorview Research Institute, a prototype of compression garments that incorporates wearable technology was developed. The core concept behind the device is the detection and storage of changes in harmless electric signals triggered by small changes in muscular volume as a consequence of muscular contraction, paired with the signals obtained from a 2-axis accelerometer. Readings from these two systems are recorded every 5 minutes. We estimated the Pearson correlation between the readings of these two detection systems. Ethics approval/participant consent were obtained. The study was funded by an Innovation grant.

Results: Two patients have been enrolled so far. 3,725 data points were obtained from the first patient and first prototype; 479 of the readings showed changes indicative of use of the compression sleeve (40 hrs of use) with a clear transition between the baseline and stretched readings (Figure 1). The correlation for the thread and the axis parallel to the ground (Axis 1: forward acceleration) was 0.69 (95% CI 0.64-0.73), and for the thread and the perpendicular axis (Axis 2: downward acceleration) was 0.33 (95% CI 0.25-0.41). The feedback and data obtained from the first patient resulted in adjustments to the device. The next device iteration, which will be piloted by the second patient, is expected to enhance the acquisition of data in compliance.

Conclusions: The use of wearable technology to monitor compliance to compression garments, which will be instrumental for pediatric and adult patients, is feasible.

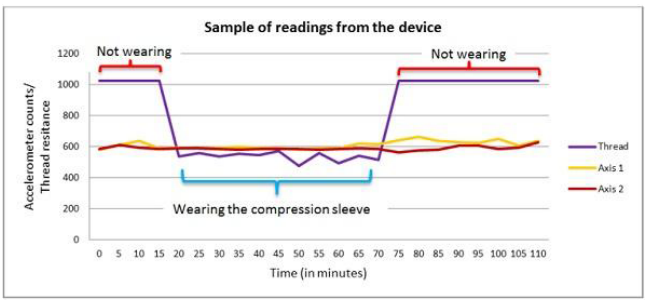

FIGURE 1 Sample of readings from the device

PB 1014 | Post-Pulmonary Embolism Syndrome Development among Patients who Needed Pulmonary Embolism Response Team Assessment

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Background: Pulmonary Embolism Response Team (PERT) initiatives are increasingly implemented, with the premise of improved outcomes after expedited, multidisciplinary, expert approach to the patient with severe PE. There is however paucity of comparative outcome data. A growingly recognized PE outcome is post-PE syndrome, defined by persistently abnormal RV function, functional status, and quality of life among PE survivors.

Aims: To quantify the incidence of post-PE syndrome after PERT activation.

Methods: We initiated a PERT registry that includes initial activation and outpatient follow up data. A follow up ECHO was ordered on patients with persistent symptoms after 3 months. Patients were classified as post-PE syndrome if the imaging right ventricular systolic pressure (RVSP) increased. Continuous variables are reported as median and categorical as percentages. We used SPSS for analysis.

Results: A total of 58 patients required activation of PERT, age 70.5, most (65.5%) were female, PESI class III and 32.7% had cancer (Table 1). The RV was dilated by CT criteria in 48.9% of the patients and 40% had troponin elevation on presentation. PERT decided to do thrombolysis in 10%. A reported 8.5% of the patients died. Among survivors, 11% had an abnormal right ventricular systolic pressure per ECHO. Right heart ECHO variables are tabulated in Table 2. Positive troponin, increased RV/LV ratios, increased RVSP at diagnosis were not associated with post-PE syndrome incidence. Patients with PESI class V were more likely to have an abnormal ECHO at follow-up, but this result was not statistically significant (OR 2.1 95%CI 0.3-13.4).

TABLE 1 Basic demographics and clinical parameters from NorthShore's PERT registry. (N=58)

Median Age	70.5
Female	38/58 (65.5%)
Active Malignancy	19/58 (32.7%)
Concomitant DVT	37/58 (66.6%)
RV/LV ratio >1	23/58 (48.9%)
Troponin Elevation	22/58 (40%)
Pro-BNP / BNP Elevation	28/58 (68.2%)

TABLE 2 ECHO Variables (N=10)

Median RSVP at Diagnosis	30 mm Hg
RV Dilation at Diagnosis	5/10 (50%)
Median RSVP at Follow Up	40.5 mm Hg
RV Dilation at Follow Up	5/10 (50%)

Conclusions: PERT activation selects more complex patients with a high incidence of post-PE syndrome. Given our results, accounting for a 20-30% of post PE syndrome reported in the literature, we estimate that a 500 patient comparative trial may define if there is clinical utility of PERT initiatives, and is needed as to justify its growing acceptance.

PB 1015 | Patients Treated with Rivaroxaban Presented Low Prevalence of Post-thrombotic Syndrome

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Background: A Post-thrombotic syndrome (PTS) is a common complication of deep vein thrombosis (DVT) and is present in about 20-50% of patients after the thrombotic episode. Poorly quality treatment with warfarin is a risk factor for PTS. Direct oral non-vitamin K anticoagulants (DOACs) are increasingly used in the prevention of recurrent DVT and the occurrence of PTS with these novel medications is not well stablished.

Aims: To compare the prevalence of PTS in patients with previous DVT treated with rivaroxaban (RIVA) or enoxaparin/warfarin.

Methods: Patients with previous proximal lower limb DVT (Doppler), treated with RIVA (5.88±3.84 months) or warfarin (8.06±4.15 months) assisted at outpatient clinic of Hemocentro de Campinas (Brazil) from 2014-2017 were included. The classification of the PTS was performed (Villalta scale), 3 to 30 months after treatment.

Results: A total of 107 patients were included, 52 RIVA (26 F/26M, mean age of 48.26 y) and 55 warfarin (45F/10M, mean age of 41.85 y). The body mass index (BMI) >30 were 30.8% and 41.8%, respectively. There were 29 (55.8%) idiopathic and 23 (44.2%) provoked DVT and 21 (38.2%) idiopathic and 34 (61.8%) provoked for RIVA/warfarin, respectively. From patients treated with RIVA 29 (55.8%) did not develop PTS whereas 23 (44.2%) presented this diagnosis (38.5% mild, 3.8% moderate and 1.9% severe). In warfarin group 29 (52.7%) did not show PTS and 26 (47.3%) developed the syndrome (29.1% mild, 10.9% moderate and 7.3% severe). Patients treated with warfarin showed an increased prevalence of moderate/severe PTS (P=0.057). After adjusting for all different factors between the groups and considering only the risk of PTS moderate/severe, warfarin treatment showed a positive impact, but not significant (P=0.078). BMI was an independent risk factor for PTS (RR=1.11; 95 % CI: 1.01-1.22, P=0.028).

Conclusions: Rivaroxaban was associated with low prevalence of PTS, but other risk factors, specially BMI can have a higher impact in development of this syndrome.

PB 1016 | Non-invasive Detection of Fresh Deep Vein Thrombosis with Diffusion Weighted Magnetic Resonance Imaging

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Background: It is important to detect fresh deep vein thrombosis (DVT) for the strategy of anti-thrombotic therapy. Diffusion weighted magnetic resonance (MR) imaging can visualize diffusion capacity of water molecules in the tissue, therefore, we hypothesized that the signal intensity on diffusion weighted MR imaging may reflect acuity of the DVT.

Aims: This study investigated whether diffusion weighted MR imaging can detect the thrombus in patients with DVT and define the thrombus age in a rabbit model of venous thrombus.

Methods: Diffusion weighted MR imaging was performed with a 1.5-T MR system in 8 patients with DVT, and we assessed signal intensity and apparent diffusion coefficient of the thrombi. Venous thrombus was induced in rabbit jugular vein by endothelial denudation and 10 minutes blood stasis with a balloon catheter. The thrombus was imaged with a 3.0-T MR system at 4 hours and at 1, 2 and 3 weeks, and the jugular veins were histologically assessed.

Results: All patients were detected DVT with diffusion weighted MR imaging, and the DVT showed high or mixed high and iso signal intensity on the diffusion sequence. The signal intensity and apparent diffusion coefficient at proximal portion of DVT were higher or smaller than those at distal portion, respectively. The rabbit venous thrombi showed time-dependent organizing reaction. The rabbit thrombi showed high signal intensity on diffusion weighted MR imaging at 4 hours, mixed high and iso signal intensity at 1 and 2 weeks, or mixed iso and low signal intensity at 3 weeks. The signal intensity was positively correlated with erythrocyte and fibrin contents, and negatively correlated with macrophage and collagen contents.

Conclusions: Diffusion weighted MR imaging can detect fresh DVT as a high signal intensity lesion in deep vein.

PB 1017 | Patients' Experience of Post-thrombotic Syndrome: A Qualitative Study with Focus Groups Aiming at a More Accurate Diagnosis

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Background: In clinical trials the Villalta scale is the recommended tool for diagnosing and grading post-thrombotic syndrome (PTS). However, limitations of the scale have been recognized. Additionally, it is our experience that the Villalta scale may have poor specificity and sensitivity in typical patients, and that diagnosis and grading of PTS can be improved.
Aims: We aimed to explore which complaints patients with chronic post-thrombotic problems of the lower limb experience, and relate these findings to the individual items of the Villalta scale.
Methods: We conducted a qualitative study with three focus group interviews with a total of 16 PTS patients within the southeastern Norway health region. The interviews were audio recorded before verbatim transcription and thematic analysis.
Results: Thematic analysis revealed four frequently occurring complaints not included in the Villalta scale: I) Venous claudication, II) symptoms related to a reduced cardiac preload, III) impaired plantar sensation, and IV) difficulty climbing stairs/walking uphill. PTS patients without ulcer did not report pain, but described discomfort, paresthesia and a burning sensation. As included in the Villalta scale, the patients experienced heaviness of the leg and pretibial edema. However, the typical worsening during the day and with prolonged standing, or relief by elevation of the leg is not included in the scale. The patients presented with various post-thrombotic skin changes, but most of them emphasized that these were not of importance. Nevertheless, skin changes constitute four out of the six clinical signs in the Villalta scale.
Conclusions: Our findings indicate that the Villalta scale does not capture some typical PTS complaints, and that some items of the scale are of less importance though weighed equally in the summary score. We conclude that a revision of the tool should be considered to improve the accuracy of PTS diagnosis and grading in clinical trials.

PB 1018 | Optimizing Treatment for Post-thrombotic Syndrome (PTS): A Systematic Review

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Background: Post thrombotic syndrome (PTS) is a burdensome and costly complication of deep venous thrombosis (DVT) that develops in 20-40% of patients within 1-2 years after DVT and is associated with reduction in the quality of life.
Aims: This review is aimed at examining the current best evidence regarding the optimal preventive and treatment strategies for PTS, particularly regarding the elastic compression stocking (ECS),catheter directed thrombolytic therapy(CDT), "venoactive" drugs and endovascular surgery.
Methods: The PUBMED, MEDLINE and the Cochrane Database of systematic reviews were searched for relevant reports of clinical trials and studies for the current best evidence regarding the efficacy of different approaches used in preventing and treating PTS. Studies were eligible for inclusion if they were randomized controlled trial(RCT) or observational studies in adult patients.
Results: We found 714 potentially eligible studies, including 27 RCTs. Most studies had small sample size (range:29-800) and there were variations in individual studies with respect to management protocols. Predominant risk factors for PTS include recurrent ipsilateral DVT, extensive DVT, sub-therapeutic INRs, obesity and advanced age. Systematic use of thromboprophylaxis in hgh- risk hospitalized patients, and the use of optimal anticoagulation of appropriate intensity and duration in the treatment of initial DVT was associated with effective prevention of PTS. Prolonged use of ECS in patients with established PTS led to reduced symptoms. Up-front CDT in conjunction with heparin to treat acute DVT in selected patients including those with low bleeding risk was associated with a significant relative reduction (P< 0.05) in the risk of PTS at 2 years Supervised exercise training was associated with reduced PTS symptoms.
Conclusions: Capitalizing on available strategies including patient and physician education, establishing hospital wide management protocols, and adherence to consensus guidelines, will ensure optimal management of PTS.

PB 1020 | Comparison of Trans-popliteal Reflux in the Limb with Deep Vein Thrombosis and the Limb without Thrombosis

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Background: Chronic venous insufficiency (CVI) leads to various consequences, including pain, swelling, edema, skin changes and ulcerations. Venous reflux is the primary cause of CVI. In literature there are no much evidence on possible damages that thrombotic events may cause to the deep veins of the lower limbs.
Aims: The aim of our study was to determinate whether deep vein thrombosis (DVT) may be cause of incompetence of the venous valves and then of the venous reflux.
Methods: In order to test the plausibility of the association between DVT and the PVI, we assessed the rate of PVI in 59 consecutive outpatients with previous proximal DVT (32 males; mean age, 57 years), and compared it with that detectable in the otherwise unaffected contralateral leg of the same patients. The presence of a retrograde flow through the popliteal valve after a standardized compression of the mid thigh, persisting after repeating the manoeuvre with a tourniquet to prevent the influence of superficial vein reflux, was considered suggestive of valve incompetence.
Results: PVI was detected in 17 patients (28.9%), and involved the leg with previous DVT in 14 cases (82.3%), the contralateral leg in 2 (11.8%), and both legs in 1 patient (5.9%). The odds ratio (OR) of PVI in the legs with previous DVT as compared to the unaffected legs was 6.40 (95% CI, 1.7 to 23.5).
Conclusions: We conclude that after an episode of proximal DVT, trans-popliteal venous reflux is detectable in the leg with thrombosis much more frequently than in the otherwise healthy leg. Our study results makes it plausible the association between DVT and the subsequent development of PVI.

PB 1022 | Inferior Vena Cava Filters: Experience of 133 Patients in a Tertiary Referral Center

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Background: Contraindication to anticoagulant therapy is the main indication for inferior vena cava filters (IVCF) insertion in patients with venous thromboembolism (VTE). An increased use of new retrievable IVCF, has been observed during the last years.

Aims: To analyze the indications for IVCF placement, clinical outcomes, retrieval rate, and causes of failed filter retrieval attempts.

Methods: From 2009 to 2015, clinical data of all consecutive patients who underwent IVCF insertion at our tertiary referral university hospital were retrospectively reviewed. The study was approved by our Clinical Research Ethics Committee. Quantitative variables are expressed as mean (standard deviation -SD-; range) and categorical variables, as percentages.

Results: In total, 133 patients underwent IVCF insertion (52.6% women), with a mean age of 62.5 (15.2; 21-93) years. Of them, 96 IVCF (72.2%) were

TABLE 2 Reasons for failure to filter removal (n=30)

Lack of planning / follow-up	13 (43.3%)
IVCF thrombosis	11 (36.7%)
Endothelial adhesion	4 (13.3%)
Residual DVT	1 (3.3%)
Patient decission	1 (3.3%)

retrievable and 37 (27.8%) permanent. There was an increasing trend in IVCF use during the study period (from 7 IVCF in 2009 to 38 in 2015). IVCF indications are shown in **Table 1.** Overall, 18 (13.5%) patients died during IVCF follow-up, 14 of them with a retrievable filter; however, no death was directly related to IVCF insertion. Among the 82 retrievable IVCF in patients who survived, successful retrieval attempt occurred in 52 (63.5%) patients, with a mean time since IVCF placement of 46.3 (23.8; 12-106) days. Reasons for failure to filter removal in the remainig 30 (36.5%) patients are shown in **Table 2.**

Conclusions: There was an increasing trend in IVCF placement, being most of them retrievable. The main IVCF indication was contraindication for anticoagulant therapy in patients with VTE. Retrievable filters could be effectively removed in almost 2 out of 3 of our IVCF patients. Lack of planning/follow-up and IVCF thrombosis were the main reasons to not remove retrievable IVCF. Applying an institutional monitoring protocol could optimize IVCF management for ensuring the filter is appropriately removed.

PB 1023 | Sex Specific Prevalence and Performance of Diagnostic Algorithms for Pulmonary Embolism

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Background: In the recommended diagnostic algorithms of pulmonary embolism (PE), clinical prediction rules are combined with D-dimer levels to rule out PE, avoiding the need for imaging. It is unclear whether the performance of these algorithms is equal in both sexes.

Aims: To compare the performance of three algorithms for PE between women and men: the Wells rule with fixed (WELLS) or age adjusted (ADJUST) D-dimer cutoff and a novel algorithm (YEARS). We also investigated two subgroups: women under 50, who do not benefit from high efficiency of the ADJUST algorithm and in whom avoiding unnecessary imaging is especially important because of high radiation sensitivity of breast tissue; and women using estrogen, since estrogen use raises D-dimer levels, possibly affecting algorithm performance.

Methods: Individual patient data were obtained from six studies (ADJUST n=1; WELLS n=5) identified by a previous systematic review and from work awaiting publication (YEARS n=1). All studies prospectively enrolled consecutive patients with suspected acute PE. Main outcomes were efficiency (proportion of patients in which the algorithm ruled out PE without imaging) and failure rate (proportion of patients with VTE during 3-month follow-up, i.e. PE missed by algorithm). Prevalence of PE was a secondary outcome. Outcomes were estimated using (multilevel) logistic regression models.

Results: No sex differences in efficiency nor failure rate were observed in any of the algorithms (Table 1), overall and in patients under 50. Prevalence of PE was lower in women in all studies. Estrogen use, adjusted for age, was associated with a lower efficiency and a higher risk of PE (Table 2).

Conclusions: Despite lower prevalence of PE in women, the three diagnostic algorithms showed no sex differences in performance, which might suggest lower specificity in women. An explanation might be sought in estrogen use, which was associated with decreased efficiency, possibly reflecting (aside from a higher PE prevalence) effects on D-dimer levels.

TABLE 1 Primary analysis. Sex specific prevalence and performance of three diagnostic algorithms for PE

	Efficiency Women	Efficiency Men	Efficiency Odds ratio	Failure rate Women	Failure rate Men	Failure rate Odds ratio	Prevalence Women	Prevalence Men	Prevalence Odds ratio
YEARS n = 3465									
Women 52.3 ± 18.5 yrs Men 54.9 ± 17.5 yrs	48.6 %	43.8 %	1.1 (0.96-1.3)	0.64 %	0.50 %	1.1 (0.4-3.0)	11.8 %	16.8 %	0.66 (0.55-0.81)
ADJUST n = 1753									
Women 66.5 ± 10.8 yrs Men 65.8 ± 10.1 yrs	31.5 %	28.8 %	1.14 (0.92-1.4)	0.63 %	(n=0)	-	17.6 %	22.5 %	0.74 (0.58-0.94)
WELLS (Five studies pooled) n = 5515									
Women 52.1 ± 18.6yrs Men 55.1 ± 17.4 yrs	29.3 %	27.3 %	1.10 (0.98-1.2)	0.55 %	0.91 %	0.60 (0.20-1.9)	20.8 %	27.7 %	0.68 (0.60-0.78)

TABLE 2 Subgroup analyses

	Women < 50 versus men < 50		Estrogen use versus no estrogen use**	
	Efficiency (%)	OR efficiency	OR efficiency	OR prevalence
YEARS	♀ 61.5% ♂ 63.5%	0.92 (0.73-1.2)	0.49 (0.38-0.65)	3.7 (2.4-5.6)
ADJUST	Not applicable*	-	0.66 (0.29-1.5)	3.2 (1.4-7.0)
WELLS	♀ 44.3% ♂ 46.2%	0.92 (0.78-1.1)	0.76 (0.61-0.93)	2.4 (1.9-3.1)

OR: odds ratio. All OR's presented with 95%CI. OR efficiency: outcome is imaging indication yes vs no. OR prevalence: outcome is VTE yes vs no.
*D-dimer adjustment was applied > 50 years. ** Adjusted for age.

PB 1024 | Clinical Course of Lemierre Syndrome: An Individual-Patient Data Meta-Analysis of 518 Subjects

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Background: Lemierre syndrome (LS) is a rare disease characterized by acute head/neck bacterial infections (often due to *Fusobacterium Necrophorum)*, neck vein thrombosis, and septic embolism. No large studies on LS have ever been performed due to its very low incidence (3 diagnoses of LS/1,000,000 person-years).

Aims: To investigate the risk of new thromboembolic events (thrombosis recurrence, extension, or new septic embolism), major bleeding, and in-hospital death.

Methods: Studies of subjects with an established diagnosis of LS were identified by a systematic search of Medline and Embase databases (2000-2016). Four investigators assessed the study eligibility, extracted data, and requested the authors of eligible articles to provide missing or additional information. Statistical analysis was based on individual-patient data (PROSPERO protocol ID: CRD42016052572).

Results: A total of 1,969 citations were identified, 467 articles included (case reports >90%), and data of 518 subjects extracted and evaluated for the present preliminary analysis of studies published between 2000 and 2015. The median age was 22 years, 56.6% were males, and jugular vein thrombosis was present in 74.9% of subjects. *Table 1* summarizes patients´ baseline characteristics and sites of thromboembolic complications detected at the time of LS diagnosis.

TABLE 1 Baseline characteristics of patients diagnosed with Lemierre syndrome and site of thromboembolic events at the time of diagnosis

Age (years), median (IQR)	22 (17-35)
Male sex, n (%)	293 (56.6)
Active cancer, n (%)	8 (1.5)
Isolation of Fusobacterium spp., n (%)	238 (45.9)
Jugular vein thrombosis, n (%)	388 (74.9)
Other neck/head vein thrombosis, n (%)	158 (30.5)
Septic pulmonary embolism, n (%)	330 (63.7)
Septic central nervous system embolism, n (%)	69 (13.3)
Septic abdominal embolism, n (%)	23 (4.4)
Septic embolism located at joints, bones, or muscles, n (%)	52 (10.0)

Initial parenteral anticoagulation was used in 243 (46.9%) subjects, of whom 90 were switched to an oral agent before discharge. Surgical treatment was performed in 201 (38.8%) subjects. During hospitalization, new thromboembolic events were reported in 35 (7.6% of valid entries; 95%CI 5.5-10.4), bleeding events in 13 (2.8; 95%CI 1.7-4.8), and death in 18 (3.6%; 95%CI 2.3-5.6) subjects.

Conclusions: LS affects young individuals and is still associated with significant in-hospital mortality rate. Although vein thrombosis is a key feature of the disease, anticoagulants are used in less than half of subjects. The risk of in-hospital recurrent thromboembolic and bleeding events appears high.

PB 1025 | Comparison of Pulmonary Embolism Severity Index Score and Hestia Criteria to Identify Pulmonary Embolism Patients for Outpatient Treatment

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Background: Guidelines recommend out of hospital treatment of low risk pulmonary embolism (PE) if home circumstances are adequate. The Pulmonary Embolism Severity Index (PESI) score is a validated risk assessment model that predicts early mortality. The Hestia criteria is a list of conditions that preclude outpatient treatment. Which tool is best to guide treatment decisions is controversial.

Aims: To quantify agreement between low risk PESI scores and eligibility for outpatient treatment per Hestia criteria in PE patients.

Methods: Elements of the PESI score and Hestia criteria were abstracted from consecutive patients with confirmed PE from May 2015 to May 2016. PESI scores were classified as very low, low, intermediate, high and very high and dichotomized into low risk (very low and low) and high risk (intermediate, high and very high). Patients were deemed candidates for outpatient treatment if they had none of the 11 Hestia criteria.

Results: Of 367 patients, 164 (44.7%) had a low risk PESI score and 97 (26.4%) were candidates for outpatient treatment per Hestia criteria; 62 (16.8%) met both criteria. 168 (45.7%) had either a high PESI score or were precluded form outpatient treatment per Hestia. 35 (9.5%) patients were deemed candidates for outpatient treatment but had high mortality risk.

Conclusions: Only 16.8% of our patients were deemed to be candidates for outpatient PE treatment and had low mortality risk using standard risk assessment tools. Nearly half of our patients were at high risk for dying or did not meet published outpatient treatment criteria. Approximately 1 in 10 of all met Hestia criteria for outpatient treatment despite having a high mortality risk. Further research is needed to define the optimal candidate for out of hospital PE treatment.

TABLE 1 Comparison of PESI score and Hestia criteria

	Low risk PESI score	High risk PESI score	Total
Hestia candidate for outpatient treatment - yes	62	35	97
Hestia candidate for outpatient treatment - no	102	168	270
Total	164	203	367

PB 1026 | Clinical Courses of Portal Vein Thrombosis (PVT) in Children

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Background: PVT is defined as thrombus in the portal vein causing of total or partial obstruction. 5-10% of PVT can lead to portal hypertension (PH) with the devastating outcomes.

Aims: The retrospective descriptive study is to report clinical courses of PVT in children from 2006-2016 in Srinagarind Hospital, Khon Kaen, Thailand.

Methods: Data of children diagnosed with PVT including sex, location, risk factors, age of developing PH, clinical presentations, treatment of PVT, and follow-up time is reviewed.

Results: 17 cases diagnosed with PVT are M:F=11:6 with median follow-up time 1 year 9 months (2 months-17 years). 13 cases were diagnosed at median age at diagnosis 1.25 months (5 days- 9 years) and 4 cases were unknown age at diagnosis. Clinical manifestations include direct hyperbilirubinemia in 7, splenomegaly 5, bleeding from esophageal varices 4, thrombocytopenia 3, abdominal pain 2, pancytopenia and no symptom in each 1. The most common risk factor of developing PVT is umbilical catheterization in 9 followed by sepsis 5, unknown etiology 4, liver abscess 3, exchange transfusion 3 and post Kasai operation 1. 11 cases developed PT at median age 7 years 10 months (range from 1year 6months-18 years 3 months). The most common locations of PVT are found in main portal, left and right portal vein in 11 cases, 10 cases and 5 cases respectively. 6 cases received anticoagulation including low molecular weight heparin 5 cases and warfarin 1 case with response from no to complete recanalization.

Conclusions: Children with PVT might progress to PH during 8 years after PVT with poor outcome in spite of adequate preventive treatment. Children with risk factors of PVT should be warranted for follow-up Doppler abdominal ultrasound at least 8 years in order to early diagnosis and proper management for PH. Anticoagulant therapy is still debated in light of prevention of PH even though it might be rapid clot resolution. The future study in order to determine the role of anticoagulation to reduce PH should be performed.

PB 1027 | The Incidence and of Catheter-related-Thrombosis during Induction Chemotherapy and Follow-up in Chinese Acute Lymphocytic Leukemia Children

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Background: As PICC is more widly used, catheter-related-thrombosis (CRT) has been a more common complication among children.

Aims: To investigate the current status of CRT of Chinese acute lymphocytic leukemia (ALL) children with PICC, and follow up the outcome of ALL children with CRT.

Methods: Collect the clinical data of the inpatients preliminarily diagnosed ALL in the Leukemia Ward of Beijing Children's Hospital with PICC from 1st March 2014 to 31st December 2014 prospectively, and follow up for the next 2 years.

Results:

① A total of 116 cases of children preliminarily diagnosed ALL with PICC were collected. Refer to the B-ultrasound on the 15th day after catheterization, the incidence of CRT is 28.4% (33/116 cases), all cases are symptom-free.

② On the 15th day after catheterization, it revealed significant statistical differences of D-Dimer between the two groups (P=0.001).

③ Multivariate Logistic regression analysis verified catheterization on right is a risk factor of CRT.

④ The B-ultrasound on the 33rd day after catheterization showed 73.1% of the cases had thrombosis reduced, 3.8% had thrombosis growth, 23.1% had no obvious change.

⑤ During the follow-up for 2 years, 61.5% of the cases had thrombosis reduce, 15.4% had thrombosis growth, 23.1% had no obvious change.

⑥ During the follow-up, there were 3 cases had leukemia relapse, and 2 of them had CRT growth or persistent existence.

Conclusions: CRT is a common catheter related complication among ALL children during induction chemotherapy, and CRT cases with symptoms are rare. Catheterization on right is a risk factor for CRT, and regular test of D-Dimer and B ultrasound contribute to detect CRT. Most of the CRT cases had thrombosis reduced without specific management. The growth or persistent existence of CRT may indicate poor prognosis of ALL, which is still need more clinical evidence to confirm.

PB 1028 | Behcet and Thrombosis in Children: Hacettepe Experience

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Background: Behcet is a multisystemic-vasculitic inflammatory disorder associated with a significant mortality and morbidity in males with early age onset. It affects any type and size of vessels which was classified as ´variable´ vasculitis. Multiple thrombosis without a known underlying disorder in a young male adults is typical for Behcet´s disorder. The frequency of vasculary involvement is reported to be 5-20% in pediatric series.

Aims: Here, we retrospectively evaluate our Behcet cases with thrombosis to define their outcome and prognosis.

Methods: We searched our database which was include 560 pediatric patients with thrombosis and find 8 cases of Behcet´s disorder with a median age 15 year-old (range 11-21 years) at the time of thrombotic event.

Results: Localization of the trombosis were DVT(n=4), intracardiac(n=2), sinovenous(n=1), thrombophlebitis of the superficial veins(n=1) and from those patients 3 had simultaneously pulmonary thromboembolism (2 cases of 4 DVT and 1 case of 2 intracardiac thrombosis). Thrombosis is the first initial presentation in 3 of 8 patients and they further diagnosed as Behcet´s disorder. Thrombotic recurrence was observed in 5 of 8 cases. During the thrombotic event they all received antiplatelet and/or anticoagulation with or without immunosupressive treatment. Six out of 8 cases, thrombosis disappered within a median 6 years follow-up time (range1- 10 years) including 2 patients with a diagnose of pulmonary artery anevrysm/ pulmonary thromboembolism; one was successfully treated with pulmonary artery embolisation and the other was still on immunosuppressive treatment. On the other hand 2 patients with thrombosis have chronic thrombosis without any sequela.

Conclusions: Despite a reported high mortality rate in Behçet´s disease with intracardiac and pulmonary thrombosis, a limited number of pediatric cases reported in this study were all alive after a considerable follow-up time.

PB 1029 | Relevance of the Pulmonary Embolism with and without Cancer of for 3 Years in an Academic Medical Center

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Background: Pulmonary embolism is high mortality disease, though the incidence of cancer associated thrombosis is not enough to reveal in Japan. Our academic medical center has been going to perform the prophylaxis of venous thromboembolism since 14 years. We experienced 73 patients of pulmonary embolism for last 3 years.

Aims: We are evaluating the feature of these cases after prophylaxis, and assessing cancer associated thrombosis.

Methods: We analyzed the feature of recent incidence after prevention of venous thromboembolism with or without surgery and cancer in our hospital. Incidence of pulmonary embolism analyzed for three years from 2013 to 2015 in our hospital. We assessed the age, gender,

D-dimer, the period of onset, risk factor, associate with a malignant disease, symptomatic or asymptomatic, provoked or unprovoked, Trousseau syndrome, paradoxical cerebral infarction, the number of IVC filter insertion, existence of a chemotherapy and mortality, etc. Then we compared the feature of our research with the JAVA study in Japan.

Results: Average age is 64.9±14.8 years old, male and female ratio is 1:0.87, BMI is 23.4±4.34, and D-Dimer is 17.5±20.3 ng/ml. medical treatment of patient is 41(56.2%)cases and patients of peri-surgical period is 32 cases (43.8%), The specialty for medical treatment is Cardiology 29 cases(40%), surgery 12 cases (16%), Obstetrics and gynecology 6 cases (8%). Thrombosis with malignant disease is 30 cases (41.1%), history of venous thromboembolism is 7 cases (9.6%). The number of Trousseau syndrome is 4, paradoxical cerebral infarction case is 1 and death case is 2. The pathological examination of peri-surgical period patient's group is mainly detected the pathological finding of the adenocarcinoma.

Conclusions: The incidence of pulmonary embolism was clearly decreasing by performing of prevention of venous thromboembolism in our hospital. Here after, we consider to be increasing incidence which is important points to manage for prevention of VTE with cancer.

PB 1030 | Pulmonary Thromboembolism in Children: Tuberculosis May Be the Underlying Problem

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Background: Pulmonary thromboembolism (PE) is a rare entity among children compared with adults and unlike adults, in whom PE is idiopathic in almost one third of the patients, children with PE has an underlying identifiable risk factor 95% of the time. Tuberculosis is one of the rare causes of PE. Deep vein thrombosis (DVT) and PE in tuberculosis is implicated to the release of inflammatory cytokines, endothelial dysfunction, increased venous stasis, decreased synthesis of anti-coagulant proteins and increased fibrinogen levels.

Aims: Here we report a 14 years old girl diagnosed with PE and tuberculosis.

Methods: She was born as the first child of a healthy mother and father with no consanguinity. She was diagnosed and followed with the diagnosis of epilepsy and motor mental retardation since she was 11 months old. When she was 14 years old she admitted to hospital because of fever, weight loss and difficulty to breathe. Thorax computed tomography (CT) showed mediastinal and hiler lymph nodes, diffuse consolidation and cavitations in the parenchyma of the lung with thrombosis in the left lower pulmonary artery. Heparin infusion started immediately and the tuberculosis culture was positive in the bronchoalveolar lavage specimen.

Results: She dignosed with PE associated with tuberculosis since there was no other risk factors shown for thrombosis.

Conclusions: Tuberculosis should be kept in mind in children with tuberculosis especially in developing countries when there is no other risk factors shown.

PB 1031 | Inherited Prothrombotic Risk Factors in Turkish Children with Hereditary Anjioedema. Single Center Experience

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Background: Hereditary angioedema (HAE)is a rare diseases. If plasma C1 inhibitor(C1-INH) is deficient, complement, quinine-bradykinin, coagulation and fibrinolytic systems activate out of control and angioedema develops, tendency to thrombosis increases.

Aims: Treatment with danazol and antifibrinolytic drugs may also situmulate the thrombosis. Therefore, prothrombotic risk factors (PRF) are important in the patients with HAE. We also planned to investigate PRF in patients with HAE.

Methods: Ten patients who were followed up at the Department of Erciyes Pediatric Immunology and Allergy were included to the study. The type and frequency of attacks, use of prophylaxy and family-history of HAE were questioned. Factor V G1691A, prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI) mutations were investigated.

Results: Mean ages of patients were 151,90±48,21 months old and nine patients (90%) had family-history. The mean value of C4 level was 4,71±1,62 mg/dl, mean value of C1-INH level was 50,10±19,22 mg/L. Eight patients (80%) had reccurrent attack (ranges:once per 2 weeks-2 months). None of them received prophylactic treatment. One patient (10%) had heterozygous F V G1691A, another one had also heterozygous protrombin G20210A mutation. The heterozygous MTHFR mutation in seven patients (70%) and homozygous MTHFR mutation in two patients (20%) were identified respectively. Furthermore, four patients (40%) heterozygous PAI mutation, another one (%10) homozygous PAI mutation had respectively.

Conclusions: A case of HAE who had heterozygous F V leiden mutation and purpura fulminans was reported in the literature. In our study, there was no clinical evidence supporting thrombosis. Our patient with homozygous PAI mutation had an attack more frequently. In HAE, it's important to be known of PRF to estimate both frequency of attack and risk of thrombosis.

PB 1187 | Relation of Stroke and Bleeding Risk Profiles to Efficacy and Safety of Edoxaban for Cardioversion of Atrial Fibrillation: An Ancillary Analysis from the Edoxaban versus Warfarin in SubjectsUndeRgoing Cardioversion of Atrial Fibrillation (Ensure-AF) Study

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Background: In the ensure-AF study (NCT 02072434), the oral factor Xa inhibitor edoxaban was compared to enoxaparin-warfarin in patients undergoing electrical cardioversion of nonvalvular atrial fibrillation (AF). This trial showed comparable low rates of major and clinically relevant non-major (CRNM) bleeding and thromboembolism in the two treatment arms.

Aims: This ancillary analysis investigated differences in relation to stroke and bleeding risk profiles, based on the CHA_2DS_2-VASc and HAS-BLED scores, respectively.

Methods: This multicenter, PROBE evaluation trial compared edoxaban 60 mg QD with enoxaparin-warfarin in 2199 patients. The primary efficacy endpoint was the composite of stroke, systemic embolic event, myocardial infarction, and cardiovascular death from randomization until end of study and the primary safety endpoint was the composite of major and CRNM bleeding. Efficacy and safety outcomes were analysed in relation to CHA_2DS_2-VASc and HAS-BLED scores, respectively. Patients were followed for 28 days on study drug after cardioversion plus another 30 days to assess safety.

Results: 1095 patients were randomized to edoxaban and 1104 received enoxaparin-warfarin. Mean age was 64.3±10 years and 64.2±11 years, respectively. The mean CHA_2DS_2-VASc score was 2.6 (standard deviation [SD] 1.5 and 1.4, respectively) and the mean HAS-BLED score was 0.9 (SD 0.8) in both arms. Efficacy and safety events were low; with only 1 event in CHA_2DS_2-VASc score 0-2 patients. There were non-significant trends towards a lower Odds Ratio (OR) for the primary efficacy endpoint in patients at high risk (CHA_2DS_2-VASc score >2) and a trend for higher ORs with HAS-BLED 0-2 or ≥3 (Table). Mean time in therapeutic range was >67%, with no difference between stroke or bleeding risk strata.

Conclusions: In the ensure-AF study, edoxaban had comparable efficacy and safety to optimized usual anticoagulation with enoxaparin-warfarin and numerically lower primary efficacy endpoint events in those at high stroke risk.

PB 1188 | Anticoagulation Treatment Patterns of Venous Thromboembolism in GARFIELD-VTE Patients

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TABLE 1 Outcomes by stroke and bleeding risk strata

	Total by Treatment No. of events / Total No. (%)			
Primary efficacy endpoint ITT Analysis Set Overall Treatment Period	Edoxaban (n=1095)	Warfarin-Enoxaparin (n=1104)	OR (95%CI)	Mean TiTR with warfarin-enoxaparin arm
CHA2DS2-VASc score				
0-1	0/252 (0%)	0/232 (0%)		71.8
2	1/305 (0.3%)	0/306 (0%)		71.0
>2	4/536 (0.7%)	11/560 (2.0%)	0.38 (0.09-1.28)	70.3
Primary safety endpoint Safety Analysis Set On Treatment Period	(n=1067)	(n=1082)		
HAS-BLED				
0-2	15/1041 (1.4%)	10/1049 (1.0%)	1.52 (0.63-3.80)	70.9
≥3	1/24 (4.2%)	1/32 (3.1%)	1.34 (0.02-109.16)	67.4

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Background: Parenteral anticoagulation overlapping with vitamin K antagonists (VKA) has been the cornerstone of venous thromboembolism (VTE) treatment. With the recent introduction of four direct oral anticoagulants (DOACs), physicians now have broader treatment choices. The non-interventional global GARFIELD-VTE registry observes real-world treatment practices and provides a contemporary snapshot of current VTE treatment.

Aims: To describe interim data on the initial anticoagulation (AC) treatment of VTE patients prospectively enrolled at 361 sites in 28 countries from July, 2014 to September, 2016.

Methods: The study was approved be the individual ethics committees of each participating site and all patients provided written informed consent. Patients with documented initial AC therapy within ±2 weeks of diagnosis (n=8017) were stratified into four treatment groups:

1. parenteral AC alone,
2. parenteral AC followed by a VKA,
3. VKA alone, and
4. DOAC with or without a heparin lead-in.

Results: Differences in therapeutic approach were observed by geographical region, patient population and site of deep-vein thrombosis (DVT) (Table). As recommended by current guidelines, patients with active cancer and pregnant women were more likely to be treated with a parenteral AC alone. AC treatment was similar in patients with DVT and pulmonary embolism, but parenteral anticoagulant alone was more frequently used for upper than lower limb DVT. Furthermore, analyses of AC treatment patterns by geographical regions found that VKA alone was more frequently prescribed in countries outside Asia, Europe and North America (Table).

Conclusions: GARFIELD-VTE provides a global perspective on AC treatment patterns for VTE, which not only varies by patient population, but also by geographic region and site of DVT.

PB 1189 | Clinical Outcomes in Fragile Patient with Venous Thromboembolism

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Background: Subgroup analyses from randomized trials suggested favorable results for the direct oral anticoagulants in fragile patients with venous thromboembolism (VTE). These findings, obtained from randomized trials with selected patients, have not been validated yet in real-life studies.

Aims: To compare the clinical characteristics, treatment and outcomes during the course of anticoagulation and after its discontinuation in fragile versus non-fragile patients with VTE.

Methods: Retrospective study using prospectively collected data from consecutive patients enrolled in RIETE registry (Registro Informatizado Enfermedad Trombo-Embolica, NCT02832245). Fragile patients were defined as those having creatinine clearance levels [CrCl] ≤50 mL/min, age ≥75 years or body weight ≤50 kg.

Results: From January 2013 to October 2016, 15079 patients were recruited. Of these, 6260 (42%) were fragile: 37% aged ≥75 years, 3.6% weighed ≤50 kg and 20% had CrCl levels ≤50 mL/min. During the course of anticoagulation (mean, 210 days), fragile patients had a lower rate of VTE recurrences (hazard ratio [HR]: 0.7; 95%CI: 0.5-0.8) and an over 2-fold higher rate of major bleeding (HR: 2.3; 95%CI: 1.8-2.8) or death (HR: 2.45; 95%CI: 2.2-2.75) than non-fragile. After discontinuing anticoagulation, fragile patients had a higher rate of VTE recurrences

TABLE 1 Treatment approach by geographic region, patient populations and site of DVT

		Parenteral alone	Parenteral+ VKA	VKA alone	DOACs ± Parenteral
Overall		17.6%	28.3%	3.7%	50.5%
Regions	Europe (n=4981)	17.3%	27.6%	2.7%	52.4%
	Asia (n=1116)	28.2%	23.0%	4.2%	44.5%
	North America (n=757)	16.4%	30.5%	2.2%	50.9%
	Other (n=1163)	9.2%	34.6%	8.3%	48.0%
Patient Populations	Active (n=710) / History (n=829) of cancer	64.2% / 36.1%	11.5% / 20.3%	1.4% / 2.8%	22.8% / 40.9%
	Pregnancy (n=115)	49.6%	23.5%	8.7%	18.3%
	Renal impairment (n=279)	21.1%	42.3%	7.2%	29.4%
Site of DVT	Upper (n=512) / Lower (n= 5500) limb	25.2% / 16.6%	22.5% / 28.9%	3.5% / 3.8%	48.8% / 51.6%

(HR: 1.59; 95%CI: 1.29-1.97), major bleeding (HR: 2.38; 95%CI: 1.09-5.33) and death (HR: 2.92; 95%CI: 2.37-3.61) than non-fragile.

TABLE 1 Clinical outcomes during and after discontinuing anticoagulant therapy

	Fragile		Non Fragile	
	N	Events per 100 patient-years	N	Events per 100 patient-years
During anticoagulant therapy	6260		8819	
Recurrent VTE	92	2.63 (2.13-3.21)	203	4.01 (3.49-4.59)
Major Bleeding	234	6.69 (5.87-7.59)	151	2.95 (2.51-3.45)
Death	783	22.1 (20.6-23.7)	465	9.02 (8.23-9.87)
After discontinuing Therapy	1848		2980	
Recurrent VTE	163	16.1 (13.7-18.7)	180	10.1 (8.71-11.7)
Major Bleeding	10	0.97 (0.49-1.73)	5	0.28 (0.10-0.62)
Death	233	22.7 (19.9-25.7)	139	7.75 (6.54-9.12)

Conclusions: In real life, 42% of VTE patients were fragile. During anticoagulation, they had fewer VTE recurrences and more major bleeds or deaths. After its discontinuation, they had more recurrences, bleeds and deaths.

PB 1190 | Comprehensive Characteristics of the Anticoagulant Activity in Relationship to the Plasma Concentration of Dabigatran

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Background: Dabigatran can be detected by a variety of coagulation assays, which have yet to be validated in clinical settings. Important problems in diagnostics include: 1) Are the assays good reflections of the concentration? 2) Most of the currently available data are derived from studies with spiked plasma samples and does not necessarily reflect the *in vivo* effect in treated patients.

3) In clinical studies, it is optimal to perform analyses batchwise at the end of the study, to reduce analytical variation. This requires stability of samples during long-term storage at -80°C.

Aims: To evaluate:

1. The correlation between the plasma concentration (liquid chromatography-tandem mass spectrometry (LC-MS/MS)) and the anticoagulant effect of dabigatran
2. The difference in anticoagulant effect between plasma samples spiked with dabigatran and *ex vivo* samples from patients treated with dabigatran etexilate
3. Stability of these samples after long-term storage at -80°C

Methods: ROTEM®, ecarin chromogenic assay (ECA), a laboratory developed diluted thrombin time (LD-dTT) and LC-MS/MS were used to measure dabigatran in plasma from healthy donors spiked with dabigatran (0-1000 ng/mL) and in *ex vivo* samples from patients treated with dabigatran etexilate. Samples were frozen and stored at -80°C, then thawed at 1, 3, and 6 months and analyzed.

Results: EXTEM clotting time (CT), ECA and LD-dTT have very high correlation with dabigatran plasma concentrations. With the exception of clot formation times (CFT) and maximum clot firmness (MCF), there were no significant differences in results between spiked and patient samples (Figure 1). Samples were stable for at least 6 months at -80°C.

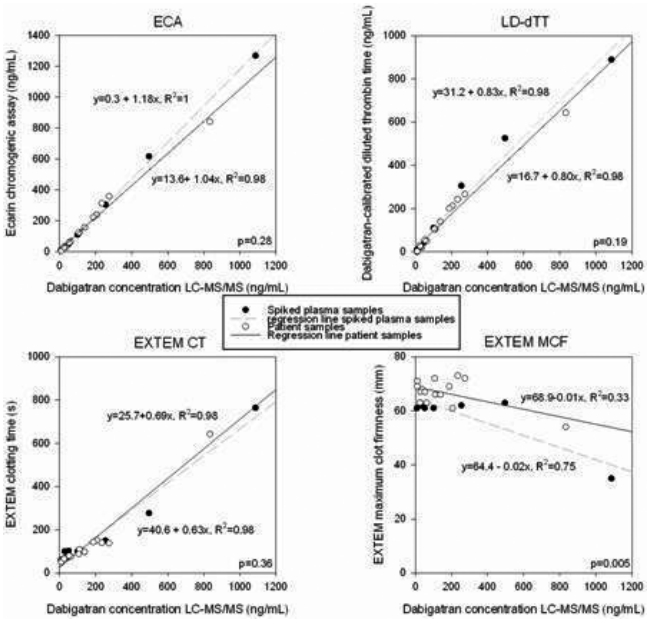

FIGURE 1 The relationship between plasma concentration and the anticoagulant effect of dabigatran. The p-value shown is for the difference between sp

Conclusions: EXTEM CT, ECA and LD-dTT are suitable for measuring the effect of dabigatran in treated patients. In general, results from spiked-plasma samples are similar to those of patient samples. Storage of plasma samples of dabigatran-treated patients for up to 6 months does not influence the measured levels.

PB 1191 | Menstrual Bleeding Patterns in Women Treated with Rivaroxaban: Data from the EINSTEIN CHOICE Trial

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Background: Recent evidence suggests that rivaroxaban increases menstrual bleeding. It is uncertain whether this effect is dose-dependent.

Aims:

1) To compare menstrual bleeding patterns in women receiving rivaroxaban 20 mg, 10 mg or aspirin in a large randomized trial.

2) To describe the frequency of changes in menstrual bleeding related-therapy in such women.

Methods: We prospectively collected data from women enrolled in the EINSTEIN CHOICE study who had menstrual bleeding. At each follow-up visit, data on last menstrual flow length and intensity (shorter/less than usual, as usual, or longer/more than usual) compared with those from before the start of antithrombotic therapy were recorded as were any actions taken to manage menstrual bleeding. Frequency was reported in percentage and differences between treatment groups were calculated.

Results: Information from 353 women was collected. Over the 5 follow-up visits, menstrual flow length increased in 12.6-18.4% of women given rivaroxaban 20 mg, in 6.0-12.8% given rivaroxaban 10 mg, and in 9.4-12.8% given aspirin. Menstrual flow intensity increased in 19.7-25.6% of women given rivaroxaban 20 mg, in 14.7-22.1% given rivaroxaban 10 mg, and in 13.4-20.6% given aspirin. The proportion of women with increased menstrual flow length and intensity was higher with 20 mg of rivaroxaban than with 10 mg of rivaroxaban or with aspirin. Women treated with 10 mg of rivaroxaban reported a mixed pattern of change in menstrual flow length and intensity compared with those receiving aspirin (Tables 1, 2). Few women required actions to manage menstrual bleeding.

Conclusions: Women treated with 20 mg rivaroxaban had increased menstrual flow length and intensity compared with those given 10 mg rivaroxaban or aspirin. Compared with aspirin, there was minimal difference in menstrual flow length and a mixed pattern of flow intensity with 10 mg of rivaroxaban.

TABLE 1 Comparison of menstrual flow length patterns across groups

Days of follow-up	Rivaroxaban 20 mg vs ASA 100 mg	Rivaroxaban 10 mg vs ASA 100 mg	Rivaroxaban 10 mg vs Rivaroxaban 20 mg
Day 30	=	---	----
Day 90	=	=	=
Day 180	++	-	--
Day 270	++++	=	----
Day 360	+++	---	----

= if difference between treatment groups is +/- 1 %, +/- if difference between treatment groups is >1% but <3 %, ++/-- if difference between treatment groups is ≥3 but <5 %, ++/-- if difference between treatment groups is ≥3 but <5 %, +++/--- if difference between treatment groups is ≥ 5 but <7%, ++++/---- if difference between treatment groups is ≥ 7%

TABLE 2 Comparison of patterns of menstrual flow intensity across groups

Day of follow-up	Rivaroxaban 20 mg vs ASA 100 mg	Rivaroxaban 10 mg vs ASA 100 mg	Rivaroxaban 10 mg vs Rivaroxaban 20 mg
Day 30	+++	-	----
Day 90	++++	+++	-
Day 180	++++	+++	-
Day 270	=	--	---
Day 360	+	-	---

= if difference between treatment groups is +/-1 %, +/- if difference between treatment groups is >1% but <3 %, ++/-- if difference between treatment groups is ≥3 but <5 %, +++/- -- if difference between treatment groups is ≥5 but <7%, ++++/---- if difference between treatment groups is ≥ 7%

PB 1192 | Absence of Interaction between Rivaroxaban, Tacrolimus and Everolimus in Renal Transplant Recipients Anticoagulated for Proximal Deep Vein Thrombosis or Atrial Fibrillation

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Background: No data is available on rivaroxaban use for the treatment of venous thromboembolism (VTE) or atrial fibrillation (AF) in renal transplant (RT) patients, not included in phase III clinical trials, and on its surmised interaction with some immunosuppressive drugs, such as tacrolimus or everolimus, which share the same metabolic pathways

Aims: To investigate a potential drug interaction in RT patients taking tacrolimus ± everolimus receiving concomitant rivaroxaban.

Methods: Five consecutive RT patients, 4 taking tacrolimus and 1 tacrolimus plus everolimus, were treated with rivaroxaban for acute VTE or AF. All patients had a stable renal function at baseline. Rivaroxaban was taken at 8.00 am, and tacrolimus and everolimus at 6.30 am. Blood samples were drawn during the first 2 weeks (days 1-5 and 8-12) both at 8.00 am and 12.00 am for Ctrough and Cpeak rivaroxaban assay, respectively (HemosIL Rivaroxaban on ACL TOP LAS 750 WERFEN-IL). Serum levels of tacrolimus, everolimus and creatinine were measured at the same time. Blood cell count, AST and ALT were determined on day 1 and 12. Both bleeding and thrombotic events were recorded.

Results: In all patients rivaroxaban levels showed a predictable trend according to its pharmacokinetics, both in samples at Ctrough (< 30-80 ug/L) and at Cpeak (54-449 ug/L). No significant variations were observed neither in tacrolimus levels (8.00 am: 2.78-9.33 ug/L; 12.00 am: 3.41-16.11 ug/L),nor in everolimus levels (8.00 am: 4.7-11.5 ug/L; 12.00 am: 8.3-17.3 ug/L), nor in creatinine levels between Ctrough and Cpeak measurements for each patient. No patient had bleeding or thrombotic events.

Conclusions: In RT recipients tacrolimus or everolimus does not seem to interact with rivaroxaban given for VTE or AF treatment. Both anticoagulant and immunosuppressive effects seem warranted, without any effect on the graft function, and with the expected antithrombotic effect without bleeding complications.

PB 1193 | Impact of Evolving ACCP Guidelines on Estimates of Venous Thromboembolism Risk in US Hospitals

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Background: The annual number of US acute-care hospital discharges at risk of VTE and the impact of evolving American College of Chest Physicians (ACCP) Consensus guidelines on identification of patients at VTE risk are unknown.

Aims: To estimate the annual number of US acute-care hospital discharges at risk of VTE.

Methods: The Y2004, Y2008 and Y2012 ACCP guidelines for prevention of VTE were applied to Y2014 US hospital discharges using Clinical Classification System codes in the National Inpatient Sample, a database of acute-care hospital discharges from the US Agency for Health Care Quality and Research.

Results: Of 35.4 million discharges from US acute-care hospitals in Y2014, 11.7 million (33%) met the Y2012 ACCP criteria for VTE prophylaxis risk, including 4.4 of 7.5 million (59%) who underwent a surgical procedure. An additional 7.3 of the 20.8 million (35%) remaining eligible discharges met criteria for VTE prophylaxis based on medical risk factors. These data allow estimation of the annual number of US hospital discharges at risk for VTE by age group (Table 1). Estimated VTE risk in patients with medical risk factors decreased by 13% (from 8.4 to 7.3 million discharges) based on 2012 vs. 2008 ACCP criteria (Table 2).

TABLE 2 Number of Discharges from US Acute-Care Hospitals in Y2014 with ACCP Guideline-Defined Risk of VTE: Comparison of the three most recent guide

VTE Risk Population	ACCP-2004 Guidelines Discharges (N)	ACCP-2008 Guidelines Discharges (N)	ACCP-2012 Guidelines Discharges (N)
Major surgery	4,416,707	4,470,702	4,440,848
Major medical illness	8,377,923	8,451,433	7,300,447
Total	12,794,630	12,922,135	11,741,295

Conclusions: One-third of Y2014 US hospital discharges met Y2012 ACCP criteria for VTE prophylaxis. Compared with estimates based on the 2004 or 2008 guidelines, a modest decrease was observed when applying Y2012 ACCP guidelines to Y2014 NIS data. This decrease reflects the ACCP's decision to adopt quantitative risk scoring to identify non-surgical discharges at sufficient VTE risk to warrant prophylaxis. Further research is needed to validate quantitative in-hospital risk models in identifying high-risk patients with medical illnesses that can benefit from VTE prophylaxis, and to evaluate the potential impact of recent evidence for continuation of prophylaxis after hospital discharge in selected patients.

TABLE 1 VTE Risk Groups vs. Age Group for Y2014 US Hospital Discharges

VTE Risk Population	At risk per ACCP-2012 (N)	Age 18–39 years, N (%)	Age 40–59 years, N (%)	Age 60–74 years, N (%)	Age ≥75 years, N (%)
Major surgery	4,440,848	446,980 (10)	1,343,020 (30)	1,644,461 (37)	1,006,385 (23)
Medical illness	7,300,447	365,330 (5)	1,256,035 (17)	2,196,985 (31)	3,482,095 (48)
Total	11,741,447	812,310 (7)	2,599,055 (22)	3,841,446 (33)	4,488,480 (38)

PB 1194 | Healthcare Resource Use in the Non-interventional XALIA Study of Rivaroxaban versus Standard Anticoagulation for Deep Vein Thrombosis

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Background: The phase III EINSTEIN DVT and EINSTEIN PE studies showed that rivaroxaban significantly reduced median hospital length of stay (LOS) compared with standard anticoagulation. The non-interventional XALIA study compared the safety and effectiveness of rivaroxaban to standard anticoagulation for treatment of venous thromboembolism (VTE) in routine clinical practice.

Aims: This XALIA substudy assessed the potential of rivaroxaban to reduce healthcare resource use, including hospital LOS and frequency of hospitalization.

Methods: Patients in XALIA aged ≥18 years scheduled to receive ≥3 months of anticoagulation for treatment of deep vein thrombosis (DVT) with rivaroxaban or standard anticoagulation were eligible. Treatment decisions were at the physician's discretion. Healthcare resource use, including hospital admission for the index DVT and initial LOS, was documented. The main analyses in this substudy used the Propensity score-matched (PMS) set of patients, with adjustment for the presence of cancer at baseline.

Results: In the safety population, 727/2619 (27.8%) rivaroxaban-treated patients were hospitalized for the index DVT, as were 1011/2149 (47.0%) of the standard anticoagulation group. In the PMS analysis, 1124 rivaroxaban-treated patients and 1124 standard anticoagulation-treated patients were included; baseline characteristics are in Table 1. In the PMS analysis, 433/1124 (38.5%) rivaroxaban-treated patients and 438/1124 (39.0%) standard anticoagulation-treated patients were hospitalized. For the index event, LOS in the PMS analysis was a least-squares mean of 2.6 days shorter with rivaroxaban vs standard anticoagulation (5.4 vs 8.0 days; geometric odds ratio=0.67 [95% CI 0.61-0.74, $p< 0.001$]).

Conclusions: In XALIA, hospital LOS was shorter with rivaroxaban than standard anticoagulation, consistent with phase III results. DVT treatment with rivaroxaban in routine clinical practice may reduce the cost per patient compared with standard anticoagulation.

TABLE 1 Baseline demographics and clinical characteristics (PMS analysis)

Characteristic N (%) unless stated	Rivaroxaban (N=1124)	Standard anticoagulation (N=1124)
Age, years, mean (SD)	60.8 (16.2)	61.2 (17.2)
Male sex	508 (45.2)	523 (46.5)
First available Creatinine Clearance <50 ml/min	64 (5.7)	82 (7.3)
DVT only	1008 (89.7)	1014 (90.2)
DVT with PE	116 (10.3)	110 (9.8)
Hospitalization for index VTE	433 (38.5)	438 (39.0)
Provoked VTE	368 (32.7)	390 (34.7)
Cancer at baseline	94 (8.4)	95 (8.5)
Previous major bleeding	23 (2.0)	24 (2.1)

PB 1195 | Oral Anticoagulation with Direct Thrombin Inhibitors Prevents the Early Events of Atrial Remodeling and the Thrombin-activated ErbB Signaling in a Rat Model of Atrial Dilation

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Background: Oral anticoagulation is a key therapy in the prevention of thromboembolism associated with atrial fibrillation. We recently published that anticoagulation with direct thrombin inhibitors (DTIs) prevent dilation, interstitial fibrosis and myocardial hypertrophy of atria from rats with heart failure (Jumeau et al JACC-BTS 1 (5) 2016).

Aims: The current study investigates the action of DTIs on the early markers of extracellular matrix remodeling and the participation of epidermal growth factors/ErbB in thrombin signals.

Methods: Heart failure associated with atrial dilation is induced by myocardial infarction (MI) in 2-months old rats, treated with either vehicle or 25 mg/kg/d dabigatran or 6 mg/kg/d S35972 for 5, 30 or 60 days. Cultures of atrial explants and cardiac cells are performed. Plasma thrombogenic potential is measured by thrombography. Soluble biomarkers are quantified by ELISA, atrial mRNAs by RT-qPCR, ErbB activation by western blot.

Results: The endogenous thrombin potential of plasma is similar 5 and 30 days post-MI and decreases at 60 days to levels remaining higher than those of controls. It is associated with increased plasma thrombospondin-1 (TSP-1). DTIs prevent this rise in soluble TSP-1 and the induction of atrial mRNAs for TSP-1 and intercellular adhesion molecule-1 from 5 days post-MI. DTIs also block the induction of mRNAs for heparin-binding epidermal growth factor (HB-EGF) and neuregulin-1, which appears together with that of myocardial hypertrophic markers 60 days post-MI. Plasma HB-EGF levels, which result from the general circulation, are similar in rats

with dilated atria and controls. However, thrombin activates release of soluble HB-EGF and expression of remodeling markers in atrial explants. To note, recombinant rat HB-EGF activates ErbB4 receptors in atrial fibroblasts but do not induce hypertrophic markers in cardiomyocytes.

Conclusions: These results show early inhibition of atrial remodeling by DTIs and suggest a role for HB-EGF in atrial fibrosis.

PB 1196 | 6-months Outcomes of Patients: Results from GARFIELD-VTE

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Background: The Global Anticoagulant Registry in the FIELD - Venous Thromboembolism (GARFIELD-VTE; NCT02155491) is a global prospective observational study, which aims to capture the treatment patterns for acute VTE and the rate and nature of VTE recurrence, VTE complications, bleeding complications, and all-cause mortality over ≥3 years.

Aims: To determine the 6-month outcomes of patients enrolled from 396 sites in 28 countries from 2014-2016.

Methods: Interim data on 8842 with a confirmed diagnosis of VTE and 6-month follow-up data were analysed. Occurrences of major outcomes were estimated using person-time event rates (per 100 person-years). Rates and 95% confidence intervals were estimated using a Poisson model.

Results: Patients were recruited from Europe (59.3%), Asia (15.0%), North America (9.9%) and the rest of the world (15.7%). The median age was 60.1 y (interquartile range: 46.0-71.5). Over 6-months follow-up, the rates (95% CI) of all-cause mortality and first occurrence of major bleeding and VTE recurrence were: 11.1 (10.0; 12.3), 2.8 (2.3; 3.4), and 3.6 (3.0; 4.3) per 100 person-years, respectively. The rates of all three major outcome events were higher in the first month of follow-up (Table) than in the subsequent 5 months. Overall, 17% of bleeds were major, requiring transfusion in 16.2% of cases. Fatal bleeding occurred in 2.2% of patients with any bleeding event (n=502). The most common cause of death at 6 months was cancer in 50.9% of cases (of these, 14.6% were due to lung cancer; 9.3% to colorectal; 8.6% to pancreatic; and 8.6% to lymphoma). A further 5.1% of deaths were due to pulmonary embolism and 1.3% due to other VTE complications.

Conclusions: Death was the most frequent major adverse outcome in patients diagnosed with VTE; although half were cancer-related. The highest event rates for death and major bleeding occurred during the first month of follow-up.

TABLE 1 Event rates during the first and 6 months of follow-up

	Event rate per 100 person-years(95% CI)	
Follow-up	1-month	6-months
All-cause mortality	15.0 (12.3 to 18.2)	11.1 (10.0 to 12.3)
Major bleeds	5.8 (4.3 to 8.0)	2.8 (2.3 to 3.4)
Recurrent VTE	3.9 (2.7 to 5.7)	3.6 (3.0 to 4.3)

PB 1197 | Outcomes, Interventions and DOAC Plasma Concentration Assay Utility in DOAC-treated Patients Presenting with Major Haemorrhage or Thrombosis - Results from the Anticoagulant Reversal and Events Study Collaborative (ARES1)

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Background: The ARES Collaborative is a large observational study in Australia and New Zealand of consecutive patients who present to hospital with haemorrhage, thromboembolism (TE) or requiring urgent anticoagulant reversal and are taking a DOAC (dabigatran, rivaroxaban, apixaban) or warfarin. The pilot study (ARES1) included case records from 287 presentations, of which 83 (28.9%) involved a DOAC.

Aims: To describe major haemorrhage (MH) and TE presentations in ARES1 amongst patients presenting on DOACs.

Methods: Demographics and site of bleed or thrombosis were reported. Outcomes assessed included interventions utilised, length of hospital stay, 30-day mortality and DOAC plasma concentration assessment within 24 hours of presentation.

Results: MH and TE accounted for 45.8% (38/83) and 14.5% (12/83) of DOAC case presentations, respectively. Most patients presenting with MH received a haemostatic agent (e.g. Prothrombinex-VF® 47.4%, FEIBA® 13.2%, FFP 26.3% and/or tranexamic acid 26.3%), however 28.9% did not receive a specific haemostatic agent.

57.9% (22/38) of patients presenting with MH and 33.3% (4/12) with TE had DOAC plasma concentrations assessed within 24 hours of

presentation. The median time from patient presentation to collection of a DOAC level was 2 hours. The majority (69.2%) of DOAC levels were within the expected on-therapy range for the respective drug. MH and TE were associated with an extended length of hospital stay (median: 6.5 days and 6 days) and a high all-cause mortality (18.4% and 8.3%), respectively.

Conclusions: MH or TE in patients taking DOACs are sentinel medical events with high 30-day mortality and prolonged hospitalisation. Most patients with MH had DOAC levels within the expected 'on-therapy' range, although a proportion had undetectable levels. Obtaining DOAC plasma levels may be critical to inform clinical management decisions in patients presenting with DOAC-related haemorrhage or thromboembolism, including the requirement for specific anticoagulant reversal agents.

PB 1198 | Direct Oral Anticoagulants in Patients with Venous Thromboembolism Usually Excluded for Clinical Trials

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Background: Based on the results from randomized clinical trials, current guidelines recommend the use of the direct oral anticoagulants (DOACs) in patients with venous thromboembolism (VTE).

Aims: We aimed to assess the use of DOACs in VTE patients that would not have been eligible for these pivotal trials, and their efficacy and safety as well.

Methods: We conducted a retrospective study that used prospectively collected data from consecutive patients enrolled in the RIETE (Registro Informatizado Enfermedad Trombo Embólica, NCT02832245) database. We considered as exclusion criteria any of the following: metastatic cancer; creatinine clearance levels < 30 mL/min; platelet count < 100,000/μL; recent major bleeding; chronic liver failure and pregnancy.

Results: From January 2013 to December 2016, 18853 patients were recruited. Of these, 3578 (19%) had exclusion criteria: metastatic cancer 48%; renal insufficiency 29%; thrombocytopenia 13%; recent bleeding 11%; liver failure 7.5% and pregnancy 3.5%. During initial therapy, patients with exclusion criteria receiving rivaroxaban (n=104) had a lower rate of major bleeding than those (n=224) on unfractionated heparin (hazard ratio [HR]: 0.18; 95%CI: 0.03-0.64) and a non-significantly lower rate than those (n=3172) on low-molecular-weight heparin (LMWH) (HR: 0.39; 95%CI: 0.06-1.30). During long-term therapy, patients on rivaroxaban (n=151) had a lower rate of VTE recurrences (HR: 0.25; 95%CI: 0.04-0.84) and major bleeding (HR: 0.38; 95%CI: 0.12-0.93) than those on LMWH (n=2071) and a similar rate than those on vitamin K antagonists (n=939). There were too few patients (n=43) with exclusion criteria on apixaban to perform comparisons.

TABLE 1 VTE recurrences and major bleeding during initial and long term therapy for rivaroxaban

	N	VTE recurrences	Major bleeding
		Events per 100 patient-years (95%CI)	Events per 100 patient-years (95%CI)
Initial therapy	2229		
Exclusion criteria	104	-	4.60 (0.77-15.2)
No exclusion criteria	2125	1.96 (1.20-3.04)	1.85 (1.11-2.90)
Long-term therapy	2499		
Exclusion criteria	151	2.41 (0.40-7.96)	4.85 (1.54-11.7)
No exclusion criteria	2348	1.87 (1.22-2.76)	1.61 (1.01-2.45)

TABLE 2 VTE recurrences and major bleeding during initial and long term therapy for apixaban

	N	VTE recurrences	Major bleeding
		Events per 100 patient-years (95%CI)	Events per 100 patient-years (95%CI)
Initial therapy	123		
Exclusion criteria	11	-	-
No exclusion criteria	112	-	2.89 (0.14-14.2)
Long-term therapy	401		
Exclusion criteria	43	10.4 (1.75-34.4)	4.98 (0.25-24.6)
No exclusion criteria	358	0.65 (0.03-3.21)	3.30 (1.21-7.31)

Conclusions: In real life, 19% of VTE patients had exclusion criteria. Those receiving rivaroxaban had fewer bleeds than those on unfractionated heparin, and fewer VTE recurrences or bleeds than those on long-term LMWH.

PB 1199 | A New Method for Determining Concentrations of Direct Oral Anticoagulants

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Background: Direct oral anticoagulants (DOACs) are commonly provided without monitoring. However, reliable assays to measure DOAC levels and activity in emergency situations are needed. We developed a test based on the inhibition of $\alpha_2$macroglobulin-thrombin (α_2M-IIa) by dabigatran (DAB) and of α_2M-factor Xa (α_2M-Xa) by rivaroxaban (RIV), making it possible to evaluate both DOAC classes in combination with thrombin generation (TG).

Aims: To quantify DOAC levels and activity in plasma.

Methods: Consenting patients using RIV (n=50) and DAB (n=28) were included. TG was performed in platelet poor plasma (5 pM tissue factor), with idarucizumab in calibrator wells for DAB samples. The new DOAC assays measured the effect of diluted plasma samples on Z-Gly-Gly-Arg-AMC conversion by α_2M-Xa or α_2M-IIa. The slopes of these curves were compared to a reference curve with known DOAC concentrations. DOAC levels were also estimated by 'classical' assays. Spearman correlation coefficients were determined.

Results: A concentration of 30 nM α_2M-Xa or α_2M-IIa was optimal to measure 50-500 nM RIV/DAB and 90 nM α_2M-Xa or 70 nM α_2M-IIa, respectively were optimal for 500-1000 nM RIV/DAB (Fig.1).

The intra- and inter-assay CV were below 2.5 % and 5% (n=2). Both the RIV and DAB assay correlated with TG parameters. The RIV assay correlated with ‚classical' assays and had a very good correlation with Biophen DiXaI. The DAB assay did not correlate with hemoclot thrombin inhibitor (probably since DAB concentrations were too low), but showed variable correlation with the other assays (Table1).

Conclusions: The new DOAC assays show good correlations with other assays that were confirmed to accurately assess DOAC levels (particularly the RIV assay with the Biophen DiXaI, which had the best correlation with mass spectrometry). Our assay can simultaneously evaluate DOAC concentrations as well as the DOACs effect on thrombin generation, providing an overview of the anticoagulation status of a patient in relation to circulating DOAC levels.

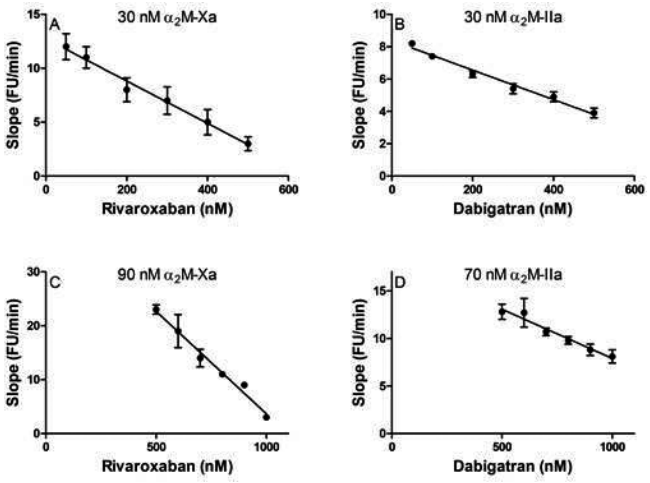

FIGURE 1 Dose-response of several DOAC concentrations at chosen α2M-Xa and α2M-IIa concentrations

TABLE 1 Correlation (Spearman) of rivaroxaban or dabigatran concentration (as determined by the new assay) with TG and other calibrated assays

	Assay	Correlation coefficient	p-value		Assay	Correlation coefficient	p-value
Rivaroxaban	Calibrated prothrombin time (ng/ml)	0.468	0.002	Dabigatran	Activated partial thromboplastin time (s)	0.581	0.002
	Dilute Russel viper venom time (s)	0.760	0.000		Dilute Russel viper venom time (s)	0.649	0.001
	Biophen DiXaI (ng/ml)	0.915	0.000		Hemoclot thrombin inhibitor (ng/ml)	0.391	0.097
	TG endogenous thrombin potential(nM.min)	-0.525	0.000		Ecarin chromogenic assay (ng/ml)	0.591	0.001
	TG Peak (nM)	-0.550	0.000		TG endogenous thrombin potential(nM.min)	-0.323	0.100
	TG lag time (min)	0.671	0.000		TG Peak (nM)	-0.354	0.201
	TG time-to-peak (min)	0.702	0.000		TG lag time (min)	0.423	0.028
					TG time-to-peak (min)	0.339	0.084

PB 1200 | Patients with Dabigatran Circulating Concentration above 200 ng/ml Have a Rebound after Neutralization by a Single Dose of Idarucizumab

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Background: Idarucizumab was recently approved for dabigatran reversal in emergent life-threatening bleeding episodes and urgent surgery or invasive procedure.

Aims: The aim of this study was to analyze all clinical and biological data available to date for idarucizumab use in real life.

Methods: From December 2015 through the February 1st 2017, a total of 27 cases related to the use of idarucizumab were published. One case was excluded because the patient received idarucizumab for dabigatran overdose without bleeding. Five cases were excluded because they were not published in English. Four cases that required idarucizumab before thrombolysis were published. We reported here 7 supplementary cases of idarucizumab use referred to our institution: 5 patients for serious bleeding and 2 who required urgent procedures. A total of 28 cases related to idarucizumab utilization were analyzed.

Results: Clinical characteristics of the 14 patients who received idarucizumab for serious bleeding and 10 patients for urgent procedures were similar to RE-VERSE AD according to age, indication of dabigatran and comorbidities. Bleeding cases had a median concentration of dabigatran lower in RE-VERSE AD Group A (84ng/ml, range 3-641) than in the reported cases (239ng/ml, range 50-3337). We also found a median concentration of dabigatran lower in RE-VERSE AD Group B (76 ng/ml, range 4-2880) than in the pre-surgical cases reported (192,5 ng/ml, range 84-1014). In all reported cases with dabigatran measure at baseline and after reversal, 60% of the patients had a rebound in circulating dabigatran concentration. The median dabigatran concentration at baseline was lower in patients without rebound (113.5, range: 50-176) than those with rebound (338 ng/mL, range; 209-3337). This rebound can appear from 7 hours to 2 days.

Conclusions: Our analysis demonstrates that a dabigatran concentration cut-off of 200ng/ml at baseline could predict the risk of rebound in patients who received a single injection of idarucizumab.

PB 1201 | Patient-reported Treatment Satisfaction with Oral Rivaroxaban: Results from the Non-interventional XALIA Study of Deep Vein Thrombosis

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Background: For venous thromboembolism (VTE) treatment, patient satisfaction improved with rivaroxaban versus standard anticoagulation in the phase III EINSTEIN DVT and EINSTEIN PE trials.

Aims: This substudy of the prospective, non-interventional XALIA study of rivaroxaban for VTE treatment assessed if this also occurred in routine clinical practice.

Methods: Patients in XALIA who received rivaroxaban or standard anticoagulation treatment were eligible for inclusion in this substudy. Treatment decisions were at the physician's discretion. Patients completed the 17-item Anti-Clot Treatment Scale (ACTS; comprising a 12-item Burdens subscale, a 3-item Benefits subscale and two global items for the respective subscales) during follow-up. The propensity score-matched set was the main analysis set; the adjusted safety analysis set (ASAF) was used for confirmatory purposes. Analyses by follow-up visit and by subgroups, including age, sex and previous VTE, were also conducted.

Results: The main PMS-ACTS analysis included 458 rivaroxaban-treated patients and 434 standard anticoagulation-treated patients. Baseline demographic and clinical characteristics were similar across treatment arms. ACTS Burdens scores were superior (indicative of lower treatment burden) with rivaroxaban versus standard anticoagulation (least-squares mean [LSM] difference of 2.4±0.4 points; $p<0.0001$); ACTS Benefits scores were numerically higher with rivaroxaban (LSM difference of 0.2±0.1 points; $p=0.2$). Similar observations occurred across follow-up visits and subgroups. Results were confirmed in the ASAF-ACTS analysis.

Conclusions: Patients who received rivaroxaban reported more favourable ACTS Burdens scores. ACTS Benefits scores numerically favoured rivaroxaban, although they were not statistically significant. The findings were observed across treatment visits and nearly all patient subgroups. Patient perception of rivaroxaban as less burdensome may positively affect treatment adherence and persistence.

PB 1202 | Comparison of Applying TTR Proportion of Tests and TTR Rosendaal to a Sample Population Attending a Warfarin Clinic

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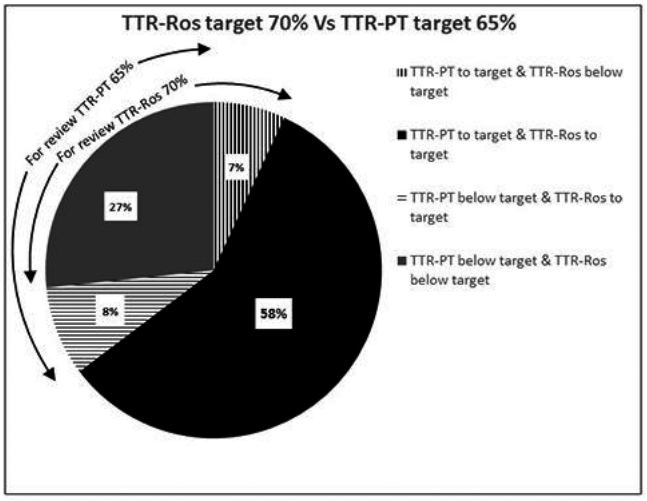
Background: Warfarin therapy is first line for long term anticoagulation but patients with labile INRs should be considered for DOAC (NICE, MMP Ireland). TTR, time in the therapeutic range, is used as a method to identify patients with poor INR control. NICE refers to two



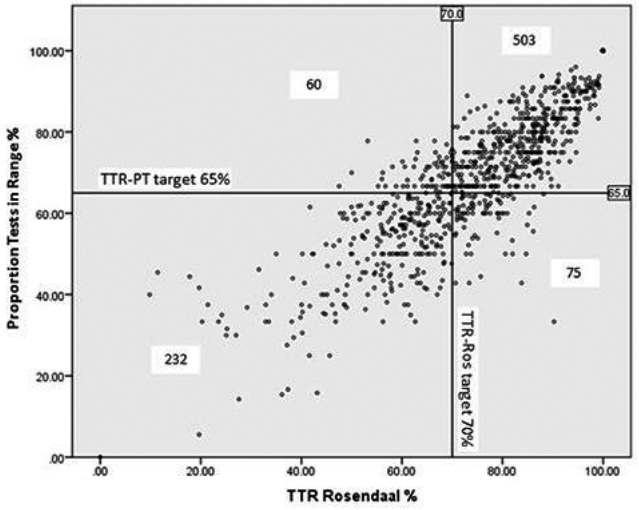


FIGURE 1 Patients considered for review using TTR-PT and TTR Rosendaal. Pie chart showing percentages and a scatter plot with the number in each quadrant

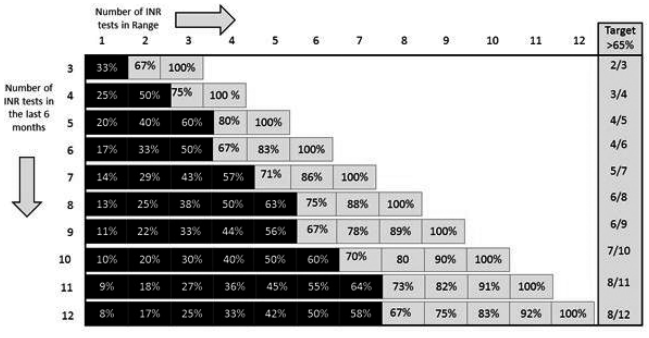


FIGURE 2 A look up chart to calculate TTR-PT with a target of 65%

methods to calculate TTR, Rosendaal and proportion of tests in range. While the Rosendaal method has been used extensively in research settings, it requires computer methods to calculate. The proportion of tests in range may be more practical clinically as it can be implemented by a lookup table or simple arithmetic and may be more easily understood by the patient.

Aims: To investigate the difference in patient selection, when TTR-Rosendaal and TTR proportion of tests in range, TTR-PT, are used on a population of patients attending the warfarin clinic at University Hospital Limerick.

Methods: A retrospective study of all INR tests performed by the clinic from June 2015 to July 2016 was conducted. 872 patients on long term anticoagulation (INR tests spanning greater than 4 months) were selected. TTRs were calculated, using a bespoke computer program, for all patients using the Rosendaal method and proportion of tests. Thresholds of TTR-Ros of 70% (MMP) and TTR-PT of 65% (NICE) were chosen to select patients for review. There is a mean difference, in our data, of 5.4(stdev 8.8) between TTR-Rosendaal and TTR-PT which was similar to that reported by Caldeira, 2015. SPSS was used to cross tabulate patients in each data set.

Results: 85% of the population are treated the same using either method (27% reviewed and 58% considered well controlled). 15% of the total population are treated differently.

Conclusions: The majority of patients are treated similarly when using TTR targets of 70%, Rosendaal, and 65% proportion of tests. TTR proportion of tests may be a more practical method to calculate TTR for patient selection for DOAC therapy.

PB 1203 | Relation between Use of Direct Oral Anticoagulants and Use of Proton Pump Inhibitors as Proxy for Gastric Complaints

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Background: Dabigatran has been linked to gastrointestinal complaints, but it is unknown whether the risk of gastro-intestinal complaints is higher in dabigatran users as compared with other direct oral anticoagulants (DOACs).

Aims: To compare the association between different types of DOACs (dabigatran, apixaban or rivaroxaban) with new use of proton pump inhibitor (PPI) as a proxy for gastrointestinal complaints in patients with atrial fibrillation.

Methods: Observational study with an active-comparator, new user study design. Anonymised dispensing data from Community Pharmacies in The Netherlands were available for 2012-2016. Patients initiating DOAC after 31-03-2012 and without PPI use within the 180 days before start of DOAC were included. The final study sample included 8231 apixaban-, 20394 rivaroxaban- and 17781 dabigatran users. The incidence of PPI initiation was examined for the 3 groups.

TABLE 1 Baseline characteristics of AF* patients included in the study

	DOAC† n=46406(100%)	Apixaban n=8231(17.7%)	Rivaroxaban n=20394(43.9%)	Dabigatran n=17781(38.3%)
Mean age (range)	68 (14-103)	69 (18-100)	68 (14-103)	69 (16-102)
Men, n (%)	27444 (59.1%)	4827 (59.2%)	11709 (57.4%)	10863 (61.1%)
Socioeconomic class** <= 90, n (%)	41193 (89.3%)	7459 (91.1%)	17972 (88.7%)	15762 (89.2%)
Concomitant medication (%)	37349 (80.5%)	6820 (82.9%)	16101 (78.9%)	14428 (81.1%)
Previous VKA use (%)	3711 (8.0%)	1105 (13.4%)	1647 (8.1%)	959 (5.4%)
Previous PPI use††, n (%)	17853 (38.5%)	3334 (40.5%)	7680 (37.7%)	6839 (38.5%)

*AF atrial fibrillation † DOAC Direct Oral Anticoagulant **According to SCP †† PPI use more than 180 days before DOAC initiation

Sensitivity analysis was performed for subjects without PPI use for more than 180 days before start of DOAC. Cox regression models were adjusted for age, sex, previous vitamin K antagonist use, socio-economic status, and concomitant drug use.

Results: The 3 groups were similar with respect to age, sex, socio-economic status, and concomitant drug use (Table). The adjusted hazard ratio (aHR) of initiating PPI for rivaroxaban, as compared with apixaban use, was 0.95 (95% CI 0.89-1.02), and these patients (on rivaroxaban/apixaban) were subsequently taken together in one reference group. The aHR for initiating PPI was 1.14 (95% CI 1.09-1.19) in dabigatran users, compared with the reference group and 1.21 (95% CI 1.14-1.29) when patients with a history of PPI use were excluded. The cumulative incidence of PPI initiation was 20.8% at 12 months for dabigatran users, and 18.7% in the reference group, which yielded a number needed to harm of 48.

Conclusions: PPI initiation occurred more often in patients treated with dabigatran than in those treated with rivaroxaban/apixaban.

PB 1204 | Development and Characterization of Neutralizing Monoclonal Antibodies Targeting the Direct Oral Anticoagulant Apixaban

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Background: Direct oral anticoagulants (DOAC) are indicated for thromboembolism prevention and treatment. Dabigatran is a thrombin inhibitor while apixaban, rivaroxaban, edoxaban and betrixaban are factor Xa inhibitors. Novel clinical research tools are required to better understand the mechanisms of action of this new generation of therapeutical molecules.

Aims: The challenge was to generate high specificity and affinity monoclonal antibodies (Mabs) against a chemical structure such as apixaban.

Methods: Apixaban molecules are conjugated to carrier proteins to obtained enhanced immune response to such reputed hapten. Eighteen weeks after immunization spleen cells from mice presenting with the highest specific serum titers are isolated for fusion following clonogenic and non clonogenic methods. Specific binding of purified Mabs to apixaban is analyzed in a competition ELISA where antibodies are pre-incubated with apixaban before being added to hapten-carrier conjugate immobilized onto microtiter plates. Prothrombin time and FXa activity assays are performed on human citrated plasmas spiked with increasing apixaban concentrations in the presence and in the absence of the selected Mabs. Isotypes and affinity were also determined.

Results: We have generated 23 clones, all secreting IgG1 isotype Mabs specific for apixaban. All Mabs presented with a total absence of reactivity against the other "xabans" as tested by ELISA. Moreover our anti-apixaban Mabs are able to neutralize the in vitro apixaban anticoagulant effect on prothrombin time (Neoplastine CI Stago) and on STA®-Liquid FXa assay (Stago) at a Mab-apixaban molecular ratio of 10:1 and 5:1, respectively.

Conclusions: We therefore report the development of various Mabs specific to apixaban and able to neutralize the anticoagulant effect of the drug. These Mabs could be very useful in diagnostic applications. Further steps are being undertaken to develop recombinant monoclonal antibodies for extended applications in clinical research and pharmacodynamic studies.

PB 1205 | Pulmonary Embolism Rule-out Criteria (PERC) Has No Added Value to the YEARS Algorithm to Rule out Pulmonary Embolism

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Background: The YEARS algorithm was designed to simplify the diagnostic work-up of pulmonary embolism (PE) and reduce the number of required CT-scans (*Figure 1*). A recent outcome study confirmed

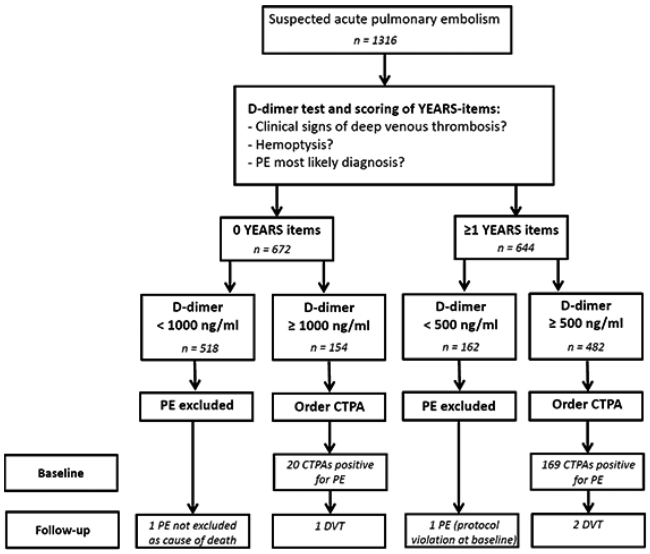

FIGURE 1 Flowchart of the YEARS algorithm with results at baseline and during follow-up of the study patients. * CTPA = computed tomography pulmonary

the safety and efficacy of YEARS (2016 ESC abstract 5727). The PE rule-out criteria (PERC) were created to exclude PE without further testing (*Table 1*). A diagnostic strategy combining both scores might save additional CT-scans, but they have never been evaluated in conjunction.

Aims: To determine the safety and efficacy of combining YEARS and PERC in a single diagnostic strategy for suspected PE.

Methods: The PERC rule was assessed in 1316 consecutive patients with suspected PE who were managed according to YEARS. We calculated the negative predictive value and number of ‚saved' CT-scans for the scenario that PE would have been ruled out without CT in the absence of all PERC items.

Results: Using the YEARS algorithm, PE was diagnosed in 189 patients (14%), 679 patients (52%) were managed without CT and the 3-month rate of VTE in patients in whom PE was ruled out was 0.38%. Only 6 of 154 patients (3.9%; 95%CI 1.4-8.2) with no YEARS items who were referred for CT would have been PERC negative, of whom none were diagnosed with PE at baseline or during follow-up (0%; 95%CI 0-64). Applying PERC in these selected patients with no YEARS-items would have led to a 0.89% absolute reduction in the number of CT-scans at baseline (0.41-1.9) without safety issues. Of the 482 patients with one or more YEARS items who were referred for CT, 51 would have been PERC negative (11%, 8.1-14), of whom 12 were diagnosed with PE, for a failure rate of 24% (14-37). Lastly, both patients managed without CT who suffered PE during follow-up were PERC negative.

Conclusions: Since combining YEARS with PERC would have yielded only a modest improvement of efficiency in patients with 0 YEARS items and an unacceptable failure rate in patients with ≥1 YEARS items, applying PERC before the YEARS algorithm cannot be recommended.

TABLE 1 The Pulmonary Embolism Rule-Out criteria (PERC)

Age ≥ 50 years
Heartrate ≥ 100 beats per minute
SO2 < 95%
Hemoptysis
Exogenous estrogen use
Trauma or surgery within four weeks
Unilateral leg swelling
Prior history of venous thrombo-embolism
The PERC rule is negative when none of above items are met - if negative acute PE is ruled out

PB 1206 | Evaluation of Different Coagulation Laboratory Methods to Monitor Apixaban, Rivaroxaban and Dabigatran Treatment

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Background: Apixaban (API), rivaroxaban (RIV) and dabigatran (DAB) are direct oral anticoagulants (DOACs) for which routine laboratory monitoring is not recommended. However, in certain clinical situations rapid measurements of these drugs are desirable.

Aims: To test different combinations of reagents and analyzers for assessment of DOACs in clinical samples against liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Plasma DAB (n=26), API (n=30) and RIV (n=36) concentration was determined by LC-MS/MS. In addition, four different analyzers CS-2100i, CS-5100, STA R Max and ACL TOP 750, in combination with various reagents (see table), were used for indirect concentration estimations.

Results: A wide range of API, RIV and DAB concentrations were determined by LC-MS/MS (2-372, 3-664 and 3-358 mg/L respectively). Pearson correlation and % bias between LC-MS/MS results and coagulation methods are shown in the table.

Conclusions: It seems that the majority of investigated combinations of coagulation analyzers and reagents for indirect determination of DOACs may be used in routine laboratory but significant bias may be observed with low values (< 50 mg/L), and therefore those results should be cautiously interpreted. It seems that Biophen® Heparin Anti-Xa on CS-5100 or STA R Max is the most reliable for anti-Xa inhibitors, while STA-ECA on STA R Max is the most appropriate for DAB. Our results

TABLE 1 Comparison of LC-MS/MS results for API, RIV and DAB with various indirect methods

Reagent	Analyzer	Apixaban R2 (high/low*)	Apixaban % Bias (high/low*)	Rivaroxaban R2 (high/low*)	Rivaroxaban % Bias (high/low*)	Dabigatran R2 (high/low*)	Dabigatran % Bias (high/low*)
STA®-Liquid-Anti-Xa	CS-2100i	0.99/0.90	15.9/46.8	0.94/0.88	25.0/39.8		
STA®-Liquid-Anti-Xa	STA R Max	0.99/0.94	-24.2/-48.2	0.95/0.91	-35.8/-46.1		
Biophen® Heparin Anti-Xa	CS-5100	0.99/0.98	-8.2/-15.0	0.99/0.87	-8.09/-7.91		
Biophen® Heparin Anti-Xa	STA R Max	0.99/0.97	-10.5/-15.2	1.00/0.93	-7,3/-9,1		
HemosIL® Liquid Anti-Xa	ACL TOP 750			0.99/0.86	73,3/99,4		
Biophen® HTI (clot)	CS-2100i					0.94/0.14	46.5/89.8
Biophen® DTI (chrom)	CS-5100					0.99/0.58	-14.2/-12.3
STA®-ECA (clot)	STA R Max					0.99/0.80	2.8/3.3
HemosIL® DTI (chrom)	ACL TOP 750					0.97/0.79	11.9/10.1

*Below 50 mg/L

indicate that the widely used method for indirect DAB determination, Biophen® HTI, probably should not be used at least for CS-2100i.

PB 1207 | Effect of Rivaroxaban on Expression of Monocyte Platelet Aggregates and P Selectin in Patients with Atrial Fibrillation

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Background: Patients with atrial fibrillation (AF) are at an increased risk of developing stroke and thromboembolism due to arrhythmia and hyperactivation of both platelets and coagulation. Activated platelets exposes on its surface glycoproteins such as P-selectin and interact with leukocytes. Binding of P-selectin to PSLG-1 expressed on monocytes mediates the formation of platelet monocyte aggregates (PMA), which contributes to inflammation and thrombogenesis. In addition, platelets activate factor (F) X to FXa on their surfaces leading to thrombin generation and further platelet activation by thrombin. Rivaroxaban is an oral direct FXa inhibitor used for prevention of thrombomembolic events in AF.

Aims: To determine the effect of rivaroxaban on the expression of P-selectin and PMA in patients with AF.

Methods: In blood samples from 74 participants, including patients using rivaroxaban (n = 33), and individuals without AF (control group, n= 41) were determined, by flow cytometry, the levels of P-selectin (CD62P+) and PMA (CD14+/ CD51/61+). Plasma levels of rivaroxaban were determined by using a chromogenic assay. Data were analyzed by Mann Whitney test and $p < 0.005$ was considered significant.

Results: The medium plasma levels of rivaroxaban in AF patients were 115.15 ng/ml. Use of rivaroxaban significantly reduced the levels of P-selectin and PMA (Table 1 and Figure 1).

TABLE 1 Levels of platelet monocyte aggregates (PMA) and P-Selectin in patients using rivaroxaban and in control group

Parameters	Rivaroxaban	Control	p Value
PMA % (± IQR)	1.85 (2.550)	3.90 (4.050)	0.000
P-Selectin % (± IQR)	1.82 (1.610)	2.73 (2.080)	0.023

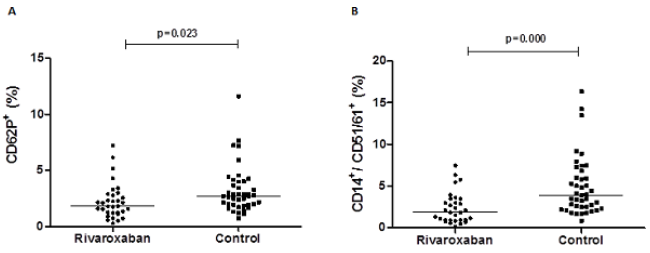

FIGURE 1 Levels of P selectin -CD62P+ (A) and platelet monocyte aggregates-CD14+/ CD51/61+ (B) in patients on rivaroxaban compared to control group

Conclusions: Rivaroxaban reduced platelet activation and its interaction with monocytes, which may contribute to decrease the inflammatory and hypercoagulable state in patients with AF.

Support: CAPES, CNPQ and FAPEMIG.

PB 1208 | Gastrointestinal Bleeding in Patients on Warfarin and Direct Oral Anticoagulant Therapy: An Observational Study in a District General Hospital

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Background: Gastrointestinal (GI) bleeding is a recognised complication of anticoagulation. Increasingly anticoagulated patients take Direct Oral Anticoagulants (DOACs) rather than Warfarin. Locally in September 2016 there were 11,678 prescriptions for Warfarin and 6,368 for a DOAC, compared to 3804 for DOACs in July 2015. Some clinical trials report increased GI bleeding on DOACs. This is consistent with our gastrointestinal physicians' anecdotal experience.

Aims: To determine the real world safety of DOAC's compared with Warfarin with respect to GI bleeding.

Methods: A systematic review of anticoagulated patients referred to the ‚Endoscopy Upper/Lower GI Request Service' between August 2015 and July 2016 was undertaken using the hospital computer system.

Results: 70 bleed events were identified, 34 on Warfarin (48.6%) and 36 (51.4%) on a DOAC. The patients in both groups had similar Charleston Index co-morbidity scores, renal function and transfusion requirements. In the Warfarin group, 8.8% of bleeds occurred within 40 days of commencing anticoagulation compared with 30.6% on a DOAC. Bleeding on Warfarin most frequently occurred secondary to gastric ulceration whereas patients anticoagulated with a DOAC had more non-specific gastric bleeding. Fewer patients (23.3%) on DOACS compared to warfarin (36.4%) remained in hospital more than 7 days.

Conclusions: The number of DOAC related GI bleeds in our local population is consistent with the rates reported in the respective clinical trials; however bleeding on DOAC's appeared to occur sooner and was less associated with a defined anatomical gastric lesion. This may make bleeding in this cohort more difficult to predict. Patients must be carefully selected and counselled about the risk of early bleeding on DOACs. There may be a role for nurse led DOAC clinics and perhaps some place for monitoring of the DOAC effect in patients at the start of treatment.

PB 1209 | Select-d Trial Qualitative Sub-study: Patient and Carer Experience of Anticoagulation for Cancer-Associated Thrombosis

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Background: Cancer patients have a 4-7 greater risk of venous thromboembolism (VTE) than the general population. VTE is the commonest cause of death during chemotherapy. Current treatment for cancer-associated thrombosis (CAT) is at least six months' low-molecular-weight heparin (LMWH). The Anticoagulation Therapy in SELECTeD Cancer Patients at Risk of Recurrence of Venous Thromboembolism (select-d) trial is a randomised, multi-centre pilot study of dalteparin (a LMWH) *vs* rivaroxaban (a direct oral anticoagulant [DOAC]) to assess VTE recurrence and optimal duration.

Aims: This qualitative sub-study explored patient/carer experience of CAT and its treatment.

Methods: Semi-structured face-to-face or phone interviews were conducted with a purposive sample of select-d participants +/- carer. Interviewers used a topic guide to explore experience of CAT and anticoagulant use. Interviews were audio-recorded and transcribed. Data were analysed using Framework Analysis and NVivo software.

Results: 37 patients and nine carers were interviewed (8 to 62 minutes long). Three main themes were identified: VTE and cancer; VTE treatment and cancer; and balancing risks and benefits. Some patients were shocked by the CAT, but others felt it was relatively insignificant in the context of cancer. Most patients were unaware of the risk of VTE. Most saw DOAC tablets as easier than injections (LWMH), but some preferred injection. There were unwanted effects with LMWH, but in the context of cancer, these were acceptable. Whilst most were happy to follow medical advice, others weighed preference on the basis of effectiveness.

Conclusions: Most patients found tablets were more convenient, but LMWH was acceptable in the context of cancer and its treatment despite the side-effects. Patients' lack of awareness of CAT is concerning. Cancer patients should be informed of VTE risk and symptoms to enable prompt help-seeking. DOACs could provide a welcome choice for patients preferring tablets. Study funded by Bayer.

PB 1210 | TRAP-induced Platelet Aggregation is Reduced in Patients Receiving Dabigatran

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Background: The availability of direct oral anticoagulants has caused a paradigm shift in thrombosis management. The direct thrombin inhibitor dabigatran seems to obstruct tenase complex by inhibiting thrombin generated in the initial phase and feedback to the amplification phase of cell-based coagulation reactions. However, it is still not fully understood if and how dabigatran impact platelet function.

Aims: This observational study aimed to assess in vitro platelet function in patients with atrial fibrillation receiving dabigatran.

Methods: Light transmission aggregometry (LTA) was performed using the international protocol for the laboratory investigation of platelet function. The antecubital venous blood was collected into tubes containing 3.2% buffered sodium citrate (anticoagulant-blood ratio 1:9) to assess platelet aggregation. Platelet aggregability was tested with platelet-rich plasma using platelet aggregometry (PACKS-4 aggregometer, Helena Laboratories, USA). Blood samples were stimulated with thrombin receptor agonist peptide - TRAP (32 μmoL).

Results: Twenty-eight patients with non-valvular atrial fibrillation were enrolled. The mean age was 71.57 ± 9.75 years (range 50-87 years), 16 patients were woman and the mean CHA_2DS_2VASc score was 3.93 ± 1.41. All patients began treatment with dabigatran as initial anticoagulant treatment. The minimum term use of dabigatran was 18 days. Dabigatran doses were 110 mg (57.14%) or 150 mg (42.86%) twice daily. The mean dabigatran concentration in sample 1 was 90.56 ± 77.61 ng/mL and in sample 2 was 143.27 ± 103.62 ng/mL. TRAP-induced platelet aggregation was analysed independently of concomitant treatment. As shown in Figure 1, the TRAP-induced platelet aggregation by LTA was significantly reduced in sample 2 compared with sample 1 (79.39% ± 13.38% vs. 90.14% ± 10.5%; p=0.000).
Conclusions: The TRAP-induced platelet aggregation was reduced in cardiovascular patients two hours after receiving dabigatran.

PB 1211 | An Oral Factor Xa Inhibitor Edoxaban Ameliorates Neointima Formation Following Vascular Injury in ApoE KO Mice

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Background: Vascular injury activates the coagulation cascade. Coagulation factor Xa and thrombin have been implicated in proliferation of vascular smooth muscle cells and neointima formation after vascular injury.
Aims: The aim of this study was to determine the effect of edoxaban on neointima formation following the carotid artery injury by ferric chloride in ApoE knockout (KO) mice.
Methods: Vascular injury was induced by the application of 10% ferric chloride to the carotid artery for 3 min in ApoE KO mice. After vascular injury, all animals were fed with high-cholesterol diets for 6 weeks. Edoxaban at 15 mg/kg was orally administered to the mice 1 hour before (n = 10) and 1 hour after (n = 9) ferric chloride injury, and thereafter 10 mg/kg edoxaban was orally administered b.i.d. for 6 weeks. As a control (n = 10), 0.5% methylcellulose was administered. Thrombus formation and neointima formation were evaluated.
Results: Ferric chloride injury of the common carotid arteries of ApoE KO mice induced thrombus formation. Thereafter, feeding with high-cholesterol diets for 6 weeks promoted neointima formation that contained smooth muscle cells, foam cells, and cholesterin crystals. Treatment with 15 mg/kg edoxaban before vascular injury almost completely inhibited thrombus formation, and chronic administration of 10 mg/kg edoxaban b.i.d significantly suppressed neointima formation. In the mice treated with edoxaban after vascular injury, there was wide interindividual variability. In some mice (4 out of 9) the neointima formation was inhibited like in edoxaban-pretreated mice, but not in the other mice. There was no statistical difference compared with control.
Conclusions: This study demonstrated that inhibition of the coagulation cascade with edoxaban ameliorated neointima formation caused by ferric chloride injury and feeding with high-cholesterol diets in ApoE KO mice. This suggests that factor Xa has a crucial role in the formation of neointima following vascular injury.

PB 1212 | Thrombelastography (TEG) for Assessing Dabigatran Reversal in a Preclinical Trauma Model

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Background: Currently, no approved point of care test for assessing direct oral anticoagulants (DOAC) is available. For the TEG device, a DOAC specific cartridge has been developed, using ecarin to detect dabigatran (DTI channel).
Aims: We used TEG in a trauma model to assess dabigatran and evaluate the efficacy of intravenous (IV) and intraosseous (IO) Idarucizumab (IDA), an approved reversal agent for dabigatran.
Methods: After ethical approval, 21 pigs received oral dabigatran etexilate and a dabigatran infusion followed by standardized trauma (blunt liver injury, femur fracture). 15 min after trauma, animals randomly received 1) IO Ringer's solution (control), 2) IO IDA, or 3) IV IDA (both 60 mg/kg). Animals were observed for 240 min or until death. Levels of dabigatran (dTT), TEG variables and blood loss (BL) were recorded. ANOVA (mean±SD) and Pearson's r were used.
Results: IO or IV IDA resulted in a 4-fold decrease in BL as compared to controls (Fig. 1). Dabigatran levels before trauma were comparable in all groups (583±139 ng/mL). A strong correlation between dabigatran levels and DTI R-time was present before trauma and after IO and IV IDA (R^2=0.90 and 0.89; Fig. 2). 15 min after IO and IV IDA, DTI R-time returned to baseline, with corresponding low dabigatran levels (0-28 ng/mL). Dabigatran levels recurred 120 min after trauma in both groups as shown by TEG, most likely caused by redistribution (1). In controls no significant correlation between DTI R-time and dabigatran levels after trauma was measured (R^2< 0.01).

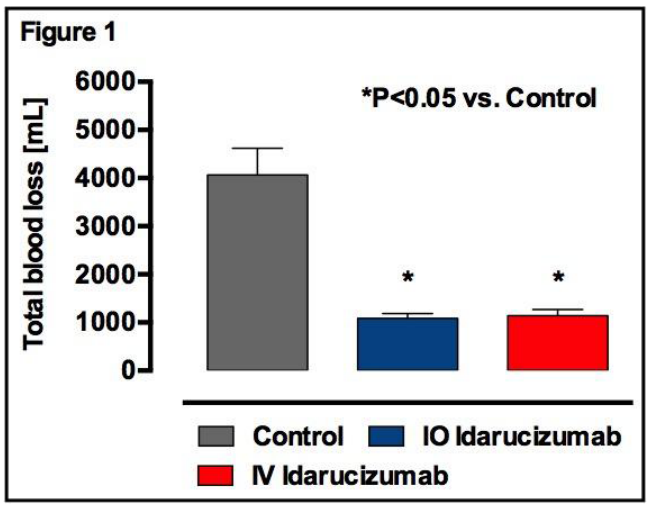

FIGURE 1 Total blood loss [mL]

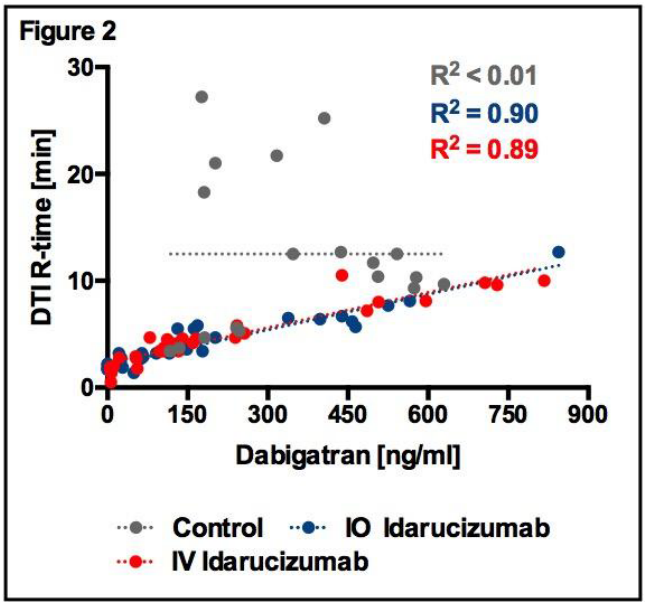

FIGURE 2 Correlation between DTI R-time [min] and Dabigatran [ng/mL

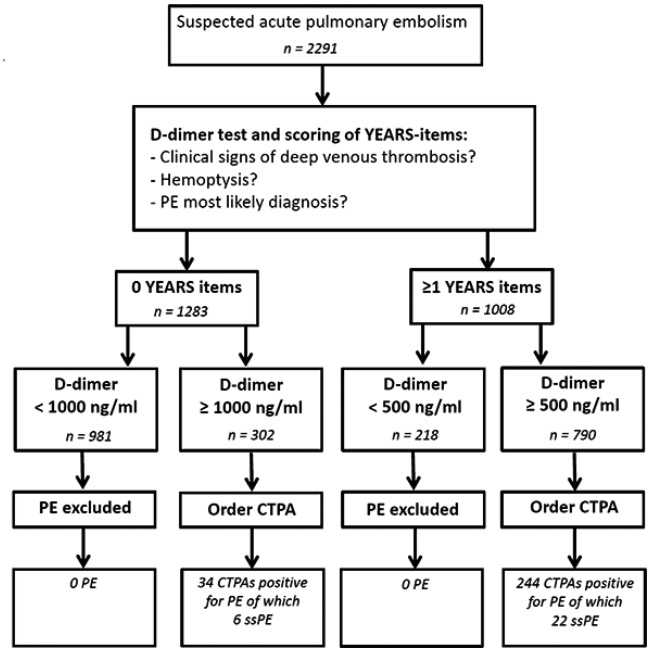

FIGURE 1 The YEARS algorithm

Conclusions: In this model, TEG was useful to assess dabigatran anticoagulation before trauma and after its reversal by IDA, as shown by strong correlations between TEG and dabigatran levels. Since TEG depends on viscoelastic quality of the clot, in controls continuous bleeding with very low coagulation factor levels was probably responsible for the poor correlation between dabigatran levels and TEG.

Reference: 1. Grottke et al. JACC 2015; 66:1518-9.

PB 1213 | Lower Prevalence of Isolated Subsegmental Pulmonary Embolism in the YEARS Diagnostic Algorithm Compared with the Conventional Algorithm for Suspected Pulmonary Embolism

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Background: The rate of isolated subsegmental pulmonary embolism (ssPE) has doubled with advances in computed tomography pulmonary angiography (CTPA) technology. The clinical relevance, i.e. the need to treat these small emboli is debated. The YEARS algorithm was designed to simplify the diagnostic work-up of PE (*Figure 1*). A recent outcome study confirmed the safety and efficacy of YEARS (2016 ESC abstract 5727). We hypothesized that this reduction for CTPA may have led to a reduction in the number of isolated ssPE diagnoses.

Aims: To investigate the prevalence of isolated ssPE in patients managed according to YEARS.

Methods: Data were collected from 2291 consecutive patients with suspected PE who were managed according to YEARS, and compared to historical data from the Christopher study, where 3306 patients were managed according to the Wells rule and a conventional D-dimer threshold of 500 ng/ml. The location and involved arteries were assessed by two reviewers.

Results: In the YEARS cohort, 10% of all PE were isolated ssPE (prevalence of all PE 12%), 51% of entire cohort were managed without CTPA. In the Christopher cohort, 17% of all PE were isolated ssPE (prevalence of all PE 20%), 32% were managed without CTPA. The prevalence of isolated ssPE was significantly lower in the YEARS cohort (absolute difference 7.2%, 95%CI 2.3-11; Odds Ratio 0.54, 95%CI 0.35-0.84). The 3-month venous thrombo-embolism rate in untreated patients was comparable: 0.35% (95%CI 0.18-0.69) in YEARS versus 0.73% (0.49-1.1) in the Christopher study.

Conclusions: The prevalence of isolated ssPE diagnoses is lower in the YEARS algorithm compared to the Christopher study for suspected PE, without compromising the safety of the algorithm. This is likely a consequence of the lower sensitivity of YEARS for ssPE due to the significant reduction in the number of necessary CTPA examinations.

PB 1214 | Renal Clearance is Able to Predict Drug Discontinuation in Patients with Atrial Fibrilation Treated with Direct Oral Anticogulants

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Background: DOACs are useful in prevention of thromboembolic events for patients with atrial fibrillation. However these drugs are no exempt of some discontinuation problems.

Aims: The objective of this study was analyze what factors could predict the discontinuation of the therapy at the beginning of the treatment.

Methods: A retrospective study was conducted of patients who were treated with DOACS in our unit from 2011-2016. Patients were followed until discontinuation drug or to the last follow. The main variables investigated were the percentage of patients discontinued the drug, and the main reason to this outcome: Exitus relate or non-related, major bleedings, thromboembolic events. Statistical analyzes were performed using SPSS 21.0 (SPSS Inc.,Chicago,IL). All the confidence interval was at 95%.

Results: A total of 514 patients were finally included with a mean age of 74(73-75) years old. Gender was female in 301(58.6%) patients. The mean of CHA2DS2-VASc and HAS-BLED was 3,9(3.8-4.1) and 2.5(2.4-2.6) points respectively. Mean follow-up was 1,2(1.0-1.3) years. Eighty two patients discontinue with the treatment until the last follow-up (15%) being the first reason exitus 33(6%). Only one patient has an exitus related with drug intake(0,19%). We analyzed what factors could predict drug discontinuation in our patients. We saw that only age and renal clearance was able to predict discontinuation $p< 0.001$ and $p = 0.02$ respectively. In multivariate analyze we showed that patients with a renal clearance between 30-50 or < 30 milliliter/minute has 4.2(1,5-11) and 3.0(1.0-9.0) times more risk of discontinuation , respectively, than patients with more than 50 milliliter/minute. Age maintained a p value less than 0.05 in multivariate study.

Conclusions: Renal clearance, that has been related with others important outcomes in oral anticoagulation treatment for AF, could be an important factor to predict drug discontinuation and be included in future predictive scales.

PB 1215 | What's the Optimal Cut-off Value of the Prothrombin Time (PT) Assay for Determining Overdose Status of Rivaroxaban?

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Background: The plasma concentration of rivaroxaban (RIV) should be known in specific clinical situations. However, the anti-factor Xa assays may not be readily available. The PT assay is simple and widely available, therefore could be a good candidate for predicting plasma RIV level.

Aims: The aim of this study is to establish the cut-off clotting time and INR of the PT assay for estimation of overdose status of RIV.

Methods: Normal pooled plasma (NPP) was prepared from 337 healthy individuals. PT assay was performed using Thromborel® S reagent with the CA-7000 coagulometer. We applicated Clinical and Laboratory Standard Institute (CLSI) EP12-A2 guideline. We set the arbitrary cut-off plasma RIV level at 275 ng/mL and the RIV-spiked NPP at 275 ng/mL was analyzed in 40 replicates to validate whether the C50 (the concentration near the cutoff that yield 50% positive results and 50% negative results) is plausible. The RIV-spiked NPPs with final concentrations of 200, 250, 275, 300, 325, and 350 ng/mL were analyzed with 20 replicates to determine cut-off value. After setting a cut-off, an imprecision study was performed using RIV-spiked NPP at 220, 247.5, 261.25, 275, 288.75, 302.5, and 330 ng/mL. They analyzed in 60 replicates to determine whether or not a particular concentration range bounds the C5-C95 interval (the range of RIV concentrations around the cut-off). The results at concentrations outside this interval are consistently negative or consistently positive.

Results: The optimal cut-off clotting time and INR value to determine RIV overdose were 13.45 and 1.19. The prepared C50 (275 ng/mL) was plausible. In the imprecision study, there is 92.5% confidence that samples with RIV level ≤247.50 ng/mL or ≥288.75 ng/mL can be expected to yield consistent results with the current PT measurement system.

Conclusions: PT test with a reliable cutoff for screening of overdose status of RIV could help physicians to determine treatment strategies in clinical settings.

PB 1216 | DOAC Reversal with Low (< 20 Units/kg) or Moderate Dose (≥20 Units/kg) FEIBA in the Urgent Management of Major Bleeding

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Background: Management of acute, major or life threatening bleeding in the presence of direct acting oral anticoagulants (DOAC) is unclear. Emergent hemostasis using concentrated clotting factors including prothrombin complex concentrates (PCC) or activated prothrombin complex concentrates (aPCC) have been explored in-vitro/ex-vivo in non-bleeding patients and case reports/small series with bleeding patients. The ideal PCC and dose is unclear, and their use has an associated risk for thrombotic events (TE). At our institution, acute major bleeding the presence of DOACs is managed with the aPCC FEIBA.

Early experience demonstrated a profound, rapid effect with doses around 8 units/kg, leading to an initial low dose (LD) with the option to titrate to effect with additional FEIBA. Moderate doses (MD) of 25-50 units/kg are considered in intracranial hemorrhage (ICH) or eminent life threatening massive bleeds.

Aims: Describe a FEIBA dosing strategy for major DOAC bleeding.

Methods: A single center retrospective analysis of consecutive DOAC patients who received aPCC. Assessed outcomes included the dose administered, post administration bleeding, new TE, or survival to discharge.

Results: 54 patients; apixaban (n=18), dabigatran (n=13) and rivaroxaban (n=23) received LD (< 20 units/kg) aPCC (mean 10±3.5 units/kg; n=32) or MD (mean 24±2.4units/kg;n=22). CNS bleeds occurred in 20 patients. 9 patients expired prior to discharge. One TE occurred with MD. Follow-up CT exams for ICH, endoscopy/colonoscopy, or interventional radiology exams did not reveal any clinically concerning active bleeding or hematoma expansion except one HD patient where CT showed slight worsening of massive multifocal ICH.

Conclusions: LD aPCC titration strategy using an option to repeat, and MD aPCC, depending on the urgency of the situation, may be a management strategy for major DOAC bleeding events. More data and experiences however are needed to determine the best approach to emergently manage major/life threatening bleeding events with DOAC therapy.

PB 1217 | Treatment Effect Estimates for Extended Treatment of VTE Using Network Meta-analysis from Clinical Trials with Rivaroxaban, Aspirin or Placebo

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Background: Four contemporary randomized trials evaluated continued antithrombotic therapy with either rivaroxaban, aspirin or placebo in patients with VTE who had completed 6-12 months of anticoagulation: EINSTEIN EXTENSION (rivaroxaban 20 mg vs. placebo; randomized: 1197), EINSTEIN CHOICE (rivaroxaban 20 mg and 10 mg vs. aspirin 100 mg; randomized: 3396), ASPIRE and WARFASA (both compared aspirin 100 mg vs. placebo; randomized: 822 and 403). None of the studies assessed all 4 treatment groups directly.

Aims: To provide hazard ratio estimates for recurrent VTE for each pairwise comparison between the treatment groups based on the evidence from the 4 trials taking into account direct and indirect comparisons.

Methods: Network meta-analysis is a method in which multiple treatments are compared using information from both direct comparisons of treatments within randomized trials and indirect comparisons across trials based on a common treatment group(s). In all 4 trials, extended treatment of VTE was investigated after completion of 6-12 months of anticoagulant treatment and all studies reported hazard ratios for recurrent VTE. Trial estimates of ASPIRE and WARFASA were combined into a single hazard-ratio by conventional meta-analysis. Hazard ratios from the whole network were calculated based on the assumption of a consistent network of treatment effects.

Results: Table 1 shows the results from the analyses from the studies and the results from the network meta-Analysis.

TABLE 1

Comparison	Hazard ratios (95% CI) within study (direct comparisons)	Hazard ratios (95% CI) from the network meta-analysis
Rivaroxaban 10 mg vs. placebo	NA	0.17 (0.09-0.32)
Rivaroxaban 20 mg vs. placebo	0.18 (0.09-0.39)	0.21 (0.13-0.34)
ASA 100 mg vs. Placebo (ASPIRE and WARFASA combined)	0.67 (0.51-0.90)	0.66 (0.50-0.87)
Rivaroxaban 10 mg vs. ASA 100 mg	0.26 (0.14-0.47)	0.25 (0.14-0.46)
Rivaroxaban 20 mg vs. ASA 100 mg	0.34 (0.20-0.59)	0.32 (0.20-0.50)
Rivaroxaban 10 mg vs. Rivaroxaban 20 mg	0.75 (0.36-1.54)	0.79 (0.40-1.56)

Conclusions: We found no evidence of treatment effect heterogeneity between ASPIRE and WARFASA (ASA 100 mg vs. placebo) or inconsistency between direct and indirect evidence in the entire network. From the network it was possible to obtain an indirect estimate for the comparison rivaroxaban 10 mg vs placebo. The treatment group comparisons obtained from network meta-analysis were consistent with the direct estimates from the studies and generally resulted in slightly narrower confidence intervals due to the information gained from the network.

PB 1218 | Is LMWH-calibrated Anti-Xa Assay Useful to Estimate Apixaban Concentration in Patients Undergoing Apixaban Treatment?

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Background: Therapy with Apixaban, a direct oral anticoagulant, is given at fixed doses without laboratory-guided adjustment, but in

particular situations it´s necessary to be measured. Chromogenic anti-factor Xa activity (aXa) using Apixaban calibrator is the preferred clinical assay. In developing countries, commercial calibrators are not always affordable, so the challenge was to use LMWH calibrators for this purpose.

Aims: To determine in patients receiving Apixaban:
a) correlation between aXa, expressed in ng/mL (aXaA), and aXa in IU/mL (aXaIU), using Apixaban and LMWH calibrators respectively,
b) influence of Apixaban on routine coagulation tests.

Methods: 10 patients taking 2.5 or 5 mg of Apixaban twice, had two blood samples taken: at peak and at trough. Plasmas were tested for PT, APTT, dRVVT and aXa (Liquid anti-Xa) using Apixaban and LMWH commercial calibrators employing the STA®-Compact Max analyzer. aXaA precision was evaluated at two levels: 90 and 228 ng/mL, intra-assay CV was 9.17% and 3.85% respectively and inter-assay CV 7.20% and 3.98%. Statistical analysis was performed with ANOVA test, correlation and linear regression with Barlett sphericity.

Results: We found a statistically significant correlation (r:0.96) between aXaA compared to aXaIU, stronger at trough (r=0.99) than at peak (r:0.96). aXaIU and aXaA assays showed a linearity up to 177 and up to 500 ng/mL of Apixaban, respectively. PT and APTT were insensitive to Apixaban neither at through nor at peak. By contrast, both dRVVT screen and confirm, were prolonged at minimal concentration of Apixaban.

Conclusions: We have shown a strong correlation between plasma Apixaban concentration and aXa activity using LMWH calibrators available in most clinical laboratories, providing a reliable assessment of Apixaban. Nevertheless, significant limitation exists using this method because therapeutic range for Apixaban exceeds that for LMWH. dRVVT could only be used to rule out significant levels of Apixaban.

PB 1219 | Calibrated Automated Thrombogram II: A New Thrombin Generation Test Able to Measure in the Presence of a Direct Thrombin Inhibitor

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Background: Thrombin generation (TG) assessed by the Calibrated Automated Thrombogram (CAT-I) reflects the overall capability of plasma to generate thrombin and is a sensitive method for assessment of a persons' coagulation profile. However, proper measurement of the thrombin calibrator in the presence of direct thrombin inhibitors is impossible due to the inhibition of calibrator by the anticoagulant.

Aims: To develop a modified TG assay (CAT-II) with an alternative calibration approach insensitive for patients receiving thrombin inhibitors.

Methods: TG was assessed by means of the CAT method as previously described. In CAT-I each measurement was calibrated against the fluorescence curve obtained in the same plasma to which calibrator was added. In the CAT-II method calibration was performed by one single measurement applying a standard concentration of thrombin in buffer to correct for substrate consumption. Correction for the color of the plasma was obtained by addition of 7-Amino-4-methylcoumarin (AMC) to every individual assessed plasma.

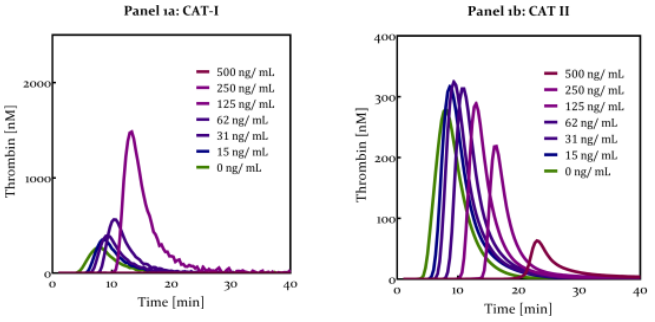

FIGURE 1 Thrombin generation curves in CAT-1 (a) and CAT-II (b) method in direct thrombin inhibitor plasma

Results: Because of inhibition of the calibrator in CAT-I by the direct thrombin inhibitor dabigatran, plasma thrombin generation is overestimated, resulting in elevated ETP and peak thrombin values with increasing dabigatran dosage (Figure 1a). Through calibration according to the CAT-II method, expected inhibition of thrombin generation was obtained with increasing levels of dabigatran (Figure 1b). In healthy individuals both methods showed good coefficients of variation (Table 1).

TABLE 1 Coefficient of variability of TG parameters in blanco measurements in CAT-I and CAT-II

TG parameter	Intra-Assay %CV CAT -I (5 pM TF)	Intra-Assay %CV CAT-II (5 pM TF)
Lag Time (min)	2.43	1.84
Peak Height(nM)	2.69	3.61
ETP (nM.min)	3.83	4.87
Velocity Index (nM/min)	5.07	5.8

Conclusions: Alternative calibration applied in the CAT-II method corrected for interference of direct thrombin inhibitors with the calibrator in CAT-I, which results in overestimated and unrealistic thrombin generations. Thrombin generation assessed by the CAT-II method in plasmas of healthy volunteers was comparable to data obtained by the CAT-I assay.

PB 1220 | Inability of Idarucizumab 5gm to Completely Reverse Excessively High Dabigatran Concentrations

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Background: Idarucizumab 5gms is a recently approved antidote that rapidly reverses the effects of dabigatran with full reversal is

suggested in 99% of patients. Occasionally, patients may present with excessively high levels where it is unclear if there is a maximal dabigatran concentration fully reversed with 5gm's of idarucizumab. Measurement of dabigatran concentrations using ecarin clotting assay are utilized to assess the amount of dabigatran present during acute reversal management.

Aims: To report 2 cases of partial reversal of excessive dabigatran concentrations with 5 gm's of idarucizumab 5gm.

Methods: Case report.

Results:

Case 1: A patient admitted for pulmonary issues but eventually required emergent surgery with a pre-operative INR of 13.2 and dabigatran level of 1360 ng/ml. 2 hours later the INR was 1.93 and dabigatran level 447ng/ml.

Case 2: A patient with an initial elevated dabigatran level of 2250ng/ml (INR > 12) that dropped to 1140ng/ml one hour after Idarucizumab was administered. Hemodialysis was initiated to remove dabigatran and subsequently stopped with a post rebound dabigatran concentration of 692ng/ml noted 2 hours prior to the second Idarucizumab dose which was administered 14 hours after the first dose. The dabigatran concentration 7 hours later (and 21 hours after the initial dose) was 552ng/ml and 465ng/ml an additional 7 hours later.

Conclusions: A 5gm idarucizumab dose appears to reverse approximately 900-1100ng/ml of dabigatran. In the setting of major bleeding or need for an emergent procedure and very high dabigatran levels, additional idarucizumab may be necessary for full neutralization. In case two, the limited drop in the dabigatran concentration may in part may have been driven by a rebound in the dabigatran level after the initial dose. Further assessments are necessary to fully understand the approach to reversing very high levels of dabigatran.

PB 1221 | The Direct Rivaroxaban: Direct Measurement of Plasma Rivaroxaban Concentration through Prothrombin Time (PT) Assay

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Background: The plasma concentration of rivaroxaban (RIV) should be monitored in specific clinical situations. However, the anti-factor Xa assays may not be readily available. The PT assay is simple and widely available, therefore could be a good candidate for measuring plasma RIV level.

Aims: The aim of this study is to develop a direct RIV measuring method with PT assay using RIV-specific calibrators, and diluted RIV-spiked normal pooled plasma (NPP).

Methods: Thromborel®S and CA-7000 coagulometer were used to PT assay. The RIV-specific commercial calibrator and RIV-spiked NPP samples (0 to 500 ng/mL) were analyzed. In addition, control material and multicalibrator spiked with RIV 0, 100, 250, and 500 ng/mL were analyzed. We calculated and compared the slopes of the calibration line and regression line of RIV-spiked plasma, control materials, and multicalibrator. To identify the effect of factor X level in relation to sensitivity for RIV, functional factor X assay was performed. The PT assay with multiple diluted NPP was performed, then comparing the change of sensitivity for RIV.

Results: The sensitivity of Thromborel®S (0.0095) for RIV in RIV-spiked NPP samples was lower than RIV-specific calibrators (0.0241). As the target INR was increase, the sensitivities for RIV in the RIV-spiked control and multicalibrator increased. The lower the factor Xa level is, the sensitivity for RIV increases. The sensitivity for RIV increased as more dilution of NPP was made (0.7-fold dilution: 0.0208, and 0.6-fold dilution: 0.0250) and the sensitivity of diluted NPP at 0.6 fold was almost same as that of the RIV-specific calibrator.

Conclusions: The direct calculation of plasma concentration of RIV using PT assay with a commercial RIV-specific calibrator material could be possible, with increase of reagent sensitivity through adequate dilution of patient plasma samples. The dilute PT assay is suggested as a simple and rapid method for evaluating the anticoagulant activity of RIV.

PB 1222 | Management of Pulmonary Embolism in a Short Stay Medical Unit: A Cohort Study

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Background: Low risk pulmonary embolism (PE) could be treated in hospital or even entirely at home, when home circumstance are adequate. However, the length of hospital stay (LOS) for PE remains long in the Italian setting (mean 11.5 days, Ministry of Health reports, 2010).

Aims: Our objective was to evaluate the management and outcome of haemodinamically stable PE patients admitted to a short stay medical unit (SSMU).

Methods: We conducted a cohort study enrolling consecutive patients diagnosed with acute PE, admitted to the SSMU and then followed at Thrombosis Center of the Ospedale di Circolo (Varese) from May 2014 to December 2016. The outcomes were LOS, symptomatic recurrent venous thromboembolism (VTE), major bleeding and mortality during hospital observation and at 1 month. The study was approved by the insitutional ethics committee.

Results: 100 patients were enrolled, with a mean age of 67.5 years (standard deviation-SD-17.5), 41% were males. According to the pulmonary embolism severity index (PESI) 37 patiens (37%) were classified at low risk of adverse outcome. Overall, the mean LOS was 5.7 days (SD 4.3). In particular, 77% of patients were discharged home

after a mean LOS of 4.5 days (SD 1.7), whereas 23% of patients were transferred to other wards, for a mean LOS of 10.0 days (SD 7.0).

Overall, 50% of patients received direct oral anticoagulants, 20% low molecular weight heparin (LMWH) with warfarin, 29% LMWH alone, 1% fibrinolysis.

During the hospital stay, no patients experienced recurrent VTE, 2 patients (2%) experienced major bleeding and 2 patients died (2%) At 30 days, one patient had recurrent VTE, whereas 2 additional patients died. All those patients were high-risk PESI and had been transferred from SSMU to other wards.

Conclusions: A reduction of LOS, as compared with the national mean, was obtained by admitting PE patients to a SSMU, with follow-up at the thrombosis center. A short hospital stay seems safe for haemodinamically stable PE patients, especially those classified at low risk by PESI.

PB 1223 | The Safety of a Nurse Led Pathway to Transition Patients with Acute Venous Thromboembolism (VTE), Treated with Rivaroxaban, into the Community

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Background: Rivaroxaban is effective treatment for patients with VTE, but the appropriate clinical monitoring of these patients after discharge is uncertain.

Aims: To audit the safety of a nurse led pathway to monitor patients with acute VTE. treated with Rivaroxaban

Methods: Prospective audit of patients treated with Rivaroxaban for acute VTE at Monash Health over 12 months. A VTE nurse identified patients at discharge then reviewed their files for suitability to receive Rivaroxaban. Patients were followed with telephone calls and text messaging and email, until clinic review. Using set criteria the nurse to identified patients at risk of major bleeding or recurrent VTE.

Outcomes:

1) The proportion of patients requiring pre-emptive intervention to prevent major bleeding or recurrent VTE.

2) Rates of major bleeding and recurrence during anticoagulation (6 weeks to 90 days).

Results: 180 patients with VTE prescribed rivaroxaban were identified. 5 patients did not come onto the pathway and 3 patients declined and 1 patient was lost to follow-up.171patients were managed on the pathway. Their average age was 54 years and 52% were male. 102 patients had pulmonary embolus (PE), 63 patients had deep vein thrombosis (29 proximal and 34 distal) and 6 patients had other VTE. Pre-emptive action was required in 11 %(95% Confidence intervals (CI) 7-17%) of patients, 5 % (95%CI2-10%) to prevent major bleeding and 6 % (95%CI 3-10%) to prevent recurrence. No 0% (95%CI: 0 to 2%) major bleeding occurred but 2 patients (1.5%(95%CI 1-4%)had possible recurrences.

Conclusions: Our nurse led pathway identified 11% of patients at risk of recurrence or major bleeding needing timely preventative physician interventions. The safety of this simple nurse led pathway is validated by the low rate of actual major bleeding 0% and recurrent VTE 1.2%.

PB 1224 | Performance of a Chromogenic Assay for the Measurement of Edoxaban Concentration in Plasma

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Background: Edoxaban is an oral direct Factor Xa inhibitor used at fixed daily dosages. Edoxaban has been approved by regulatory authorities for use as a direct oral anticoagulant (DOAC) without the need for routine blood monitoring. Nevertheless, the quantification of edoxaban plasma anti-Xa activity may be desired, or necessary, in specific clinical situations including emergency surgery or severe active bleeding. A chromogenic assay has been developed to provide rapid and automated measurements of edoxaban activity in plasma.

Aims: The purpose of this study was to assess the performance of this assay in comparison to results obtained by the reference standard of liquid chromatography-mass spectrometry (LCMS).

Methods: 65 plasma samples were obtained from clinical studies sponsored by Daiichi-Sankyo (DSI). The chromogenic assay for edoxaban quantitation in plasma was performed using proper assay methodology, calibrators and quality controls. The results obtained from the chromogenic assay were compared to LCMS measurements performed by the DSI reference site.

Results: Samples in the method comparison showed LCMS results ranging from 19 to 408 ng/mL *versus* 25 to 393 ng/mL for anti-Xa results. The correlation between the chromogenic Anti-Xa assay and LCMS was excellent (r^2 = 0.99). Inter-laboratory coefficient of variation (CV) were determined for two edoxaban levels: 7.06% and 2.92% for edoxaban level equivalent to 42 ng/mL and 103 ng/mL respectively.

Conclusions: The comparison between the chromogenic assay for measuring edoxaban plasma concentration and the LCMS method demonstrates excellent overall correlation between both methodologies. The rapid and automated chromogenic assay permits haemostasis laboratories the ability to quickly measure edoxaban plasma concentrations which may be useful in serious or emergency patient situations.

PB 1225 | The Perioperative Anticoagulant Use Surgery Evaluation (PAUSE) Study

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Background: The optimal perioperative management of patients who are receiving direct oral anticoagulants (DOACs) is uncertain. The ongoing PAUSE Study is recruiting patients with atrial fibrillation (AF) who are taking a DOAC (dabigatran, rivaroxaban or apixaban) and require an elective surgery/procedure to demonstrate the safety of a perioperative standardized DOAC interruption protocol. This protocol, adjusted by type of DOAC, renal function and related bleed risk (see figure), aims to demonstrate acceptably low rates of perioperative major bleeding (MB) and arterial thromboembolism (ATE). This study aims to recruit 3,300 patients (1,100 for each DOAC), of whom 1/3 will have a high bleed risk surgery/procedure.

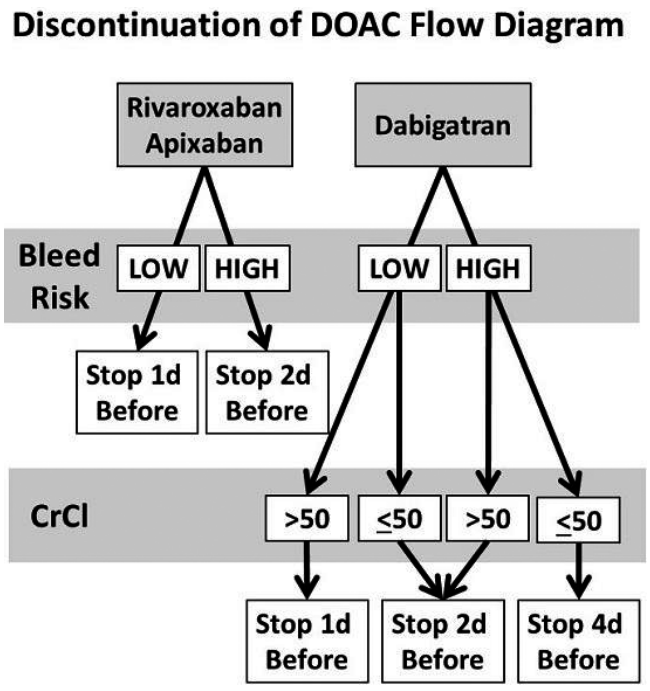

FIGURE 1 Standardized DOAC preoperative interruption protocol (Based on DOAC type, risk of bleed and CrCl)

Aims: To review the recruitment profile of the first 1,500 patients recruited into PAUSE to assess the distribution among the 3 DOACs and bleed risk classification. Should the target recruitment in one DOAC cohort and/or high bleed risk procedure categories be under-represented, selective recruitment may be implemented.

Methods: This international, observational, prospective cohort study, with 3 parallel cohorts for each DOAC, at ~20 centres expects to recruit 3,300 AF patients receiving a DOAC and having an elective surgery will be recruited for the study in a non-selective manner. The first 1,500 patient recruitment profile will be reviewed to evaluate whether current recruitment practices are favorable to meeting target recruitment.

Results: To date, 1,500 patients have been recruited into PAUSE over a 2-year period with 32.7% of patients having a high bleeding risk surgery/procedure. There are 28.3% of patients in the dabigatran, 35.5% of patients in the rivaroxaban, and 36.2% of patients in the apixaban cohorts.

TABLE 1 PAUSE Study Recruitment Profile - Type of DOAC and Surgery/Procedure Bleed Risk

Type of DOAC	Patients having HIGH RISK for BLEED procedures	Patients having LOW RISK For Bleed procedures	Total Recruited
Dabigatran	145	279	424
Rivaroxaban	167	366	533
Apixaban	179	364	543
Total	491	1009	1500

Conclusions: The PAUSE Study recruitment, to date, demonstrates symmetric patient recruitment in the 3 DOAC cohorts and has attained the targeted proportion of patients having a high bleeding risk surgery/procedure. Selective recruitment is not required.

PB 1226 | Assay Availability for Measurement the Anticoagulant Effect of Direct and Indirect Xa Inhibitors

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Background: Although routine coagulation monitoring of the direct oral anticoagulants is unnecessary, there is an urgent need for readily and rapidly available tests to measure them. This need will increase with the introduction of reversal agents such as idarucizumab for dabigatran and andexanet alfa for rivaroxaban, apixaban, and edoxaban. Idarucizumab is already licensed and andexanet is undergoing regulatory review and could be approved later this year.

Aims: There have been recent calls for the urgent adoption in the hospital setting of assays that can accurately and rapidly determine the concentration of these drugs. We describe how rapid access to assays based on anti-Xa activity has been achieved in two of our hospitals.

Methods: Our laboratories validated the Hyphen Biomed [Hyphen] LRT reagent against the Hyphen Heparin 6 reagent for the indirect-Xa inhibitors, and against the Hyphen DiXaI reagent for rivaroxaban. New assays were created for apixaban and edoxaban using the Hyphen LRT reagent. Calibrators and controls for all assays were from Hyphen, except for edoxaban, where they were obtained from Diagnostica Stago.
Results: All assays comply with the requirements of ISO15189, except edoxaban where no inter-laboratory comparisons are yet available.
Conclusions: Through the use of a single reagent set with different calibration curves for each drug, training for staff on all shifts is achievable. The stability of the liquid reagents on board the analyser also means that reagents can be left on the analyser; when a request is received, relevant controls are run and the assay is performed. In this way assays are available immediately for heparin (low molecular weight and unfractionated), rivaroxaban, apixaban, edoxaban, danaparoid and fondaparinux with less than one hour turnaround time. Hospital laboratories should consider the use of liquid reagents for anti-Xa activity assays in order to enable rapid evaluation of all direct and indirect Xa inhibitors using commercially available calibrators and controls.

PB 1227 | Evaluation of Prescribing Practices and Medication Persistence in Patients Initiated on Direct Oral Anticoagulant Therapy at a Tertiary Care Academic Medical Center

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Background: Direct oral anticoagulants (DOACs) are preferred over warfarin due to favorable efficacy and safety comparisons as well as patient convenience. However, concerns remain over gaps in appropriate prescribing and reduced medication persistence resulting in suboptimal outcomes.
Aims: To assess the appropriateness of DOAC prescribing and medication persistence in patients following discharge.
Methods: A retrospective analysis of patients initiated on a DOAC during hospitalization between January 1, 2016 to October 31, 2016 was conducted. Data was abstracted from the EMR in order to evaluate clinical outcomes and medication persistence at 1 month and 3 months post-discharge.
Results: A total of 281 patients were identified. Primary indications were treatment of acute VTE (63%) and atrial fibrillation (26%). Rivaroxaban was used in 77% of the patients and apixaban 19%. Based on current dosing recommendations, 14 patients (5%) received incorrect initial dosing. The most common error was providing induction doses of a DOAC when an acute VTE was not present (8/14 patients). At 1 month, 29% of patients were lost to follow up. Only 142 patients (50%) had follow-up documentation in the EMR regarding anticoagulation therapy in the first 3 months. In patients with documented follow-up, 96% remained on therapy for a minimum of 1 month. Major adverse events occurred in 11 patients
(7 hemorrhagic, 3 thromboembolic, 1 other) which led to 7 or 11 patients discontinuing therapy. Of the documented patients, 88% remained on DOAC therapy at 3 months.
Conclusions: Our results demonstrate concerns regarding medication persistence with a high percentage of patients newly started on DOAC therapy being lost to follow-up within 1 month and a small but significant percentage of inappropriate dosing. Clinical outcomes may be adversely affected by these factors. These results support the concept that follow up with an anticoagulation clinic can aid in the transition of care and appropriate use for patients newly started on DOAC therapy.

PB 1228 | Selection of Long-term Anticoagulation Therapy after Acute Pulmonary Embolism in Real Life

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Background: In patients with acute pulmonary embolism (APE), two classes of oral anticoagulants are recommended for long-term treatment - vitamin K antagonists (VKAs) and direct oral anticoagulants (DOACs). Clinical trials indicate that DOACs are non-inferior and possibly safer than VKAs (ESC Guidelines, 2014).
Aims: To assess the factors for long-term oral anticoagulant class selection after APE in real-life practice.
Methods: The prospective cohort study included 147 consecutive patients from a single centre from June 2014 till October 2016 presenting with symptomatic APE. Statistical analyses were conducted using SPSS 23.0. Pearson's chi-square test, Student's t-test or Mann-Whitney U test were used.
Results: 136 patients survived until discharge. As their long-term treatment 134 received oral anticoagulants. DOACs were prescribed to 92 (68.7%) and VKAs to 42 (31.3%). Statistically significant factors in favour of VKA selection were gender (male: 54.8% in VKA group vs 35.9% in DAOC group, p=0.040), chronic heart failure (59.5% vs 39.1%, p=0.028), chronic lung disease (23.8% vs 7.6%, p=0.009), creatinine clearance (CrCl, Cockcroft-Gault) < 60 mL/min (39.0% vs 22.2%, p=0.046). However, in favour of DOACs were higher body-mass index (BMI) (kg/m2, median (IQR): 26.8 (24.4-30.4) vs 29.5 (25.7-34.6), p=0.016) and presence of transient risk factors for venous thromboembolism (recent surgery, immobility, hormonal therapy, recent travel) (16.3% vs 38.0%, p=0.011).
Higher Charlson Comorbidity Index (CCI) values were more present in VKA group (mean (SD): 4.8 (2.3) vs 3.7 (2.8), p=0.029). Presence of cancer was similar between both groups.
Conclusions: Male gender, chronic heart failure, chronic lung disease, CrCl < 60 mL/min, and overall higher burden of comorbidities (CCI)

are associated with preference of VKAs for long-term treatment of APE in real-life practice. Patients with higher BMI and transient risk factors more often receive DOACs.

PB 1229 | Impact Analysis of Prognostic Stratification for Pulmonary Embolism: The iAPP Study

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Background: Pulmonary Embolism Severity Index (PESI) is an extensively validated prognostic score on the risk of adverse outcomes after acute pulmonary embolism (PE). However, there is no evidence that adopting PESI changes physicians behaviour, improves patients outcomes and/or reduces costs.

Aims: To demonstrate that the use of PESI will help physicians to correctly identify PE patients at low-risk of adverse outcomes, thus discharging them earlier and reducing the length of hospital stay (LOS).

Methods: The iAPP study (Impact Analysis of Prognostic Stratification for Pulmonary Embolism) is a multicenter randomized controlled trial, enrolling consecutive adult outpatients with an objective diagnosis of acute PE. Within 48 hours from diagnosis, treating physicians are centrally randomized, for every patients, to stratify PE prognosis by formally calculating PESI and reporting it in the clinical record form or to routine practice. Randomization is stratified by the intented treatment choice.

The primary outcome is the median LOS. Secondary efficacy outcomes include the proportion of patients undergoing complete home-treatment, post-discharge visits to emergency department or hospital re-admissions. Safety outcomes include mortality, PE complications, hospital-acquired infections or iatrogenic complications.

To find a statistically significant difference ($p< 0.05$) between the median LOS of the two groups, with an α error of 0.05 and a statistical power of 80%, 220 patients for each group need to be enrolled. The study was approved by the ethics committee of the participating centers. ClinicalTrials.gov identifier NCT03002467.

Results: The study is ongoing. Enrollment started in September 2016 and thirty patients from five active centers were enrolled until January 2017. We estimate that 2 years are necessary to reach the sample size.

Conclusions: The iAPP study is expected to provide information on the impact of PESI in clinical practice, in terms of changing physicians behaviour on the duration of hospitalization.

PB 1230 | Do the Direct Oral Anticoagulants (Doacs) Make Managing Anticoagulant Therapy during the Peri-operative Period Easier?

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Background: The introduction of the DOACs into clinical practice is leading to significant number of patients being prescribed these agents. With their increased use, all too often, anticoagulation services are being asked to manage these agents during the peri-operative period.

Aims: To describe how many patients prescribed DOACs were referred to the anticoagulation service at King's College Hospital, London between April 2016 - December 2016, whether patients required bridging with low molecular weight heparin (LMWH), and what the outcomes were for the patients evaluated.

Methods: All patients referred to the service for peri-operative management advice had a proforma plan completed. This contained amongst other things, indication for use, DOAC prescribed, renal function, when to stop and re-start the DOAC, along with if any patients required bridging with LMWH therapy when the DOAC was stopped. These proformas were retrieved and reviewed retrospectively, following which the outcomes from the medical notes and patients were collated.

Results: During the evaluation time period 28 patients were referred; 16 males, average age 65.68 (SD 14) years of age. The hospital guidelines on managing DOAC therapy were followed in 100% of cases. One patient experienced a minor bleed and a further patient had to be taken back to theatre, following the development of a haematoma. None of the DOAC patients required bridging therapy with LMWH. During the same period, 36 warfarin patients were referred to our service for peri-operative advice, of which 17 patients required enoxaparin bridging therapy.

Conclusions: Managing DOACs during the peri-operative period is much less resource intensive, compared to warfarin therapy, with benefits observed on clinic personnel time saved.

PB 1231 | Limits of Sensitized Rotational Thromboelastometry for Rivaroxaban and Apixaban Detection

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Background: Direct oral factor Xa (FXa) inhibitors are increasingly used for the prevention and treatment of thromboembolic diseases.

Their detection may be essential in emergency situation involving invasive procedure, bleeding, or before thrombolysis in acute ischaemic stroke. Target-specific assays (anti-Xa) quantifying the drugs in plasma are out of reach in emergency.

Aims: Rotational thromboelastometry (ROTEM) is performed with whole blood and available in a number of operating rooms and intensive care or emergency units but the marketed ROTEM assay only detects high amounts of inhibitors. Since thrombin generation assay and clot waveform analysis are adequately sensitive to FXa inhibitors, we investigated the potential of ROTEM.

Methods: We first verified that sensitized ROTEM has the potential to detect low level of FXa inhibitors in platelet poor plasma. We studied the interferences of platelet count, fibrinogen level, and haematocrit on inhibitor detection. We tested whole blood spiked with FXa inhibitors from healthy volunteers. Finally we evaluated the method with whole blood from 66 patients.

Results: We established that, independently, neither platelets nor fibrinogen or haematocrit preclude FXa inhibitor detection. We established what would be the lowest amount of FXa inhibitor detected in whole blood taking into account the inherent variability of samples having haematocrit comprised between 30 and 60%. Regrettably sensitized ROTEM failed to safely detect FXa inhibitors in patients.

Conclusions: Study suggests that in real life conditions sensitized ROTEM is not relevant in clinical practice for emergency FXa inhibitor detection.

PB 1232 | Choice of Anticoagulation in the Obese

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Background: The optimal anticoagulation strategy in the obese is unclear. There is limited available data to guide the use of direct oral anticoagulants (DOACs) and low molecular weight heparin (LMWH) in this patient group.

Aims: Examine anticoagulant choice, drug specific anti-Xa levels and clinical outcomes in this group.

Methods: We identified patients with venothromboembolism (VTE) and weight >120kg or BMI >40kg/m^2 attending Western Sydney Haematology Services in 2016. We collected demographic data, weight, height, indication, anticoagulant choice, drug monitoring, dose adjustments and clinical outcomes.

Results: We identified 21 VTE patients with a median weight of 136kg (range 119-208). 8 were given DOACs as initial anticoagulant (5 rivaroxaban, 3 apixaban) and 7 subsequently received DOACs during follow-up. Anti-Xa levels on rivaroxaban 20mg daily were: peak 65-96ng/ml (n=3) and trough 13-32ng/ml (n=2). Anti-Xa levels on apixaban 5mg BD were: peak 97-141ng/ml (n=3) and trough 39-48ng/ml (n=2). 10 patients received protracted enoxaparin therapy monitored by Anti-Xa levels (n=8) and 4 required dose adjustments. Median dose to achieve therapeutic Anti-Xa (0.6-1.2U/ml) was 0.87mg/kg (range 0.66-1.1). 3 patients had recurrent thromboses while anticoagulated, 2 on rivaroxaban (troughs 13ng/ml and 23ng/ml) and 1 when warfarised previously. Bleeding complications occurred in 1 on enoxaparin and 2 on rivaroxaban (menorrhagia).

Conclusions: There is limited data on the appropriate use of LMWH and DOACs in obese patients. Although measurement of DOAC drug levels in obese patients has been recommended by ISTH, there is limited data available on clinical correlates of DOAC drug levels or therapeutic ranges. Compared with available published DOAC ranges, peak levels appear to be reduced in the obese population, however trough levels appear appropriate. The clinical significance of this is yet to be determined. We have commenced a multi-centre prospective registry of obese patients requiring anticoagulation.

PB 1233 | Antithrombotic Effects of Edoxaban, a Direct Factor Xa Inhibitor, and Antiplatelet Agents and their Combination Effects on Stent Thrombosis in Rats

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Background: Stent thrombosis is an uncommon but serious complication after percutaneous coronary intervention and femoropopliteal endovascular intervention in patients with peripheral artery disease.

Aims: The aim of this study was to determine the antithrombotic effects of a direct factor Xa inhibitor edoxaban alone or in combination with antiplatelet agents in a rat model of stent thrombosis.

Methods: Bare metal stents (4 per rat) were inserted in an ex vivo arterio-venous shunt in rats. Stent thrombosis was induced by exposing to arterial blood for 30 min. Protein content of the thrombus was measured. Edoxaban and antiplatelet agents (aspirin, clopidogrel, and ticagrelor) were orally administered 30 min and 2 hours before the stent exposure to blood, respectively. As a control, 0.5% methylcellulose was administered.

Results: In this rat stent thrombosis model, edoxaban (0.3 - 3 mg/kg), aspirin (1 - 100 mg/kg), clopidogrel (1 - 30 mg/kg), and ticagrelor (0.3 - 3 mg/kg) exerted significant and dose-dependent antithrombotic effects. The maximum inhibition by each compound was 86% (3 mg/kg edoxaban), 83% (100 mg/kg aspirin), 94% (30 mg/kg clopidogrel), and 89% (3 mg/kg ticagrelor). The combination of submaximum doses of edoxaban and aspirin or edoxaban and clopidogrel significantly potentiated the antithrombotic effects compared with these drugs alone.

Conclusions: This study demonstrated that a direct factor Xa inhibitor, edoxaban, significantly and dose-dependently inhibited the formation of stent thrombosis in a rat model. The effect of edoxaban was comparable to that of antiplatelet agents, aspirin, clopidogrel,

and ticagrelor. The concomitant use of edoxaban and antiplatelet agents exerted the combination effects. These results suggest that edoxaban alone and in combination therapies with edoxaban plus aspirin and edoxaban plus clopidogrel are promising options for prevention of stent thrombosis.

PB 1234 | Dental Extractions on NOACs withouTStopping Therapy (DENTST) Study: Interim Analysis

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Background: International guidelines recommend warfarin be continued for dental extractions, as bleeding rates are not high; instead, discontinuation can result in venous thromboembolism (VTE). There is currently no published data from clinical trials to guide the management of patients on new oral anticoagulants (NOACs) requiring dental intervention.

Aims: To determine the safety of performing dental extractions on patients taking NOACs. Secondary aims are to identify factors associated with increased bleeding risk and to determine if there is a safe lower limit of NOAC drug level below which dental extractions may be safely performed.

Methods: This is a prospective cohort study with 3 groups: patients on NOAC, warfarin, or no anticoagulant. The study has local ethics approval and informed consent is obtained. Participants do not withhold their anticoagulant. Blood tests are measured immediately prior to extraction. After extraction, Surgicel is placed in the socket, the socket then sutured and pressure applied with gauze. The gauze is weighed before and after haemostasis is achieved, and blood loss estimated by laboratory analysis. Bleeding complications are assessed at 48 hours and 7 days.

Results: Recruitment commenced in February 2016 with a target completion of November 2018. Data from 41 participants available at the time of abstract submission is summarised in tables 1 and 2. There have been 2 episodes of clinically relevant non-major bleeding: both were delayed bleeding requiring unanticipated dental review in patients on warfarin with therapeutic INRs. The difference in weight of gauze appears to indicate that patients on NOACs bleed less than warfarin. Interim blood loss analysis suggests this is typically low and not clinically significant.

TABLE 2 Anticoagulant drug levels

Oral anticoagulant	Warfarin	Apixaban	Dabigatran	Rivaroxaban
Assay	INR	Anti-Xa	Dilute thrombin time	Anti-Xa
Median drug level (range)	2.2 (2.0-2.4)	136.1 ng/ml (37.3-238.7)	122.3 ng/ml (68.5-188.1)	137.0 ng/ml (11.0-487.0)

Conclusions: Interim results suggest that bleeding outcomes in patients on NOACs are comparable and perhaps less than patients on warfarin. NOAC interruption may be unnecessary for dental extractions.

PB 1235 | Patients on a Fixed Dose of DOAC: What Percentage is in the Therapeutic Window?

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Background: Adequate antithrombotic medication ideally modulates the function of the clotting system to a certain therapeutic window (TW) to minimize thrombotic recurrence while maintaining adequate hemostasis, i.e. prevent bleeding. For anti-vitamin K (AVK) treatment

TABLE 1 Patient details and outcomes

Oral anticoagulant	Warfarin	All NOACs	Apixaban	Dabigatran	Rivaroxaban	No anticoagulant
Patient number	6	22	12	4	6	13
Total number of teeth extracted	10	48	17	15	16	27
Total number of roots extracted	15	67	27	18	22	48
Any bleeding	3 (50%)	5 (23%)	3 (25%)	1 (25%)	1 (17%)	1 (8%)
Major bleeding episodes	0	0	0	0	0	0
Clinically relevant non-major bleeding episodes	2 (33%)	0	0	0	0	0
Minor bleeding episodes	1 (17%)	5 (23%)	3 (25%)	1 (25%)	1 (17%)	1 (8%)
Difference in weight of gauze per tooth (grams)	3.24	2.00	2.91	1.80	1.29	1.82
Difference in weight of gauze per root (grams)	2.16	1.42	1.83	1.50	0.94	1.03

the TW is roughly an international normalized ratio (INR) of 2-4. For heparins and direct oral anticoagulants (DOACs), the TW is unknown. Therefore, their dosage can only be based on the outcome of clinical trials using standard dosages.

Ample evidence exists that the thrombin generating capacity (TGC) of plasma, measured as the area under the thrombin generation curve (Endogenous Thrombin Potential, ETP) is an adequate surrogate parameter for both thrombotic- and bleeding tendency. An INR of 2-4 corresponds to a TGC of 33-66% of the normal mean, and TGC < 30% is the clinical limit of a bleeding phenotype in congenital bleeding disorders. We therefore assume 66%>TG>33% as a tentative TW for all anticoagulant treatment.

Aims: To estimate the amount of patients on DOACs within the TW.

Methods: Plasmas from 60 healthy volunteers were spiked with low molecular weight heparin (LMWH), unfractionated heparin (UFH) or DOACs at concentrations that inhibit TGC in pooled normal plasma by 40%. In plasma of 48 patients on rivaroxaban samples were harvested 2±0.6 h after intake. The concentration of the drug was measured by its inhibition of factor Xa activity and the ETP was assessed by Calibrated Automated Thrombinography.

Results: The individual response of normal plasmas to an IC40 concentration of heparin and DOACs proved to be highly variable (Table).

TABLE 1

	Normal plasma	N	CV (%)	Above TW	Below TW
Spiked Plasma	None	60	18.0	99	0
	UFH	60	27.8	14	9
	LMWH	50	29.0	15	10
	DOAC-IIa	40	23.9	10	6
	DOAC-Xa	40	21.5	10	5
Patient Samples	DOAC-Xa	22	27.0	14	9

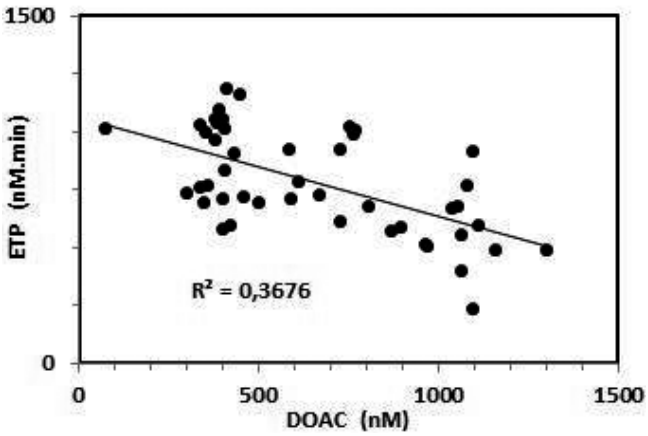

FIGURE 1

The level of the direct anti-Xa inhibitor measured in patient samples had a CV of 46% and a poor correlation was found between inhibition of ETP and the DOAC levels (Fig. 1).

Conclusions: Inter-individual variation of both pharmacokinetics and pharmacodynamics of the tested DOACs is so high that maintenance within the TW is only possible by personalized dosage guided by the effect of the drug on the TGC.

PB 1236 | Comparing the Performance of Different Assays for Monitoring Dabigatran Plasma Concentrations in Real-practice

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Background: Patients under therapeutic doses of dabigatran, a direct thrombin inhibitor, do not require routine coagulation monitoring. However, it may be important in certain clinical situations to assess anticoagulant activity. Most guidelines recommend to perform activated partial thromboplastin time (APTT) and thrombin time as screening assays, and dilute thrombin time (dTT) or ecarin chromogenic assays (ECA) to determine dabigatran plasma concentration.

Aims: Compare the results obtained with ECA, used in our laboratory, and two dTT assays, and correlate ECA with APTT. This will enable us to choose the best test for our hospital.

Methods: Blood samples were collected from 18 patients taking therapeutic doses of dabigatran, admitted to our emergency room. Plasma dabigatran concentrations were determined with STA®-ECA II (Diagnostica Stago) and compared with 2 methods of dilute thrombin time: Hemoclot® Thrombin Inhibitor (Hyphen Biomed) and a dilute thrombin time assay based on STA®-Thrombin. APTT was determined with Pathromtin® SL (Siemens). All tests were performed on STA-R® Max coagulation analyser (Diagnostica Stago).

Results: ECA showed high correlation with dTT assays, across a broad range of dabigatran levels. The correlation between ECA and Hemoclot® was very strong (R=0,980; R^2=0,960), as well as between ECA and dTT based on STA®-Thrombin (R=0,994; R^2=0,987). Both dTT assays values were similar, with excellent correlation (R=0,994; R^2=0,989). The correlation between ECA and APPT was strong (R=0,814; R^2=0,663). ECA showed to be more sensitive and useful for detecting very low dabigatran levels (limit detection level: 15 ng/ml) than dTT assays (limit detection level: 50 ng/ml).

Conclusions: This study showed that dTT and ECA accurately identify a broad range of dabigatran levels. The lower limit detected by ECA is 15 ng/ml, which can be important for patient needing high-risk surgery. Facing these results we decide to maintain ECA as the quantitative test for dabigatran in our hospital.

PB 1237 | Total Thrombus-formation Analysis System (T-TAS) as a Potential Tool for Assessing Comprehensive Hemostatic Function in Patients Taking Dabigatran

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Background: Patients with non-valvular atrial fibrillation (NVAF) have an increased risk of stroke. To reduce this risk, patients with NVAF are recommended to take anticoagulants. Among these, direct oral anticoagulants (DOACs) are now widely used. DOACs are used as fixed doses without laboratory monitoring, however, it is still possible that some monitoring tools may be useful for identifying patients with high risk of bleeding complications. Recently, we have developed a flow channel-based total thrombus-formation analysis system (T-TAS®) that quantitatively analyzes the process of mixed thrombus formation involving platelets and fibrin.

Aims: In this study we analyzed correlation and discrepancy between conventional coagulation tests and T-TAS.

Methods: Blood samples were taken from patients with NVAF before initial dabigatran dosing (baseline), 2 hours after initial dosing (2h post-dose) and before the day 7 dosing. Dabigatran levels were measured by diluted thrombin time (Hemoclot®), and thrombotic abilities were measured by T-TAS and conventional coagulation tests.

Results: A total of 19 cases were evaluated in this study. Dabigatran levels on 2h post-dose correlated with the PT ratio to baseline (R=0.667), the APTT ratio to baseline (R=0.829), and the T-TAS occlusion time ratio to baseline (R=0.899). A part of patients showed discrepancy between T-TAS occlusion time and APTT, and elder patients exhibited this tendency.

Conclusions: T-TAS is a novel tool that reflects the anticoagulant activity of dabigatran. This tool may provide additional information as to comprehensive hemostatic function in patients taking dabigatran. Further studies are needed to elucidate whether T-TAS is useful for identifying patients with high risk of bleeding complications.

PB 1238 | Evaluation of Dabigatran Concentrations in Patients Plasma. Comparison of Four Commercially Available Assays

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Background: Dabigatran is a new direct oral anticoagulant that inhibits specifically thrombin. It achieves strong anticoagulation that is more predictable than warfarin. Even though the routine monitoring of dabigatran is not recommended, there are circumstances in which the evaluation of its plasma level could be valuable such as emergent surgery, and occurrence of bleeding episode in one of the treated patients.

Aims: To evaluate the clinical performances of four commercially available assays for dabigatran in clinical materials.

Methods: We evaluated 145 plasma samples obtained from 108 patients. Indication of blood collection was emergent surgery, routine purpose, and bleeding complications including one episode that required the use of the antidote idarucizumab. Dabiigatran levels were measured using three clotting assays: the DG DTI assay performed on the QSmart analyzer, the HemosIL Direct Thrombin Inhibitor Assay and the Hemoclot Thrombin Inhibitors, the two latter were performed on the ACL TOP 700 analyzer, and one chromogenic assay (STA-ECAII assay) performed on the STAR Max analyzer. Assays were calibrated using the specific lyophilized calibration plasmas obtained from the reagent manufacturer, and the same applied for the control plasmas.

Results: The within-run and between-run precisions evaluated using control lyophilized samples, in the low and high ranges of concentrations, were below 10% for the four assays. Plasma dabigatran levels were measured in the range from below the detection limit of the techniques to 1250 ng/mL. The correlation coefficients between test results obtained using the four methods were above 0.98 in all cases with mean bias, calculated according to Bland-Altman, below 7.0%.

Conclusions: Test results obtained using the four evaluated assays for dabigatran are in good concordance, and their performances allow their use in clinical routine to evaluate the drug concentration in the plasma from treated patients.

PB 1239 | Comparison of the LMWH Tinzaparin and the Direct Inhibitors of Factor Xa and Thrombin on the Kinetics and Qualitative Characteristics of Blood Clot. An in vitro Thromboelastometric Study

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Background: Fibrin clot structure and firmness are major determinants for thrombus early lysis in patients with venous thromboembolism (VTE).

Aims: The comparison of the effect of tinzaparin, rivaroxaban and dabigatran on the clot formation kinetics and quality.

Methods: Citrated whole blood samples from healthy volunteers were spiked with clinically relevant concentrations of rivaroxaban or dabigatran (10 to 500 ng/ml) or tinzaparin (0.05 to 0.8 anti-Xa IU/ml). Combination of rivaroxaban and dabigatran at clinically relevant concentrations were used. Whole blood thromboelastometry, triggered by low tissue factor concentrations (5 pM) was assessed with

TABLE 1 Patient characteristics, management of bleeding and biological measurements for major bleeding events

Gender, age (years)	CLcr (ml/min)	DOAC (dosage, indication)	Time after last intake (hours)	Site of bleeding	DOAC plasma concentrations (ng/ml)	Management	Length of stay (days)	Outcome at 90 days
F, 70	Unknown	Rivaroxaban 15 mg OD, SPAF	22	GI bleeding	Biophen DiXaI: 237.5	RBC (3 units)*	Unknown	Unknown
F, 87	48	Rivaroxaban 20 mg OD, VTE	38	GI bleeding	Biophen DiXaI: 70.8	RBC (2 units)*	3	Alive
F, 67	53	Rivaroxaban 20 mg OD, SPAF	27	GI bleeding	Biophen DiXaI Low: 58.3	RBC (3 units)*	1	Alive
F, 74	73	Apixaban 5 mg BID, SPAF	5	IC bleeding	Biophen DiXaI: 298.0	-	9	Alive
F, 77	61	Rivaroxaban 15 mg OD, SPAF	27	IC bleeding	Biophen DiXaI: 139.4	PCC 2500 IU (40 IU/kg)	43	Alive
F, 76	33	Dabigatran etexilate 110 mg BID, SPAF	53	GI bleeding	HTI Low: 19.0	RBC (1 unit)	34	Alive
F, 90	60	Rivaroxaban 15 mg OD, SPAF	27	IC bleeding	Biophen DiXaI: 86.5	-	1	Alive

* Transfusion before blood sampling. BID: twice daily, CLcr: creatinine clearance (according to Cockroft-Gault equation), Biophen DiXaI: Biophen® Direct Factor Xa Inhibitors, GI: gastrointestinal, HTI: Hemoclot Thrombin Inhibitor®, IC: intracranial, OD: once daily, PCC: prothrombin complex concentrates, RBC: red blood cells, SPAF: stroke prevention in atrial fibrillation, VTE: venous thromboembolism

the ROTEM®; Clotting Time (CT), Clot Formation Time (CFT), α-angle, Maximum Clot Firmness (MCF) were analyzed.

Results: Rivaroxaban and dabigatran, prolonged the CT but did not significantly alter the CFT, the α-angle and the MCF. At equivalent gravimetric concentrations dabigatran effect was more pronounced compared to rivaroxaban. When the two agents were combined no amplificatory effect on CT was observed. The α-angle and MCF were not significantly affected. Tinzaparin induced a significant prolongation of CT and CFT and reduced the α-angle and MCF values as compared to the control. Tinzaparin impact was significantly more potent than the DOAC combination used even at the higher studied concentrations.

Conclusions: When coagulation is triggered by physiologically relevant concentrations of TF, rivaroxaban and dabigatran prolong the CT, did not alter fibrin polymerization kinetics nor clot firmness. The combination of rivaroxaban and dabigatran did not alter clot formation process suggesting that the important effect of tinzaparin on clot formation quality is not linked to its anti-Xa or anti-IIa activity association but most probably is related to a direct interference with fibrin network and others antithrombin dependent inhibitory effects.

PB 1240 | Measurement of Direct Oral Anticoagulants Levels in Patients Admitted for Bleeding Events: A Prospective Study

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Background: Although direct oral anticoagulants (DOAC) do not require close laboratory monitoring, their measurement remains useful in specific clinical situations. A therapeutic range has not been defined yet, but DOAC plasma concentrations were shown to correlate with bleeding outcomes.

Aims: To describe DOAC plasma concentrations in patients with bleeding events, in relation to clinical and medication data.

Methods: As part of a prospective observational study conducted in the emergency departments of 2 hospitals, we collected 14 plasma samples from DOAC-treated patients admitted for bleeding events. Routine clotting assays were performed as well as specific assays using CE approved procedures (calibrated chromogenic anti-Xa assays for apixaban and rivaroxaban and diluted thrombin time and ecarin chromogenic assay for dabigatran). Clinical data were collected on admission, at discharge and at 90 days. Bleeding severity was assessed using ISTH definition. The study was approved by the Ethics Committees.

Results: Patients (median age 75 years) were mainly treated with rivaroxaban (10/14), for stroke prevention in atrial fibrillation (10/14). The most frequent site of bleeding was gastrointestinal (6/14). Seven bleedings were major (table 1).

Conclusions: A large range of DOAC plasma concentrations was observed, with above on-therapy levels estimated in around half of patients admitted for bleeding events. Among the factors contributing to bleeding, potential drug interactions are frequent in anticoagulated patients and should be screened.

PB 1241 | Direct Oral Anticoagulants for Pulmonary Embolism in Patients Initially Admitted to Sub Intensive Care Unit for Intermediate High Risk Index Event

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Background: Pulmonary Embolism (PE) is a potential life-threating cardiovascular emergency with an early mortality rate varying up to 25% depending on the severity of presentation.

DOACs (Direct Oral Anticoagulants) have been developed to address limitations associated with traditional anticoagulant therapy. Anyway due to the scarce representation of intermediate high risk patients in clinical study, parenteral anticogaulants overlapping with oral vitamin k antagonists, still represent the mainstay of treatment in many Sub Intensive Care Unit (SICU).

Aims: The aim of our pivotal register is to obtain a reliable estimate of the risks and benefits of DOACs treatment in PE patients initially admitted to SICU.

Methods: Consecutive patients admitted for intermediate high risk PE to the two SICU of our hospital in the last 24 months were retrospectively evaluated. For patients who started DOAC, informations on recurrent venous thromboembolic and major bleeding events were collected at discharge and at the 3rd and 6th month follow up visits.

Results: 7 of the 77 patients admitted to the two SICU died during hospital stay (9.1% mortality). To 55 of the remaining 70 patients (78.6%) DOAC was prescribed after a period of parenteral therapy variable from 0 to 24 days (median 3 days). Moreover 7 patients (12.8%) were thrombolysed before starting any other therapy.

At the time of the index event the mean age was 70.1 (SD±16.5) while the median PESI (Pulmonary Embolism Severity Index) scored 104.0 (range 24-181) and signs of ventricular dysfunction on an imaging test were present in 40 patients (72.7%).

Of the 55 patients discharged on DOAC, 37 started Rivaroxaban, 17 Apixaban and 1 Dabigatran: up to February 1st 2017 no recurrent venous thromboembolic and 2 major bleeding events were observed in the 6 months period of active therapy.

Conclusions: DOACs appear to be a reasonable alternative for patients admitted to SICU for intermediate high risk PE after an initially favorable evolution.

PB 1242 | Oral Anticoagulant Associated Bleeding Events - A Single Centre Experience

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Background: We report our single centre experience of patients presenting with major bleeding whilst on an oral anticoagulant (OAC: warfarin or DOAC) and their clinical outcomes, reported to the ORANGE registry.

Aims: Based on primary care OAC prescription data, as a denominator, we aimed to determine the incidence of major bleeding in real-life terms.

Methods: During a 32 month observation period (2013-2016) we documented 186 major bleeding events - defined as bleeding leading to hospitalisation and one or more of the following: death, transfusion of ≥ 2 units red blood cells, drop in haemoglobin of ≥ 20g/L, bleeding into a critical organ, administration of plasma or coagulation factor based therapies.

Results: Based on prescription data during the study period, those prescribed an OAC rose from 1.44% to 2.0% of our hospital catchment population (circa. 320,000) with those receiving a DOAC increasing from 6.2% to 38.7%. The total number of anticoagulated patient years was calculated as 14,921y. Of the 186 patients who developed major bleeds the median age was 78y with 53% female. The overall OAC-associated bleed rate was 1.25%/y. Table 1 details bleed characteristics by OAC type and incidence rates (bleeds/100 patient years), with 95% CI calculated by Fisher's exact test. No bleeds were recorded during 150 patient years dabigatran treatment. The majority of bleeds on rivaroxaban and apixaban (87% and 66% respectively) were encountered within the first year of therapy with that agent, compared with 10% for warfarin. We observed significantly lower overall and gastrointestinal bleed rates with apixaban compared with warfarin and a similar non-significant trend for intracranial bleeds and 30-day post bleed mortality.

Conclusions: Despite the small cohort size, this real-world data is consistent with results from clinical trials reporting a lower incidence of bleeding events with apixaban, but not with rivaroxaban, when compared to warfarin.

TABLE 1 Bleeding rates by OAC

	Warfarin	Rivaroxaban	Apixaban	All OACs
Patient years f/u	11461	1196	2114	14921
Bleeds: n, incidence [95% CI]	162, 1.41 [1.20-1.65]	15, 1.25 [0.702-2.069]	9, 0.43 [0.195-0.808]	186, 1.25 [1.074-1.439]
Intracranial bleed: n, incidence [95% CI]	69, 0.60 [0.468-0.762]	5, 0.42 [0.136-0.976]	6, 0.28 [0.104-0.618]	80, 0.54 [0.425-0.667]
GI bleed: n, incidence [95% CI]	63, 0.55 [0.422-0.703]	6, 0.50 [0.184-1.092]	3, 0.14 [0.029-0.415]	72, 0.48 [0.378-0.608]
30d mortality: n, incidence [95% CI]	34, 0.30 [0.205-0.415]	4, 0.33 [0.091-0.856]	1, 0.05 [0.012-0.264]	39, 0.26 [0.186-0.357]

PB 1243 | Influence of Direct Oral Anticoagulants in Fibrinolysis Testing

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Background: The effect of Direct Oral Anticoagulants (DOACs) on routine tests of haemostasis is now well known; the effect on special tests (factor assays or thrombophilia testing) begins to be reported. However, there are few studies on the effect of DOACs on fibrinolysis tests.

Aims: Evaluate the influence of the direct thrombin inhibitors and direct FXa inhibitors, on fibrinolysis.

Methods: We have studied plasma samples from 16 patients on dabigatran, 17 on rivaroxaban and 22 on apixaban: TAFI, PAI-1, Plasminogen (PLG), antiplasmin (AP) activity and FXIII antigen were evaluated. The DOAC concentration was determined in all samples. All the reagents were supplied by Stago (France) and run on a STA Compact Max.

Results:

Dabigatran: 8 samples had concentration < 50ng/mL (4 below detection limit) and 8 above 50ng/mL. TAFI level was low (median 54%) in 10 samples but showed no correlation with Dabigatran level (p< 0.2). FXIII was not analysed.

Rivaroxaban: 12 samples had concentration < 90 ng/mL and 5 above; 1 sample had TAFI level 52% and 2 with low FXIII.

Apixaban: 5 samples with level below 90 ng/mL, and 16 with level >90ng/mL; 4 samples had low FXIII. None of the parameters presented significant correlation with the DOAC concentration. All samples had normal PLG, AP levels, except for one sample with dabigatran with undetectable PAI-1.

Conclusions: There was a marked decrease on TAFI from patients on dabigatran, not observed on patients taking rivaroxaban or apixaban. Correlation between dabigatran concentrations and the decrease on TAFI was not found, however the number of samples analysed was low. These results reflect the inhibition of thrombin that is the activator of TAFI and acts directly on its regulation. The other parameters studied seems not to be affected by DOAC.

PB 1837 | Natriuretic Peptides for Detection of Heart Failure in Patients with Atrial Fibrillation? An IPD Analysis of Opportunistic Screening Studies from the Community

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Background: Heart failure (HF) increases the risk of atrial fibrillation (AF), and the vice versa. Early detection of concurrent heart failure in patients with AF has important clinical consequences. However, both diseases share similar symptomatology and elevated natriuretic peptide levels, making it difficult to identify AF patients in need for additional diagnostic testing for heart failure, in particular for primary care physicians.

Aims: To assess the diagnostic value of amino-terminal pro B-type natriuretic peptide (NTproBNP) for uncovering heart failure in patients with atrial fibrillation.

Methods: Individual patient data from four opportunistic HF screening studies in older high-risk persons from the open population (≥60 years and type 2 diabetes, ≥65 years and chronic obstructive pulmonary disease, ≥65 years and shortness of breath, and ≥65 years and multimorbidity) were used. All participants underwent an extensive clinical assessment, blood testing, electrocardiography, and echocardiography. Presence or absence of HF was established by an expert panel following the criteria of the European Society of Cardiology on HF. We used a two-stage mixed effects regression meta-analysis to calculate discrimination; efficiency; the proportion of missed cases; and sensitivity, specificity and predicted values.

Results: In 1,941 individuals with median age 72 (IQR 67-78) years and 49.7% male, 196 (10.1%) cases had atrial fibrillation. Heart failure was uncovered in 82 (43%) patients with AF. Median NTproBNP levels of AF patients with and without uncovered HF were 744 pg/mL and 211 pg/mL, respectively. Fourty-three (21.9%) AF patients had NTproBNP value below 125 pg/mL. At this recommended exclusionary cut-point, the sensitivity was 93%, specificity 36%, and the positive value and negative predictive value 52% and 87%, respectively.

Conclusions: In older high-risk AF patients from the community the prevalence of unrecognized HF is very high (42.5%), and straightforward echocardiography should be considered.

PB 1838 | Direct Oral Anticoagulants (DOAC) vs Low Molecular Weight Heparin (LMWH) as Anticoagulant Therapy in Patients with Non-valvular Atrial Fibrillation (NVAF) and Concomitant Active Cancer

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TABLE 1 Demographic characteristics

Demographic characteristics	DOAC (n=96)	LMWH (n=55)	p value
Gender (male)	52 (54.1%)	30 (54.5%)	0,964
Age (years)	78,73 ± 1,5	74,88 ± 1,94	0,002
Median CHA2DS2-VASc (Median HAS-BLED)	4 (3)	4 (2)	
Arterial hypertension	78 (81.2%)	42 (76.3%)	0,483
Diabetes mellitus	30 (31.2%)	16 (29%)	0,780
Cardiac failure	31 (32.2%)	20 (36.3%)	0,613
Myocardial ischemia	13 (13.5%)	9 (16.3%)	0,643
Vasculopathy	5 (5.2%)	5 (9%)	0,387
Previous stroke	14 (14.5%)	6 (10.90%)	0,506

TABLE 2 Cancer subtypes and chemotherapy agents

Cancer subtypes	DOAC (n=96)	LMWH (n=55)	p value	Chemotherapy agents	DOAC (n=52)	LMWH (n=40)	p value
Breast cancer	16 (16.6%)	10 (18.1%)	0.814	Antimitotic agents	3 (5.7%)	10 (25%)	0.011
Prostate cancer	11 (11.4%)	3 (5.4%)	0.178	Antimetabolite agents	9 (17.3%)	12 (30%)	0.155
Lung cancer	6 (6.2%)	11 (20%)	0.020	Hormonotherapy	21 (40.3%)	4 (10%)	0.0002
Digestive tract cancer	15 (15.6%)	9 (16.3%)	0.905	Alkylating agents	3 (5.7%)	4 (10%)	0.461
Urothelial cancer	8 (8.3%)	4 (7.2%)	0.813	Platinum based agents	2 (3.8%)	5 (12.5%)	0.140
Lymphoproliferative syndromes	17 (17.7%)	8 (14.5%)	0.606	Tyrosine kinase inhibitors	13 (25%)	6 (15%)	0.225
Plasmatic cell disorders	7 (7.2%)	5 (9%)	0.701	Monoclonals antibodies	17 (32.6%)	19 (47.5%)	0.147
Others	26 (27%)	12 (21.8%)	0.463	Immunomodulation inhibitors	2 (3.8%)	9 (22.5%)	0.008
				Others	10 (19.2%)	12 (30%)	0.235

Background: Appropriate anticoagulation management of NVAF in active cancer is of growing clinical concern, especially in those receiving chemo-immunotherapy where LMWH are still recommended despite that there is no indication for primary prevention of stroke in patients with NVAF. Nowadays, DOAC are broadly used for primary prevention of stroke. However there is no data about active cancer patients with NVAF who need a personalised approach.

Aims: To assess the safety and effectiveness in both cohorts according to DOAC or LMWH treatment and to determine the rate of anticoagulant-associated clinically relevant bleeding-free survival in our unit.

Methods: We consecutively included patients with active cancer and NVAF treated with DOAC or LMWH (2011-2016). Demographic, laboratory, cancer diagnosis, and NVAF diagnosis data were collected. All active cancers were included. Pharmacological interactions check-up was performed prior election of treatment. Bleeding events were classified according to ISTH criteria.

Results: Among 151 patients, 96 (63.6%) were treated with DOAC (10 Dabigatran, 34 Rivaroxaban, 40 Apixaban and 12 Edoxaban) and 55 (36.4%) with LMWH. Mean follow-up (FU) were 10.8 and 8.5 months (DOAC vs LMWH,p:0.56). Demographic characteristics and cancer subtypes and drugs are summarised in tables 1 and 2, respectively.

Patients undergoing chemotherapy were 54% vs 73% (DOAC vs LMWH,p:0.01). Most frequent previous anticoagulant treatment was acenocumarol (DOAC 54% vs LMWH 51%,p:0.69). During FU, no stroke was reported and 9 (9.3%) vs 7 (12.7%) major bleeding events (DOAC vs LMWH,p:0.53) occurred. Bleeding-free survival rates were not statistically different between both cohorts. There were 2 thromboembolic events in the LMWH group. All reported mortality was disease-related.

Conclusions: The safety and effectiveness of DOAC treatment for NVAF in patients with active cancer have a similar profile to LMWH. Anticoagulation units play a crucial role in offering the best personalised therapy.

PB 1839 | Thrombin Generation and International Normalized Ratio in Patients with Atrial Fibrillation Using Rivaroxaban or Warfarin

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia and predisposes patients to an increased risk of stroke. So, one of the main strategies for the treatment of AF is the use of oral anticoagulant, rivaroxaban or warfarin for prevention of stroke and systemic embolism. International normalized ratio (INR) has been traditionally used for control doses of warfarin. Thrombin is the central enzyme in blood coagulation and an increased *ex-vivo* thrombin generation (TG) can be considered a marker of thrombogenic potential in plasma.

Aims: To evaluate the effect of warfarin and rivaroxaban on the hemostatic system by using TG assays and its correlation with INR, in patients with non-valvular AF.

Methods: TG using calibrated automated thrombogram (CAT) system was performed in plasma samples from patients using rivaroxaban (n= 36) or warfarin (n= 37), and in individuals without AF (control group, n= 41 for rivaroxaban and n= 36 for warfarin). The medium plasma levels of rivaroxaban were 115.15 ng/ml. TG tests were performed using 10 pM of tissue factor and prothrombin time/INR was performed according to conventional methodology. Mann-Whitney and Spearman tests were used.

Results: Use of rivaroxaban (Table 1) and warfarin (Table 2) reduced endogenous thrombin potential (ETP) and peak and prolonged lag time, compared to controls. A significant inverse relationship between INR and ETP (r= -0.739, p=0.000) and peak (r= -0.758, p=0.000) and a positive correlation for lagtime (r= 0.482, p=0.003) were found in patients using warfarin. For patients on rivaroxaban, no correlation between TG and INR was found.

TABLE 1 Parameters of thrombin generation in patients using rivaroxaban compared to control group

Parameters	Rivaroxaban	Control	p value
Lagtime_(min) (± IQR)	3.13 (1.670)	1.33 (0.665)	0.000
ETP_(nM•min) (± IQR)	1227.5 (356.4)	1606.0 (409.5)	0.000
Peak_(nM) (± IQR)	158.6 (95.9)	386.8 (102.1)	0.000
ttPeak_(min) (± IQR)	7.30 (3.912)	3.33 (0.985)	0.000

TABLE 2 Parameters of thrombin generation in patients using warfarin compared to control group

Parameters	Warfarin	Control	p value
Lagtime_(min) (± IQR)	2.93 (1.800)	1.64 (0.7075)	0.000
ETP_(nM•min) (± IQR)	598.8 (310.1)	1577.4 (490.3)	0.000
Peak_(nM) (± IQR)	114.95 (78.4)	379.6 (127.9)	0.000
ttPeak_(min) (± IQR)	5.00 (2.490)	3.50 (1.330)	0.000

Conclusions: Warfarin and rivaroxaban provided effective anticoagulation measured by TG. As expect, INR correlated with TG parameters only in patients under warfarin therapy. Contrarily, in patients using rivaroxaban, INR was not sensitive for assessing anticoagulation status.

Support: CNPq, CAPES. and FAPEMIG

PB 1840 | Variation of Renal Function during Treatment with Direct Oral Anticoagulants and Risk of Major Bleeding in Patients with Non-balvular Atrial Fibrillation

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Background: Chronic kidney disease is a risk factor for major bleeding in patients with atrial fibrillation (AF) on treatment with warfarin.

Aims: To assess the effect of variations of creatinine clearance on the risk for major bleeding (MB) during treatment with direct anticoagulants (DOACs) in patients with non-valvular AF.

Methods: Consecutive AF patients were prospectively followed since they received the first DOACs prescription. Periodic assessments of creatinine clearance (by means of both the Cockcroft-Gault and Chronic Kidney Disease Epidemiology Collaboration equations) were performed and the incidence of MB recorded. A joint survival model was used to estimate the association between variation of creatinine clearance and the risk for MB.

Results: During a mean follow-up of 575 days in 449 patients, 44 MBs were observed (6.1% patient-year). Variations lower than 30% in creatinine clearance were common, regardless of baseline values. Decreases in creatinine clearance greater than 30% were more common in patients with baseline values lower than 60 ml/min and rarely occurred in patients with baseline values higher than 90 ml/min.

Variation of creatinine clearance over time was inversely and independently associated with the risk for MB; in particular, every 1 ml/min decrease in creatinine clearance was associated with 2% increase in the risk for major bleeding (HR 0.98, 95% CI 0.97-0.99; p< 0.001). No association was found between diabetes, chronic heart failure or treatment with different DOACs and the risk for major bleeding.

Conclusions: Variation of renal function over time is associated with the risk for major bleeding in AF patients treated with DOACs. Identification of patients and intervening clinical settings likely to be associated with variation in renal function is essential to reduce the risk of major bleeding associated with DOACs and to make this treatment further safe.

PB 1841 | Global Public Awareness about Atrial Fibrillation

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Background: Atrial fibrillation (AF) is an important cause of ischemic stroke that often remains undetected until stroke occurs. Awareness of the risk factors and symptoms of AF is important because effective stroke prevention in such patients is available. However, the extent of public awareness of AF is uncertain.

Aims: Compare global awareness of AF with awareness of other thrombotic and non-thrombotic disorders.

Methods: In collaboration with Ipsos-Reid, we conducted an internet-based survey from Sep 22 to Oct 13, 2016 in 10 countries: Argentina, Australia, Canada, Germany, Japan, Thailand, Netherlands, Uganda, United Kingdom, and United States. Participants were selected from

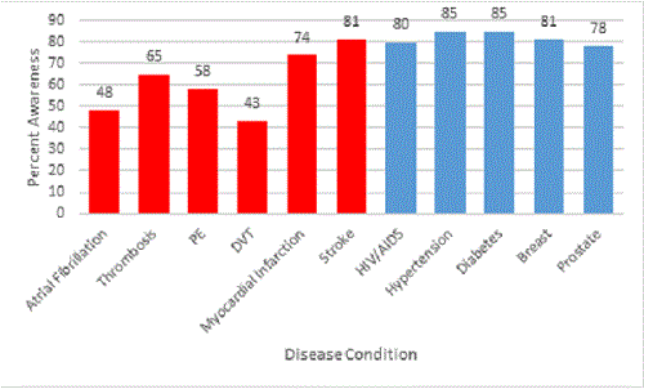

FIGURE Average overall awareness of selected health conditions. Thrombosis-related conditions are in red, whereas non-thrombotic conditions are in blue

survey panels in weighted, age-stratified categories (40-60, 61-74, and ≥75 years). The survey included 11 questions about demographics and AF awareness and took ≤5 minutes to complete. Awareness of other thrombotic disorders (thrombosis, pulmonary embolism, deep-vein thrombosis, heart attack and stroke) and non-thrombotic disorders (hypertension, HIV/AIDS, diabetes, breast cancer, and prostate cancer) was also assessed. Proportions and 95% confidence intervals (CI) were calculated.

Results: In a total of 6,312 participants, overall awareness of AF was 48% (95% CI 46%-50%), which was lower than all other thrombotic and non-thrombotic disorders except deep-vein thrombosis (43%, 95% CI: 41%-45%) (Figure). Across countries, AF awareness ranged from 25% to 69%; US-specific awareness was 67% (95% CI 62%-72%). Knowledge of AF risk factors ranged from 8%-52% (Table) and awareness AF leads to stroke was 36%-46%. Among those reporting awareness of AF, 82% correctly identified palpitations as an AF symptom.

Conclusions: Global awareness of AF is low. Although US awareness was higher than that in most other countries, possibly reflecting direct-to-patient marketing, awareness about AF was lower than that of other disorders. Increased awareness of AF is essential to reduce the burden of stroke.

TABLE 1 Distribution of participants' recognition of risk factors for atrial fibrillation

Risk Factors	%	95% CI
High blood pressure	52	50, 53
Smokin	46	44, 47
Obesity	43	41, 45
Heart failure	37	35, 38
Drinking too much alcohol	31	29, 32
Age over 75 years	27	26, 29
Diabetes	20	18, 21
Hyperactive thyroid	9	8, 10
Asthma	8	7, 9

PB 1842 | VKA Treatment and Bleeding Rate of Patients Aged Older than 90 Years: Results from a Prospective Multicentre START Register Study

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Background: Oral anticoagulant therapy (OAT) is increasingly used for prevention of stroke in atrial fibrillation (AF). Bleeds are the major concern for prescription, especially in very old patients.

Aims: We evaluate the history of patients aged≥90 years (yrs) enrolled in the multicentre prospective observational START Register to evaluate the quality of anticoagulation and the incidence of bleedings.

Methods: Patients' demographic and clinical data were collected as electronic file in anonymous form in the web site of START-Register (ClinTrials Gov Identifier: NCT02219984). The study included 4579 AF patients naïve to anticoagulation. In this study the analysis is limited to183 patients aged ≥90 yrs (97 starting treatment a≥90 yrs, 86 patients became ≥90 yrs during follow-up). The overall exposure to anticoagulants for each patient was calculated in relation to aging, before and after his/her 90th birthday. Patients characteristics and incidence of bleeds were recorded.

Results: Clinical characteristics of patients are listed in table 1. The total follow-up was 183 patients-yrs(pt-yrs), 119 patients were females(65%) 141 (77%) were treated with vitamin K antagonist (VKA) and 43 (23%) with direct oral anticoagulants (DOACs). We recorded 8 major bleedings (rate 4.37x100 pt/yrs), 6 bleeds occurred among VKAs patients (rate 4.42 x100 pt/yrs) and 2 among DOACs patients (rate 4.24); 3/8 were cerebral (rate 1.64 x x100 pt/yrs) 2 occurred in VKAs patients and 1 in DOACS patients.

TABLE 1 Patient's chracteristics

	N (%)
Heart failure	47 (25.7)
Hypertension	157 (85.8)
Diabetes mellitus	27 (14.8)
Previous Stroke/TIA	34 (18.6)
Coronary artery disease	26 (14.2)
CHA2DS2VASc score (mean±SD)	4.5±1.2
HAS-BLED (mean±SD)	2.9±0.9
Time in Therapeutic Range (IQR) (%)	56 (37-75)
Antiplatelet treatment	17 (9.3)

Conclusions: In this group of AF patients aged ≥90 yrs on anticoagulation for stroke prevention the rate of bleedings is elevated both on VKAs and on DOACs, suggesting a careful evaluation of clinical benefit of OAT in very advanced age.

PB 1843 | Low Performance of the CHA2DS2-VASc Rule for Predicting Stroke Risk in Atrial Fibrillation Patients: A Systematic Review and Meta-analysis

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Background: The CHA2DS2-VASc decision rule is widely recommended for estimating stroke risk in patients with atrial fibrillation (AF). It has seen a rapid introduction into practice guidelines, though subsequent validation studies show ambiguous and conflicting results.
Aims: To
1) review existing studies validating CHA2DS2-VASc in AF patients not (yet) anticoagulated,
2) meta-analyze estimates of stroke risk per score, and
3) explore sources of heterogeneity across the validation studies.
Methods: We performed a systematic literature review and random effects meta-analysis of studies externally validating CHA2DS2-VASc in AF patients not on anticoagulants. To explore between-study heterogeneity in stroke risk, we stratified studies to the clinical setting in which patient enrollment started, and performed meta-regression.
Results: In total 16 studies were evaluated with over one million person-years of follow-up. In studies recruiting AF patients in hospitals, stroke risk for a score of zero, one and two were 0.4% (approximate 95% prediction interval (PI) 0.03 to 2.3%), 1.2% (95% PI 0.1 - 3.5%) and 2.2% (95% PI 0.4 - 5.7%), respectively. This was consistently higher than studies recruiting patients from the open general population, with risks of 0.2% (95% PI 0.03 - 1.2%), 0.6% (0.04 - 1.8%) and 1.5% (95% PI 0.2 - 4.4%) for score zero to two respectively. As an illustration of heterogeneity, our findings implicate that for a CHA2DS2-VASc score of 1 there is still a probability of 36% to 83% that patients have a stroke risk below 1% per year; the commonly used threshold below which the benefits of anticoagulant treatment do not outweigh its bleeding risks. Heterogeneity could not be fully explained by meta-regression.
Conclusions: The CHA2DS2-VASc score is unable to accurately predict stroke risk in patients with AF. Therefore its current role in contemporary practice guidelines could be questioned.

PB 1844 | Improving Appropriate Use of NOACs in AF: Success of Online CME

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Background: Evidence-based anticoagulant therapy is the cornerstone of stroke prevention in atrial fibrillation (SPAF); however, patients are often inappropriately treated, resulting in morbidity and mortality that might otherwise be preventable.
Aims: Determine whether a video-based online continuing medical education (CME) activity can improve the knowledge and competence of cardiologists and primary care physicians (PCPs) regarding understanding and appropriate use of non-vitamin K antagonist oral anticoagulants (NOACs) in management of SPAF.
Methods: An online CME activity was developed as a 25-minute roundtable discussion with 3 leading experts on the role of NOACs in the setting of SPAF. The effects of education were assessed using a linked pre-assessment/post-assessment study design. For all questions combined, the McNemar's chi-square test was used to assess differences from pre- to post-assessment. *P* values are shown as a measure of significance; *P* values < .05 are statistically significant. Cramer's V was used to calculate the effect size (> 0.3 are large, 0.16-0.3 are medium, and < 0.16 are small).
Results: Comparison of individually linked pre-assessment question responses to the respective post-assessment question responses demonstrates statistically significant improvements for both cardiologists (n = 184; *P*< .05; V=0.2) and PCPs (n=250; *P*< .05; V=0.243). Following the education, 15% of cardiologists and 33% of PCPs selected that they had improved confidence in prescribing NOACs for a newly diagnosed patient with AF at risk for stroke.
Conclusions: The significant improvements observed for thislonline CME roundtable discussion demonstrate the benefits of educating the large target audience base. This assessment of physicians' knowledge identified education gaps that support the need to develop additional CME activities on SPAF management, including appropriate use of NOACs to optimize safety while preventing further strokes.

TABLE 1 Percentage of Participants With Correct Response by Question (Pre- and Post-Assessment Questions)

	Cardiologists (n=184)	Cardiologists (n=184)	PCPs (n=250)	PCPs (n=250)
Topic	Relative percent change; P value	Pre-assessment vs Post- assessment	Relative percent change; P value	Pre-assessment vs Post- assessment
Real-world evidence for NOACs on major bleeding/mortality	68%; <0.05	17% vs 28%	162%; <0.05	12% vs 30%
TTR data from the real-world setting for patients on VKA therapy	67%; <0.05	27% vs 45%	65%; <0.05	29% vs 48%
Stroke reduction efficacy of NOACs based on real-world registries	96%; <0.05	28% vs 55%	153%; <0.05	20% vs 50%

PB 1845 | Optimisation of Anticoagulation Therapy for Stroke Prevention in Atrial Fibrillation Using a Virtual Clinic Model

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Background: Atrial fibrillation (AF) is the leading and most preventable cause of embolic stroke. Anticoagulation is the current gold standard for the prevention of strokes in patients with AF with $CHA_2DS_2VASc \geq 2$. Quality and Outcome Framework (QOF) data for two Clinical Commissioning Groups (CCGs) in London revealed that of 2493 patients with AF and $CHADS_2$ score ≥ 2, only 1546 (62%) were anticoagulated. In the cohort of 947 high risk patients who were not anticoagulated the CCGs could expect to see 48 strokes per annum, up to 33 of which could be prevented by oral anticoagulation.

Aims:

- To ensure all patient considered at risk are offered appropriate anticoagulant therapy
- To identify reasons why patients who are at risk are not being prescribed anticoagulation
- To educate practice staff on the use of stroke risk assessment tools, bleeding risk assessment tools and the role of anticoagulation in stroke prevention in AF

Methods: Specialist pharmacist led virtual clinics were conducted with general practitioners at each practice. GP engagement was secured through the GP delivery scheme/prescribing incentive scheme. Patients with a CHA_2DS_2VASc score ≥ 2 who were not currently anticoagulated were identified and reviewed and an action plan for each patient was agreed and documented.

Results: Data from the two CCGs (Jan 2017) shows that 83% and 78% of AF patients are now anticoagulated, an increase from 73% and 72% respectively prior to the virtual clinics.

1292 patients were initiated on anticoagulation equating to the prevention of approximately 43 strokes.

The following were identified as barriers to anticoagulation:

- Misconception of bleeding risk vs stroke risk for certain patient groups
- Lack of review for patients who had historically refused warfarin
- Housebound patients unable to attend anticoagulation clinics for initiation

Conclusions: The use of specialist pharmacist-led virtual clinics led to a significant increase in the proportion of AF patients with a $CHA_2DS_2VASc \geq 2$ being appropriately anticoagulated.

PB 1846 | Evaluation of Antithrombotic Management in Patients with Atrial Fibrillation at a Tertiary Care Academic Medical Center

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Background: Atrial fibrillation (AF) affects an estimated 5 million people in the United States. Oral anticoagulation (OAC) is the preferred therapy for reducing the risk of stroke, however several studies demonstrate that the prescribing rates of OAC in these patients have remained lower than anticipated, especially in those who are at high risk for stroke.

Aims: To assess the antithrombotic management of AF patients in an academic medical center and its concordance with recommended evidence-based practices, and to explore potentials barriers to prescribing OAC.

Methods: Retrospective data analysis of 1198 patients with atrial fibrillation admitted between June 1 - December 31, 2015. Patients charts were reviewed for documented comorbidities to calculate risk of stroke using the CHADSVASC score, to evaluate OAC agent prescribed, and documented reasons for not prescribing OAC in eligible patients.

Results: OAC was prescribed in 759 (63%) eligible patients, 175 (14.6%) had documented reasons for not using OAC, and 264 (22.0%) were candidates for OAC without a documented reason. Physician specialty in cardiology and CHA2DS2-VASc score were positively associated with antithrombotic prescribing. Conversely, age, female gender and HAS-BLED score were negatively associated with antithrombotic prescribing. History of bleeding, fall risk, and patients declining therapy were the most common documented reasons for not prescribing OAC.

Conclusions: OAC use in eligible patients with AF remains suboptimal. Further research is needed to address barriers to prescribing OAC is patients who are candidates and would benefit from such therapies.

TABLE 1 Agreed patient recommendations from virtual clinics (n = 1348)

Referral for consideration of anticoagulation	Anticoagulation contraindicated	Anticoagulation not indicated	Referral for confirmation of diagnosis	Anticoagulation declined-review yearly	Other (e.g. Left Atrial Appendage Occlusion Device, patient uncontactable)
455 (33.8%)	111 (8.2%)	525 (38.9%)	79 (5.9%)	80 (5.9%)	98 (7.3%)

PB 1847 | Recent Years Trends in Real-life Antithrombotic Therapy for In- and Out-patients with Atrial Fibrillation in Moscow

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Background: Stroke prevention strategy in atrial fibrillation (AF) patients had changed past decade. Registries are the useful instruments for study how it´ve changed their real-life management.

Aims: To investigate a real-life use of antithrombotic drugs in AF patients.

Methods: The registry of 1624 in- and out-patients with AF, observed in 2 hospitals and 3 out-patient clinics of Moscow at 2009-2015.

Results: 99,1% of patients had indications for tromboembolic events prophilaxis with anticoagulants. HAS-BLED score median was 2,0 (2,0-3,0).

Retrospective analysis showed, that 9,0% of out-patients've took warfarin (66,7% of them had INR values available, 30,9% of them had INR target rate); 3,4% - new oral anticoagulants (NOACs), 17,7% - antiplatelets (AP). 33,2% of in-patients were prescribed warfarin (42,0% of them had INR data available, 39,7% of them achieved INR target rate); 1,9% - NOACs, 42,6% - AP. So only 5,3% of out-patients and 7,4% of in-patients have got adequate thromboprophylaxis.

388 patients hospitalized in 2014-2015 with AF were observed prospective. Before hospital admission 14,7% of them took warfarin (47% of them had INR data available, 44,4% of them had target rate); 11,9% - NOACs, 26% - AP (so adequate thromboprophylaxis have got 14,9% of patients). At discharge 78,6% of patients were prescribed warfarin (82% of them had INR data, 31,8% of them - at target rate), 7,5% - NOACs, 4,1% - AP (adequate stroke prevention have got 28,1% of patients). 84,6% of patients were recommended NOACs as warfarin alternative. In 13 (10-14) months after discharge telephone contacts with 176 of these patients were obtained. 25,6% of interviewed patients continued to use warfarin, 33,5% - NOACs, 34,1% - AP.

Conclusions: Warfarin and NOACs use in Moscow at 2009-2015 increased (but remains low), of AP - decreased. Financial reasons limitate NOACs further growth and warfarin reduce. So the greatest effort required for improving monitoring of warfarin treatment by INR, especially in out-patient clinics.

PB 1848 | Should we Abandon the APTT for Monitoring the Anticoagulant Effect of Unfractionated Heparin?

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Background: The activated partial thromboplastin time (APTT) is commonly used to monitor unfractionated heparin (UFH) but may not accurately measure anticoagulation. The anti-Xa assay is less prone to confounding factors and may be a better assay for this purpose.

Aims: To determine the relationship between anti-Xa activity and APTT in patients on intensive care.

Methods: Anti-Xa (chromogenic Liquid anti-Xa assay), and APTT (SynthASil,HemosIL®), [IL] were performed in parallel on 3543 samples from 475 patients (infants [n= 165], children 1-16 years [n= 60] and adults [n= 250]) receiving UFH in intensive care units in a single centre over 6 months. Patients with coagulation factor deficiencies and lupus anticoagulant were excluded.

Results: Table 1 shows anti-Xa levels and corresponding APTT values. Samples with anti-Xa activity within the therapeutic range (0.3-0.7IU/mL) had a wide range of APTT: (mean 62, range 29-250 s). The correlation between APTT and anti-Xa was poor in all 3 age groups; r values and 95% confidence intervals (CI) were 0.29(0.16-0.41), 0.38(0.20-0.52) and 0.34(0.29-0.39) for infants, children and adults respectively. For infants with anti-Xa of 0.3-0.7IU/mL; 10 (5%), 60 (31%) and 123 (64%) had an APTT in the sub-therapeutic, therapeutic (60-100s) and supra-therapeutic ranges respectively. Children had the highest concordance (48%) between anti-Xa of 0-3-0.7 and APTT values of 60-100s.

Adults with anti-Xa of 0.3-0.7 IU/ml but sub-therapeutic APTT had significantly higher fibrinogen levels: mean [CI] 4.5g/l [2.8-7.5] compared to those with therapeutic range APTT; 3.5 [1.5-5.5] and supra-therapeutic APTT; 3.3 [1.0-5.5], (p=0.02).

TABLE 1 Anti-Xa levels and corresponding APTT values

	Anti-Xa IU/mL	No.(%) of samples in the sub-therapeutic range (APTT <60s)	No.(%) of samples in the therapeutic range (APTT 60-100s)	No.(%) of samples in the supra-therapeutic range (APTT >100s)	Total
Infants (<1year)	<0.3	85(34%)	100(40%)	64(26%)	249
	0.3-0.7	10(5%)	60(31%)	123(64%)	193
	>0.7	2(12%)	4(23%)	11(65%)	17
Children (1-15 years)	<0.3	98(76%)	23(18%)	8(6%)	129
	0.3-0.7	20(17%)	55(48%)	40(35%)	115
	>0.7	2(20%)	3(30%)	5(50%)	10
Adults (16-88 years)	<0.3	1370(84%)	251(15%)	17(1%)	1638
	0.3-0.7	640(56%)	432(38%)	64(6%)	1136
	>0.7	12(21%)	23(41%)	21(38%)	56

Conclusions: There is a poor correlation between anti-Xa and APTT in all age groups. The majority of samples from infants with anti-Xa of 0.3- 0.7IU/ml had a supra-therapeutic APTT, whereas adults tended to have a sub-therapeutic APTT. These results confirm the limitation of APTT in monitoring UFH. Some of the variation in adults may be accounted for by fibrinogen level.

PB 1849 | Evaluation of the 4T Score and Rapid Anti PF4/Heparin Antibodies Detection for Heparin Induced Trombocytopenia (HIT) Diagnosis in Clinical Practice. A Multicenter Study

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Background: Incidence of HIT is 1-5% in patients receiving heparins, and 30 to 50% of them develop venous or arterial thrombosis. The 4T score (4Ts) is used as a clinical pretest score.

Aims: To perform an external validation of 4Ts in patients with HIT suspiction, b) To evaluate patients' characteristics, c) To evaluate factors associated to HIT development

Methods: An observational transversal cohort multicenter study was performed. We included 204 consecutive patients (>18 years old) with anti PF4/Heparin Antibodies (APF4/HEP) test requested from January 2014 and December 2016. APF4/HEP were measured by an immunoturbidimétric assay (HemosIL HIT AB Instrumentation Laboratory), Patients risk was categorized as low, moderate and high according to the 4Ts blinded calculated by independent hematologists upon clinical records. Descriptive statistics, chi2, t test as well as ROC curves were calculated by using Stata13 program.

Results: Characteristics of the 175 patients finally included (29 were excluded because of no HIT suspicion or scarce clinical data) are summarized in table 1.

TABLE 1 Clinical characteristic of patients with and whitout APF4/HEP

Characteristic	APF4/HEP + (n = 28)	APF4/HEP - (n = 147)	OR (95%CI)	p value
Age Median (range)	64 (18-84)	65 (18-90)		0,84
Sex (F/M)	11/17	72/75		0,34
Type of inpatient acceptance	clinical 19 (68%) surgical 9 (32%)	clinical 105 (72%) surgical 40 (28%		0,62
Oncological patients, n (%)	8 (29%)	42 (29%)		1
Inpatients area	clinical 16 (57%) // ICU 9 (32%) // CCU 3 (11%)	clinical 90 (61%) // ICU 22 (15%) // CCU 33 (22%)		0,11
Type of Heparin	UFH 17 (61%) // LMWH 10 (36%)	UFH 51 (35%) // LMWH 96 (65%)		0,002
Trombosis, n (%), OR (CI 95)	Stroke 4 (14%) // AMI 1 (4%)// VTE 10 (36%)	Stroke 1 (0,68%)// AMI 0// VTE 27 (18%)	Stroke 24(2-254)// VTE 2,47 (1-6)	<0.001// 0,021 // 0,022
4Ts	Low 3 (11%)// Intermediate 14 (50%)// High 11 (39%)	Low 65 (44%)// intrmediate 73 (50%)// High 9 (6%)		<0.001

APF4/HEP were more associated to UFH than LMWH, and thrombosis was more prevalent in the group with APF4/HEP positive (VTE and Stroke).

APF4/HEP positive prevalence according 4Ts was 3/68 [4,4% (IC95 0-12)] for low risk; 14/87 [16% (IC95 9-25)] for intermediate risk and 11/20 [55% (IC95 32-77) for high risk, p 0.000].

ROC AUC for 4Ts: 0,75 (0.65 a 0.86). Sensitivity and specificity were 90% and 44% for a 4Ts of 4; 39% and 94% for a 4Ts of 6. Positive predicting value for an intermediate or high 4Ts were 0,24 (IC95 0,16-0,33) and 0,55 (IC95 0,32-0,76). Negative predicting value of low 4Ts was 0.96 (IC95 0.87-0.99).

Conclusions: 4Ts was a good pretest tool to select patients to be studied and safely excluding patients with 4Ts ≤ 3. Performing a rapid test in patient with intermediate and high risk to exclude HIT diagnosis seems to be useful to avoid stop heparin and start other antithrombotic therapy particularly in critically ill patients.

PB 1850 | Risk of Thromboembolic Disease in Renal Transplant Patients

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Background: Venous thromboembolic disease (VTE) is a complication in renal transplant (Tx) recipients and a source of morbi-mortality.

Aims: To evaluate incidence and prevalence of VTE; and risk factors associated with thrombosis after renal tx

Methods: Retrospective cohort study including all patients(px) with renal Tx at our institution between Jan 2010-Jan 2016. A nested case control study was performed to identify transient risk factors. Primary endpoint: diagnosis of VTE or venous graft thrombosis from time of Tx till death, second renal Tx or loss to follow up. Per institutional protocol px received prophylaxis with unfractionated heparin 5.000-10.000 units/day beginning 12-24 hours after Tx; continued with enoxaparin 20mg/day once creatinine < 2mg/dl for 3 months; then low dose aspirin for one year. Study was approved by IRB. Descriptive statistics, t test and chi2 and multivariate analysis with logistic regression were performed (Stata 13 software)

Results: We included 181 patients. Prevalence of VTE was 15%, most during first 3 months of Tx (32% in first month). Lower limb DVT was the most frequent event (82%), 65% of them homolateral to transplanted kidney. Factors associated with increased risk of VTE in univariate analysis are listed in Table 1.

TABLE 1 Factors associated with increased risk of thrombosis in univariate analysis

Variable	OR (CI 95%)	p Value
Age > 50 years	3,9 (2-9)	0,0009
Personal History of DVT	4,7 (2-13)	0,0013
Elevated FVIII	3,87 (1-11)	0,0060
Hyperhomocysteinemia	2,6 (1-6)	0,0195
LAC	0,58 (0,2-2)	0,3444
Reoperations	6,43 (2-25)	0,0019
1-2 Thrombotic risk factors	6,15(1-47)	0,082
3 o > Thrombotic risk factors	16,1(1,6-156)	0,017

Hemoglobin level and creatinine clearance were lower in case group vs control group. Use of mycophenolate was associated with lower incidence of VTE (0 vs 21% p=0.011). In multivariate analysis age over 50, personal history of VTE and number of thrombophilic risk factors were statistically significant.

Conclusions: Age over 50y, personal history of VTE and more than one laboratory-measured thromboembolic risk factor identifies px at increased risk of VTE after renal tx. Individualized VTE risk factor assessment and differentiated prophylaxis strategies should be considered for these px, in order to optimize outcomes.

PB 1851 | Validation of Risk Assessment Models for Venous Thrombosis in Hospitalized Medical Patients

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Background: Medical patients are at risk for developing venous thrombosis (VT). However, regardless of current evidence, international guidelines differ on thromboprophylaxis regimens for medical patients. Consequently, many hospitals create their own thromboprophylaxis policy. To assist in these decisions, risk assessment models (RAMs) have been developed to predict the risk for VT. Of all RAMs currently available, only a few have been externally validated, all showing moderate discrimination.

Aims: Our objective was to externally validate all developed RAMs till date, in one dataset of medical patients.

Methods: A literature search was performed to find all eligible RAMs. We used data from a large population based case-control study (MEGA) into aetiology of a first VT (4956 cases with VT and 6297 controls without). To identify hospitalized medical patients, the MEGA study has been linked to the Central Bureau for Statistics database of the Netherlands with information on all hospital admissions. Written informed consent

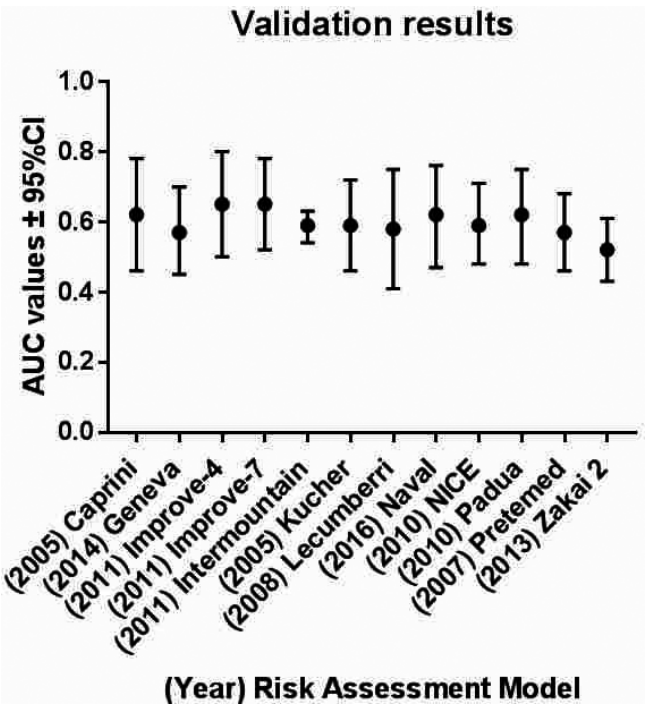

FIGURE 1 Validation results of the performance of all Risk Assessment Models for VT in hospitalized medical patients

was obtained for all participants. 516 cases and 40 controls met the inclusion criteria (medical patient, hospitalized for at least one day within three months before VT/control date, no surgery). The discriminative performance of each RAM was assessed by calculating the c-statistic, sensitivity and specificity and negative and positive predictive values.

Results: The literature search provided 12 RAMs. None showed good discrimination (Fig 1. Area Under the Curve range 0.52-0.65) or a high sensitivity with a high specificity. The negative predictive value was high in all RAMs (range 98.8-99.4%) and the positive predictive value low (range 1.2-3.4%), meaning that in those who had a positive screening test, the probability of getting VT was at best 3.4%.

Conclusions: The discriminative performance of currently available RAMs in medical patients to predict VT seems limited. Our results underline the importance for the development of a new and better RAM for medical patients at risk for VT.

PB 1852 | In ECMO Patients Anticoagulated with Heparin the Dose-response Relationship Disappears when the Dose is Adjusted by ACT but it is Preserved with Anti-Xa Activity

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Background: Extra-corporeal membrane oxygenation (ECMO) has become standard supportive therapy during critical cardio-respiratory situations. Anticoagulation is mandatory to prevent obstruction of the circuit. The optimal heparin infusion rate is estimated for each patient according to a dose-response relationship that is usually measured with the activated clotting time (ACT).

Aims: To test if ACT gives a wrong estimate of the dose-response relationship compared to anti-Xa activity during ECMO.

Methods: We performed a prospective cohort study on 65 consecutive patients treated with ECMO and unfractionated heparin between January 2014 and April 2015 in the intensive care unit of the Rangueil University Hospital in Toulouse, France. ACT was measured with Hemocron®Low Range for a target 180-220 sec. Anti-Xa activity was measured for a target 0.2-0.4 UI/ml (verified for a range 0.3-0.7 UI/ml) with STA-Liquid anti-Xa on the STAR® coagulometer. We studied the dose-response relationship over time with a generalized mixed effects model. The study protocol was approved by our institutional ethics and research committee (n°11-0214).

Results: ACT results are independent (p=0.55) of the daily amount of heparin administered (IU/kg/day) while anti-Xa activity results show a clear quadratic dose-response relationship (p< 0.001).

ACT does not predict the risk of overdosage (p=0.82) while anti-Xa activity shows that this risk diminishes over time (p< 0.001) and directly relates to heparin (p=0.05). The risk of underdosage is less pronounced with ACT than with anti-Xa activity (table 1).

TABLE 1 Effect of ACT and anti-Xa activity on the risks of over and under dosage during heparin treatment

Risk	Test	Risk factor	OR	95% confidence interval	p-value
Overdosage	Anti-Xa activity	Time (12h periods)	0.65	0.57-0.74	<0.0001
Overdosage	Anti-Xa activity	Heparin dose (IU/kg/day)	1.001	1.000-1.003	0.05
Overdosage	ACT	Time (12h periods)	0.99	0.91-1.08	0.823
Overdosage	ACT	Heparin dose (IU/kg/day)	1.00	0.999-1.001	0.956
Underdosage	Anti-Xa activity	Time (12h periods)	1.12	1.03-1.21	0.006
Underdosage	Anti-Xa activity	Heparin dose (IU/kg/day)	0.997	0.996-0.998	<0.0001
Underdosage	ACT	Time (12h periods)	1.02	0.94-1.11	0.562
Underdosage	ACT	Heparin dose (IU/kg/day)	0.999	0.997-1.000	0.039

Conclusions: This study confirms that ACT gives a wrong estimate of the dose-response relationship during ECMO and challenges this test as the reference method for adjusting heparin infusion rates.

These results should be confirmed by independent studies. If this is the case, a dose response study that considers the risks of thrombosis and bleeding using anti-Xa activity for dose adjustments needs to be performed.

PB 1853 | AVAIL-MoNa-Study: Audit of Venous Thromboembolism Evaluation and Management Thromboprophylaxis in Moroccan Hospitals - National Level

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Background: Venous thromboembolism (VTE) is a common clinical problem that is associated with substantial morbidity and mortality.
Aims: The aim of this study was to describe the clinical practice in VTE prophylaxis in university and peripheral hospitals in Morocco.
Methods: This was a national, cross sectional, multicenter, observational study assessing the management ofthe VTE risk in selected Moroccan hospitals (4 university hospitals and 3 peripheral hospitals). The thromboembolic risk of the patients selected was assessed according to the American College of Chest Physicians guidelines.
Results: A total of 1318 patients were analyzed: 467 (35.5%) medical and 851 (64.5%) surgical. Mean age of the patients was 52.6±16.5 years and 52.7% were female. 51.1% of the patients had at least one risk factor for VTE and 10.3% had two risk factors according to ACCP guidelines. Medical patients were more likely to present risk factors than surgical patients (53.6% vs 50.7%, respectively). A total of 54.8% patients without contraindication to thrombophylaxis were considered to be at risk of VTE according to ACCP guidelines and were eligible for thrombophylaxis. Thrombophylaxis was prescribed to 66.8% of these patients. On the other hand, TP was prescribed for 42.9% of patients who according to recommendations should not receive it. In total, treatment prescription was concordant with the recommendations for 62.4% of the patients and not concordant for 37.6% of them. The concordance between the recommended and the prescribed prophylaxis was poor for the total population (kappa = 0.239; p < 0.001) as well as when considering surgical and medical patients alone (kappa=0.318 for medical patients and 0.147 for surgical patients; p < 0.001).
Conclusions: New strategies are required toaddress TP appropriately in hospitalized patients, including simpler assessment of the risk of VTE, at least in hospitals where complex strategies based on computerized reminders and alerts are not implementable.

PB 1854 | A Post Hoc Analysis of Dalteparin versus Vitamin K Antagonist (VKA) for the Treatment of Cancer-associated Venous Thromboembolism (VTE) in High-risk Patients with Metastatic Disease and Recent Antineoplastic Treatment

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Background: Cancer-associated VTE is associated with higher rates of recurrent VTE (rVTE) and bleeding events than seen in non-cancer patients. Variation in risk for rVTE and bleeding also exists between subgroups of cancer patients. Discriminating between low- and high-risk cancer patients is crucial to optimize the risk-benefit of patient-centered care, especially in the era of direct oral anticoagulants.
Aims: This exploratory, hypothesis generating analysis aimed to help improve the treatment of cancer-associated VTE in high-risk patients with metastatic disease and recent antineoplastic treatment.
Methods: This post hoc analysis used data from the pivotal CLOT study to compare the efficacy and safety of dalteparin vs. VKA for the treatment of cancer-associated VTE in patients with metastatic disease and recent antineoplastic treatment (chemotherapy, radiation, and/or surgery within prior 6 months) at baseline. Significance was set at 5%. No multiplicity adjustments were made. Patients gave informed consent; local independent ethics committees/review boards reviewed the study.
Results: 15/171 (8.8%) dalteparin patients and 35/177 (19.8%) VKA patients experienced ≥1 symptomatic rVTE (p=0.0048). In these patients, 25/171 (14.6%) dalteparin patients and 30/174 (17.2%) VKA patients experienced ≥1 any bleeding episode (p=0.4315); 11/171 (6.4%) dalteparin patients and 3/174 (1.7%) VKA patients experienced ≥1 major bleeding episode (p=0.0498). Most major bleeds occurred during the first month of treatment: 8/11 (72.7%) dalteparin; 1/3 (33.3%) VKA. Overall 6-month mortality was 162/348 (46.6%): 82/171 (48.0%) dalteparin; 80/177 (45.2%) VKA.
Conclusions: High-risk cancer patients with metastatic disease and recent antineoplastic treatment showed a 58% relative risk reduction of rVTE when treated with dalteparin vs. VKA. Dalteparin was associated with a small, but statistically significant increase in the incidence of ≥ 1 major bleeding events in these patients.
Funding: Pfizer

PB 1855 | Tinzaparin for the Treatment of Superficial Vein Thrombosis of the Lower Limbs

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Background: The optimum duration of anticoagulation to treat superficial vein thrombosis (SVT) of the lower limbs is a matter of debate.
Aims: To investigate the optimum duration of treatment with tinzaparin in patients with lower limb SVT.
Methods: Consecutive patients with SVT were treated with subcutaneous tinzaparin (Innohep™, LEOPharma A/S, Ballerup, Denmark). Those with thrombi measuring less than 5 cm on Duplex or reaching the saphenofemoral junction (last 3 cm of the great saphenous vein) were excluded. The composite primary endpoint of the study was recurrent thrombosis, defined as occurrence of clinically evident SVT recurrence, deep-vein thrombosis or pulmonary embolism. Patients were stratified into three groups by the duration of treatment: group 1 (≤30 days) and group 2 (31-60 days), which run in parallel and patients received mostly an intermediate or therapeutic dose and also a subsequent group 3 where patients received an intermediate dose

(131iu/Kg) for 90 days. Duration of follow-up was 120 days after initiation of treatment.

Results: A total of 147 patients (101 females and 46 males) with a median age of 58.2 years were studied (group 1, n=60, group 2, n=38 and group 3, n=49). Recurrent thrombosis occurred in 15/147 patients (10.2%), including 10 cases of recurrent SVT, four cases of deep-vein thrombosis and one case of pulmonary embolism. Recurrent thrombosis rates were significantly lower in group 3 (0%) compared to groups 1 (18.3%) and 2 (10.5%) (Table and Figure). Similar results were obtained for recurrent SVT only.

TABLE 1 Recurrence-free rate in relation to treatment duration

	30-day event-free rate (%±s.e.)	60-day event-free rate (%±s.e.)	90-day event-free rate (%±s.e.)	120-day event-free rate (%±s.e.)	p value
Group 1 (N=60)	93.3±0.032	85.0±0.046	81.7±0.05	81.7±0.05	0.002
Group 2 (N=38)	100±0.0	97.4±0.026	89.5±0.05	89.5±0.05	0.021
Group 3 (N=48)	100±0.0	100±0.0	100±0.0	100±0.0	REF

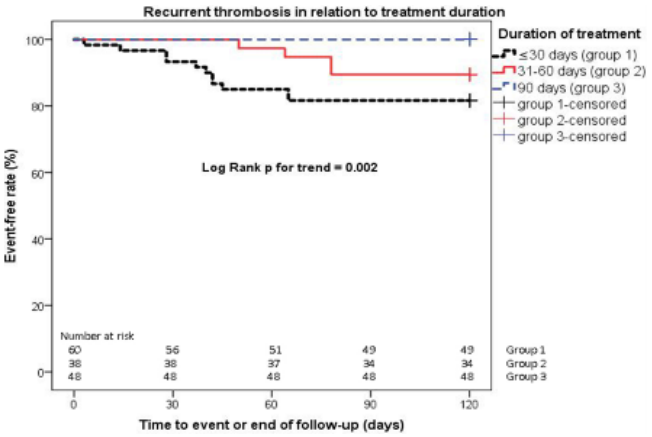

FIGURE 1 Recurrent thrombosis in relation to treatment duration

Conclusions: The incidence of recurrent thrombosis in patients with SVT of the lower limbs is high for the first three months and as a result treatment of shorter duration is inadequate. Future randomized trials should compare firstly the effectiveness of tinzaparin administered for the three-month risk period identified by our study against a 30-45 day regimen per current recommendations, and secondly the optimum dose of tinzaparin.

PB 1856 | Structural and Haemostatic Features of Pharmaceutical Heparins from Different Animal Sources: Challenges to Define Thresholds Separating Distinct Drugs

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Background: Heparin is widely employed in medicine as anticoagulant and antithrombotic agent. Its use is essential for procedures of extracorporeal circulation during cardiovascular surgeries and hemodialysis. The tissue sources used for preparation of pharmaceutical-grade heparins have changed over time, from dog liver, where heparin was originally described, to bovine liver, then to bovine lung and, lastly, to porcine intestinal mucosa, which is currently the most commonly used source. The current production of heparin mostly based on a single animal source raises a "supply insecurity", which lead the FDA - USA to evaluate the reintroduction of lung or intestine bovine heparins.

Aims: The objective of the present study was to investigate if heparins obtained from different sources are distinct drugs and which are the analytical thresholds separating these distinct heparins.

Methods: We performed herein a systematic analysis on the physicochemical properties, disaccharide composition, in vitro anticoagulant potency and in vivo antithrombotic and bleeding effects of several batches of pharmaceutical grade heparins obtained from porcine intestine, bovine intestine and bovine lung.

Results: Each of these three heparin types unambiguously presented differences in their chemical structures, physicochemical properties and/or haemostatic effects. We also prepared derivatives of these heparins with similar molecular weight differing exclusively in their disaccharide composition. The derivatives from porcine intestinal and bovine lung heparins were structurally more similar with each other and hence presented close anticoagulant activities whereas the derivative from bovine intestinal heparin had a higher proportion of 6-desulfated α-glucosamine units and about half anticoagulant activity.

Conclusions: Our findings reasonably indicate that pharmaceutical preparations of heparin from different animal sources constitute distinct drugs, thus requiring specific regulatory rules and therapeutic evaluations.

PB 1857 | The Effect of Prolongation of Initial Enoxaparin Therapy to One Month on the Risk of VTE Recurrences and the Recanalization of Deep Veins

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Background: Standard therapy of VTE pts consists of at least 5 days initial therapy with parenteral anticoagulants followed by long-term oral anticoagulants therapy. Prolongation of LMWH therapy over 5 days is an alternative treatment, which can be recommended in VTE pts with cancer only. Need of prolongation of initial LMWH therapy in all VTE pts is not yet clear.

Aims: To determine the effect of prolongation of initial enoxaparin (En) therapy to 1 month on the risk of VTE recurrence and recanalization of deep veins.

Methods: Sixty-four pts (38 men, age 54±14 yrs.) with deep vein thrombosis (DVT) and/or pulmonary embolism (PE) were studied. First group pts (n=32) received UFH for at least 5 days followed by long-term warfarin (W) therapy (INR 2,0-3,0). Second group pts (n=32) received En (1 mg/kg twice daily) for 30 days and then long-term W therapy (INR 2,0-3,0). Compression ultrasonography was performed at baseline and after 1, 3, 6, and 12 months. We analyzed the presence or absence of signs of occlusive thrombosis in deep veins. Follow-up period was 12 months. Endpoints were DVT/PE recurrences.

Results: In all pts, the rate of DVT recurrence during 12 months was 14%. There were no PE recurrences. The rate of DVT recurrence was significantly lower in En group (3,1% vs 25%; p=0,026). Univariate Cox regression showed that prolongation of En therapy to 1 month significantly decreases the risk of DVT recurrence during 12 months (HR 0,12; 95% CI 0,02-0,99; p=0,049). The Kaplan-Meier curve showed that pts received En had lower cumulative risk of DVT recurrence (chi-square = 5,6; p (log rank test) = 0,018). Univariate Cox regression also showed that prolongation of En therapy increases the probability of recanalization of occlusive thrombosed veins during 12 months (HR 2,52; 95% CI 1,06-5,97; p=0,036).

Conclusions: Our pilot study showed that prolongation of initial En therapy to 1 month decreases the risk of DVT recurrence and increases the probability of recanalization of occlusive thrombosed veins during 12 months.

PB 1858 | Validation of the Pulmonary Embolism Severity Index (PESI) and Simplified PESI in Asian Patients with Acute Pulmonary Embolism

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Background: Pulmonary embolism (PE) is common, with mortality ranging from 2% to 95%. The Pulmonary Embolism Severity Index (PESI) and simplified PESI (sPESI) are risk stratification tools developed to predict mortality in outpatients presenting with PE and identify a low risk group that can potentially be treated without hospitalisation. Both PESI and sPESI have been extensively validated in Caucasians, but limited data exists about their validity in Asians.

Aims: To assess the validity of the PESI and sPESI scores in an Asian population.

Methods: Consecutive outpatients presenting with acute PE to our institution and diagnosed by computed tomographic pulmonary angiogram (CTPA) from 2013 to 2016 were recruited. Clinical characteristics and outcomes were extracted from a comprehensive electronic patient records system. For each patient, the PESI and sPESI were calculated, and patients were stratified into class I-V for PESI and low or high risk groups for sPESI respectively. The primary end-point was 30-day all-cause mortality. Area under the receiver operating curves (ROC) was used to assess the performance of these scores. This study was approved by our institutional ethics review board.

Results: 119 patients (mean age 58±16 years, 46.2% males) were recruited. 30-day mortality data are shown in Table 1. Overall mortality was 6.7%. ROC curves are shown in Figure 1. The area under the ROC curve was 0.87 (95% CI 0.79-0.95) for the PESI and 0.69 (95% CI 0.48-0.90) for the sPESI scores. The negative predictive value for 30-day mortality was 100% and 95.8% for the PESI class I/II and sPESI low risk groups respectively.

TABLE 1 30-day all-cause mortality data for patients stratified by PESI class and sPESI risk

PESI Class	Number Dead	Number Alive	Total	30-Day All-Cause Mortality (%)
Class I (low risk)	0	21	21 (17.6%)	0
Class II (low risk)	0	21	21 (17.6%)	0
Class III (high risk)	0	28	28 (23.5%)	0
Class IV (high risk)	2	18	20 (16.8%)	10
Class V (high risk)	6	23	29 (24.4%)	20.7
Total / Overall	8	111	119	6.7
Simplified PESI				
Low Risk	1	23	24 (20.2%)	4.2
High Risk	7	88	95 (79.8%)	7.4

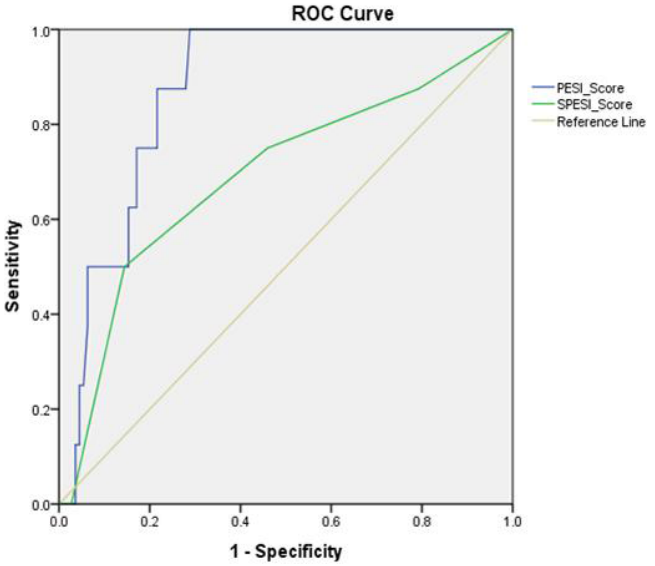

FIGURE 1 ROC curves for PESI and sPESI scores and 30-day all-cause mortality

Conclusions: The PESI score accurately predicts 30-day mortality in Asian patients, but sPESI, whilst easier to apply, is less discriminatory. The negative predictive value for overall 30-day mortality in class I/II PESI was 100% and reliably identifies low risk PE patients that may be treated safely in the outpatient setting.

PB 1859 | Help the Aged? Are we Failing to Protect Patients with Dementia from the Risks of Developing Hospital Associated Venous Thrombo-Embolism?

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Background: In 2016 dementia became the leading cause of death in the UK. Increasing age in the UK population is predicted to raise the number of hospital inpatients with dementia. Hospital associated Venous Thrombo-Embolism (VTE) has been reported at a district general hospital in patients with dementia declining Low Molecular Weight Heparin (LMWH) thrombo-prophylaxis.

Aims: To determine if patients with a diagnosis of dementia miss more doses of appropriately prescribed LMWH prophylaxis compared to those without dementia and if this is a result of patients declining medication.

Methods: A retrospective audit of adult patients admitted to an acute hospital between March and May 2016 identified 189 study patients by a standard dementia screen. Electronic Prescribing (EPMA) requires VTE risk assessment and records prescribing decisions, analysis of which determined the total number of doses of LMWH prophylaxis prescribed, missed and declined by patients. A control group (n=177) of similar sex, age and hospital length of stay was studied along with the number of prescribed and missed doses of LMWH prophylaxis for the hospital population over the same time period.

Results: Patients with dementia missed 167 (9.0%) doses of prescribed LMWH prophylaxis compared to 76 (4.2%) in the control group. Of the missed doses in the study group 115 (6.2%) were recorded as declined, statistically more than both the control (1.8%) and the hospital population as a whole (4.0%). 12.7% of study group patients assessed as at risk of VTE were not prescribed any prophylaxis compared to 5.4% of all hospital patients.

TABLE 1 Prescribed and missed doses of LMWH VTE prophylaxis

	Study Group (n=189)	Control Group (n=177)	Hospital Population
Prescribed Doses of LMWH prophylaxis	1854	1789	29,049
Total Missed doses	167 (9.0%)	76 (4.2%)	1817 (6.3%)
Declined Doses	115 (6.2%)	32 (1.8%)	1171 (4.0%)
Mean Age	85.4	83.5	n/a
Mean Length of Hospital Stay (days)	10.3	10.6	n/a
Female %	50.2	55.6	n/a

Conclusions: Following admission to hospital patients with dementia identified as at risk of VTE are less likely to be prescribed prophylaxis. When LMWH prophylaxis is correctly prescribed for these patients most missed doses are due to the patient declining. These factors may contribute to failure of VTE prevention strategies and a greater risk of hospital associated VTE, prompting the need to consider alternative care pathways.

PB 1860 | The Efficacy and Safety of Anticoagulations in Cerebral Vein Thrombosis: A Systematic Review and Meta-analysis

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Background: Anticoagulation with unfractionated heparin (UFH) or low molecular weight heparin (LMWH) is the mainstay of treatment of acute cerebral vein thrombosis (CVT) patients with or without intracranial hemorrhage (ICH).

Aims: We conducted a systematic review and meta-analysis to determine the efficacy and safety of LMWH compared to UFH in the acute treatment of CVT.

Methods: An electronic search of MEDLINE and Google Scholar was performed. Randomized controlled trials (RCT) reporting on the efficacy and safety of anticoagulation for acute treatment of CVT were

included. Outcomes of interest included mortality, disability, new ICH and pulmonary embolism (PE).

Results: Overall, 4 RCT were included in the meta-analysis. Two trials compared anticoagulation (one trial UFH and one trial LMWH) to placebo. The use of anticoagulation therapy was associated with an odd ratio (OR) for mortality and disability of 0.31 (95% confidence interval (CI) 0.07 to 1.45) and 0.3 (95% CI 0.09 to 1.01), respectively. Three new ICH were observed among patients receiving placebo and no patient had a PE complication. The other two trials compared LMWH to UFH. LMWH was associated with an OR for mortality and disability of 0.21 (95% CI 0.02 to 2.44) and 0.5 (95% CI 0.11 to 2.23), respectively. There were no new events of ICH or PE in these trials. Combining the result from all the 3 RCT that assessed LMWH showed that LMWH was associated with lower mortality (OR 0.25 (95% CI 0.07 to 0.85)). The OR for disability was 0.46 (95% CI 0.16 to 1.32).

Conclusions: In this study, LMWH treatment for CVT appeared to be safe and effective for the management of CVT.

PB 1861 | Study of Bioaccumulation of Tinzaparin in Renally Impaired Patients when Given at Prophylactic Dose: The STRIP Study

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Background: Hospitalized patients with severe chronic kidney disease (CKD) are among the highest risk patients for venous thromboembolism (VTE). Although low-molecular-weight heparins (LMWH) are effective and safe for VTE prophylaxis, LMWH are rarely used in this population because of concerns about bioaccumulation leading to increased risk of bleeding.

Aims: To assess whether bioaccumulation occurs after repeated daily subcutaneous administration of prophylactic doses of tinzaparin in patients with severe CKD.

Methods: Patients with severe CKD (defined by an estimated glomerular filtration rate (eGFR) between 6 and 30 ml/min/1.73 m2) were included in this prospective single-center observational cohort study. Informed consent was obtained for all subjects and the study was approved by the local Research Ethic Board and Health Canada. Tinzaparin was injected s.c. once daily as per local guidance for medical thromboprophylaxis. The dose used was 3500 IU or 4500 UI, depending on patient's body mass index. Peak anti-Xa activity blood levels (anti-Xa) were measured 4 hours after injection on day 2 (or 3), 5 and 8. A through anti-Xa was obtained before the dose injected on day 5. Primary outcome was the comparison of peak anti-Xa on day 2/3 and day 5.

Results: 28 patients with a mean (SD) eGFR of 17.6 ml/min/1.73m2 (6.6) were enrolled; 14 patients were excluded because they were discharged before testing or their treatment/sampling was interrupted (n = 10) or their eGFR rose above 30 ml/min/1.73m^2 (n = 4). Of the remaining 14 patients, none had quantifiable through anti-Xa (< 0.10 IU/ml). Overall peak anti-Xa levels ranged from < 0.1 to 0.33 UI/ml. There was no significant increase in anti-Xa levels over time (Figure), supporting an absence of tinzaparin bioaccumulation. No thrombotic or bleeding adverse events were documented during the study period.

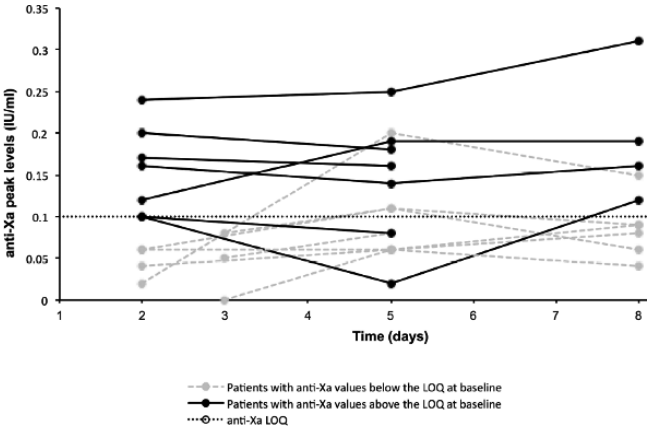

FIGURE 1 Time course of anti-Xa peak levels (UI/ml) in patients (n=14) with severe CKD

Conclusions: This study shows that prophylactic administration of tinzaparin in patients with severe CKD does not result in bioaccumulation.

PB 1862 | Thromboembolic Events Following Splenectomy: Risk Factors, Prevention, Management and Outcomes

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Background: Thromboembolic events following splenectomy are not uncommon. However, the role of thromboprophylaxis and risk factors for thrombosis, as well as the clinical course and outcomes are not well characterized.

Aims: To investigate the risk factors, incidence, clinical presentation, management and outcomes of thrombotic events following splenectomy.

Methods: A retrospective review of individuals who underwent splenectomy between January 2006 and December 2015.

Results: Overall, 297 patients underwent splenectomy [open splenectomy (n=199), laparoscopic splenectomy (n=98)]. Mechanical (thigh-length pneumatic compression stockings) and pharmacologic thrombo-prophylaxis (40 mg enoxaparin daily, starting 12 hours after surgery until discharge) was provided for all patients. One hundred

and sixteen patients (39%) also received an extended course of enoxaparin for 2-4 weeks after discharge. Twenty-three patients (7.7%) experienced thrombotic complications following splenectomy, including 16 cases (5.4%) of portal-splenic mesenteric venous thrombosis (PSMVT), 5 (1.7%) pulmonary embolism and 2 (0.7%) deep vein thrombosis. Longer operative time (mean operative time of 369 vs. 273 minutes, P=0.02) was independently associated with thrombosis. Post-splenectomy thrombocytosis was not associated with thrombosis (P=0.41). The overall thrombosis rate was significantly lower in patients who received an extended course of anticoagulation following splenectomy (3.4% vs. 10.5%, P=0.02). Complete resolution of PSMVT was observed in most cases (n=13, 81.3%) with no *recurrent thrombosis during a mean follow up of* 33±24 months.

Conclusions: Thromboembolic complications, mainly PSMVT, are common following splenectomy. Longer operative time was associated with thrombosis. As significantly lower rates of thrombosis were found in patients who received an extended course of anticoagulation, we support its use for at least two weeks after discharge.

PB 1863 | Low and High Responders to Unfractionated Heparin Can Be Distinguished in Vitro by Inhibition of Thrombin Generation

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Background: Unfractionated heparin (UFH) is the parenteral anticoagulant of choice in the critical care setting. Proper selection of an initial dose of UFH is important. However, the anticoagulant response varies widely and some patients present with heparin resistance, defined as an inadequate responsiveness to standard doses of heparin.

Aims: We aimed to assess whether the *in vitro* response to UFH measured by thrombin generation can be predicted by (a combination of) biological parameters.

Methods: Thrombin generation was determined in platelet-poor plasma from 91 healthy subjects spiked with 0.07 IU/mL UFH. The variability (%CV) of the inhibitory effect of UFH within the population was calculated. Responsiveness to UFH was expressed as the sum of the inhibition (%) of the peak and endogenous thrombin potential (ETP). Antithrombin (AT) activity was determined as described by Kremers et al. (2015).

Results: The CVs of the % inhibition of ETP and peak height by UFH were 48% and 56%, respectively. Interestingly, instead of a normal bell-shaped distribution, two partly overlapping populations of low- and high responders to UFH could be distinguished in the frequency distribution graph.

In the low-responder group there were significantly more women (p = 0.0328), who were older (p=0.0022) and had lower AT activity (p=0.0023). Mixing equal volumes of plasma from low and high responders resulted in correction of the low response to the theoretical average in most but not all cases.

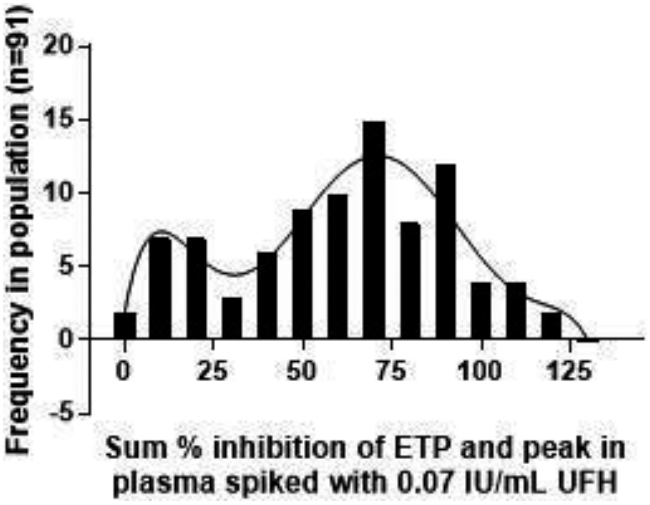

FIGURE 1 Frequency distribution of TG inhibition by UFH

Conclusions: Addition of a fixed concentration of UFH causes a highly variable inter-individual inhibition of TG. Two populations of low- and high responders to UFH can be distinguished based on TG parameters, with significant differences in age, sex and AT activity. Whereas correction of a low response upon mixing with high responders' plasma may be attributed to AT deficiency, an additional, currently unknown cause underlies heparin resistance in plasma that does not correct upon mixing.

PB 1864 | Should Anticoagulation Therapy for Cancer Associated Pulmonary Embolism (PE) Be Modified in Individuals with Brain Metastasis or Thrombocytopenia?

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Background: There is little guidance for managing cancer associated pulmonary embolism (PE).

Aims: Cases of cancer associated PE were reviewed at a UK teaching hospital, to compare rates of mortality and intracranial bleeding (ICH) in those with brain metastasis or thrombocytopenia (platelets< $100x10^9$/L).

Methods: Cases were retrieved from a PE database between July 2012-July 2013 and electronic medical records reviewed. Groups were compared using the chi-squared test.

Results: There were 135 cases, 63 (47%) male with a median age of 68 years. The most common primary cancer sites were: lung, 28 cases; oesophagus, 12; prostate, 9; and colorectal, 9. A primary brain malignancy was present in 3. 78 (58%) had metastatic disease at PE diagnosis and the overall mortality 12 months post PE was 84% (114).

Anticoagulation was given to 124 (95%); 113 with tinzaparin. In 5 cases the anticoagulant was unrecorded. 6 were not anticoagulated: 2 required multiple procedures, 1 presented with an ICH, 1 died prior to anticoagulation and in 2 cases there was no reason recorded. 92 received long-term anticoagulation; 23 stopped following a median duration of 6 months (range 5-36) and in 9 cases the duration was unrecorded.

17 (13%) had brain metastasis, either at PE diagnosis (14) or following anticoagulation (3). Two ICHs occurred, one within a brain metastasis and one prior to anticoagulation in a case with a primary brain malignancy (14% vs. 0% p = 0.012). There was no significant difference in mortality 12 months post PE (93% vs. 83%, p = 0.694).

Thrombocytopenia was present in 19 cases (14%) in the 3 months prior to the PE. Only 1 case was not anticoagulated, this was due to the need for recurrent procedures. No ICH occurred in this group and the mortality rate 12 months post PE was similar to those without thrombocytopenia (95% vs. 82% p=0.306).

Rate of recurrence was 3% (4), none of which were thrombocytopenic or had brain metastasis.

Conclusions: There appears to be an association between brain metastasis and ICH.

PB 1865 | Comparison of the Injection Site Reactions Elicited by Two Subcutaneously Injected Heparins, Nadroparin Calcium and Enoxaparin Sodium

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Background: Subcutaneous administration of low-molecular-weight heparins (LMWHs) often causes problems such as pain at the injection site. This can lead to problems with treatment compliance. The vital need for anticoagulation treatment compliance to prevent the high risk of morbidity and mortality prompted the primary objective of this clinical trial.

Aims: The primary objective was to assess local administration site signs of intolerability, specifically pain intensity, after a single subcutaneous injection of two widely used LMWHs, nadroparin calcium and enoxaparin sodium. Secondary objectives included monitoring safety, as well as changes in physical examination and laboratory findings.

Methods: A five-week double-blind, randomised, placebo-controlled, single-centre, phase IV clinical trial was conducted in 15 healthy volunteers. The trial was conducted according to the Declaration of Helsinki and international and local guidelines on good clinical practice. The trial was approved by an independent research ethics committee and individual informed consent was obtained prior to the commencement of any trial-related procedures. Perceived pain was assessed using a visual analogue scale (VAS) and an 11-point numeric rating scale (NRS). Measurements were taken at 1, 3, 5, 10, 15 and 20 min post-administration.

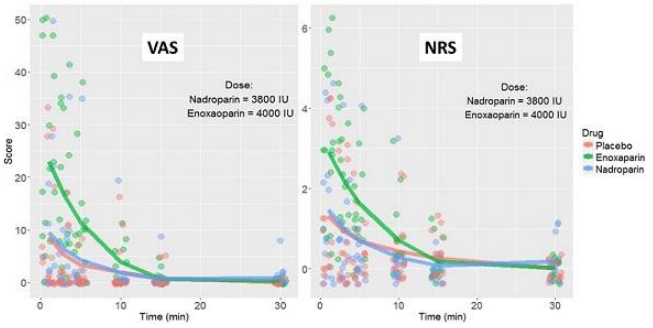

FIGURE 1 Individual and mean pain scores following study drug administration

Results: Enoxaparin administration was significantly more painful, compared to either placebo or nadroparin administration. Compared to placebo, enoxaparin was also associated with a significantly greater burning sensation. Statistically, no distinction could be made between the study drugs with respect to perceived pruritus.

Conclusions: Administration of LMWHs in the form of nadroparin, as compared to enoxaparin, results in less subjective pain experienced by participants, which may improve compliance to anticoagulation treatment utilised to prevent the high risk of morbidity and mortality.

PB 1866 | The Incidence of Adverse Events in Patients Treated with Therapeutic Dose Enoxaparin in Relation to Body Mass Index

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Background: Low Molecular Weight Heparin (LMWH) has widely replaced its parent and oldest anticoagulant compound "Heparin" due to its numerous advantages. However, LMWH are not devoid of adverse effects which may be serious. Since the therapeutic dose of LMWH "*Enoxaparin*" is determined by a weight-based regimen, the incidence of adverse events may be increased when using higher doses.

Aims: To assess the incidence of adverse events in acutely thrombosed patients treated with therapeutic dose Enoxaparin in relation to Body Mass Index (BMI).

Methods: A prospective study conducted through a 6 months period in 2014 including 181 patients (98 males, 83 females) with acute thrombotic disorders (i.e. acute venous thromboembolism, acute coronary syndrome excluding those received thrombolytic, and acute cardiac thromboembolism) admitted to the coronary care unit in Al-Hussein Medical city in Karbala-Iraq. All patients had normal renal functions and were received weight-based LMWH in the form of *Enoxaparin*. They were subdivided according to their BMI into 3 groups; normal, overweight and obese. Any evidence of adverse events were followed and recorded. Statistical analysis was done using Fisher exact test.

Results: Bleeding was the most common adverse event occur in 15 out of 181 patients (8.3%), categorized as Major Bleeding comprising 2.2% overall, occur in 2% of normal BMI, 1.6% of overweight, & 2.9%

of obese patients, and Minor Bleeding comprising 6.1% overall, occur in 5.9% of normal BMI, 6.5% of overweight, & 5.8% of obese patients. No death was reported related to bleeding. Other adverse events were thrombocytopenia in 2.6%, and fever in 1.1% overall.

TABLE 1 Incidence of Adverse Events in Relation to Body Mass Index (BMI)

Adverse Events	BMI <25 N=51	BMI 25-<30 N=61	BMI ≥30 N=69	P-value
Major Bleedings	1 (2.0%)	1 (1.6%)	2 (2.9%)	0.97
Minor Bleedings	3 (5.9%)	4 (6.5%)	4 (5.8%)	0.96
Thrombocytopenia	1 (2.0%)	2 (3.2%)	2 (2.9%)	0.92
Fever	1 (2.0%)	0 (0%)	1 (1.4%)	0.63
Death related to Bleeding	0 (0%)	0 (0%)	0 (0%)	1.0
Death related to Thrombosis	2 (3.9%)	2 (3.3%)	2 (2.9%)	0.95

Conclusions: The incidence of bleeding and other adverse events was not found to be increased in relation to BMI when using weight-based therapeutic dose *Enoxaparin*, which may indicate the rationale use of this regimen in patients with acute thrombotic disorders.

PB 1867 | Management of Venous Thromboembolism in Patients with Glioma

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Background: Venous thromboembolism (VTE) is a common complication among patients with glioma. However, data on the safety of therapeutic doses of anticoagulation is scarce in this patient population.

Aims: The purpose of this study is to evaluate the risk of intracranial hemorrhage (ICH) in glioma patients receiving therapeutic anticoagulation for VTE treatment.

Methods: We conducted a case-control study including glioma patients with and without acute VTE from Jan 2010 to March 2015. Controls were matched based on age, gender and tumor grade.

Results: 569 patients with glioma were identified, 76 (13.3%) developed acute VTE. Of the 70 patients treated with full dose anticoagulant therapy, 14 (20%) patients had a major bleeding including 11 (15.7%) ICH. The odds ratio for ICH in patients with glioma and VTE who were treated with anticoagulation compared to the control group was 7.5 (95% CI, 1.6-34.9) P=0.01. Overall survival was similar for VTE and control group (36 vs. 42 months, P= 0.93).

Conclusions: Therapeutic anticoagulation is associated with a 7-fold increase risk of ICH in glioma patients. Data emerging from this study support the need for high quality studies to evaluate the risk of ICH in patients with glioma and VTE.

PB 1868 | Resistance of Liquid Anti-Xa Assay (IL) to Platelet Factor 4 Interference

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Background: Platelet Factor 4 (PF4) can bind to both unfractionated and low-molecular weight heparins (UFH and LMWH), thereby neutralizing heparin activity. Low levels of PF4 (~0.005 µg/mL) usually present in plasma have a negligible effect on anti-Factor Xa (Xa) assays, however, improper plasma sample preparation can lead to platelet activation and release of high levels of PF4. Such PF4 levels in plasma samples have the potential to interfere with anti-Xa assays causing erroneous results and increased risk of patient mismanagement.

Aims: This study evaluates PF4 interference on heparin measurements made with the Liquid Anti-Xa Assay (Instrumentation Laboratory (IL)), a global assay for both UFH and LMWH.

Methods: Heparinized plasma samples (UFH or LMWH) ranging from 0 - 2 IU/mL were prepared by spiking UFH or LMWH international standards into normal plasma. Various concentrations (0 - 2 µg/mL) of PF4 were added to these samples, which were analyzed using the Liquid Anti-Xa kit (IL) on an automated coagulation analyzer (IL). The heparin recoveries for samples with and without PF4 were compared to assess the inhibitory effect of PF4 on the assay results.

Results: All UFH and LMWH samples activities covering the heparin concentration range of 0.2 - 2 IU/mL were resistant (decrease in activity <10%) to the presence of PF4 up to 0.2 µg/mL and 0.5 µg/mL respectively, which is 40 and 100 fold higher than normal PF4 levels. Sample recoveries with UFH or LMWH concentration of 0.6 IU/mL and higher were resistant to PF4 concentrations up to 0.5 µg/mL or 1.0 µg/mL, respectively. At PF4 concentrations of 0.5 µg/mL or higher, UFH and LMWH sample activities at ~0.2 IU/mL were the most affected with a change in heparin activity >10%.

Conclusions: Our results demonstrate that the Liquid Anti-Xa Assay (IL) is insensitive to PF4 presence up to 0.2 µg/mL or 0.5 µg/mL in UFH or LMWH plasma samples respectively, which is 40 or 100 fold higher than normal PF4 levels.

PB 1869 | A Matched Case-control Study to Determine Early Post-operative Haematological Complications after Renal Transplantation in Chronic Renal Failure Patients Receiving Therapeutic Anticoagulation

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Background: Chronic Renal Failure (CRF) patients often have associated pro-thrombotic conditions requiring the use of anticoagulation peri- and post-operatively after renal transplantation. This can lead to early haematological complications in this group of patients who also have platelet dysfunction.

Aims: To determine the haematological complications and outcomes after renal transplantation in chronic renal failure patients with therapeutic anticoagulation compared to their matched controls.

Methods: Twenty-two renal transplant cases on therapeutic anticoagulation were analysed and matched with 22 controls (renal transplants without therapeutic anticoagulation) to determine the risk of haematological complications including, bleeding, re-explorations, upper gastro-intestinal (UGI) bleeding, and deep vein thrombosis (DVT). Controls were matched on gender, age, induction agent, pre-transplant dialysis and donor factors (type, gender and age).

Results: Although there was no observed increased risk of bleeding complications in the anticoagulated group (5 versus 3, p=0.70), all 5 cases returned to theatre compared to 0 controls (p=0.024). The rate of delayed graft (kidney) function was significantly greater in the study group (p=0.011). However, renal function at 3,6 and 12 months was comparable between the 2 groups, as was the incidence of DVT, UGI bleed, blood transfusion and mean length of stay.

TABLE 1 Complications, function and graft loss between cases and controls

Characteristic	Cases (n=22)	Controls (n=22)	Significance
Haematological Complications (%):	6 (27)	3 (14)	P=0.47
Return to theatre (%):	5 (22)	0 (0)	P=0.024
Blood transfusion (%):	5 (22)	2 (9)	P=0.187
Graft loss (%):	2 (9)	0 (0)	P=0.244
eGFR: 3 Months (mean, variance); 6 Months (mean, variance); 12 Months (mean, variance):	(45.62, 354.95); (48.35, 305.50); (51.88, 405.36)	(44.27, 173.54) (43.35, 223.50) (44.63, 194.58)	P=0.39; P=0.17; P=0.11
Mean length of Stay:	13.8	14.5	P=0.403
Primary Function (%):	14 (62)	21 (95)	P=0.011

Conclusions: This study demonstrates an increased risk of post-operative bleeding requiring surgical intervention in CRF patients on therapeutic anticoagulation, resulting in delayed graft function. Thus, careful postoperative monitoring of anticoagulation in this group of patients with platelet dysfunction is advised to minimise the post-operative comorbidity.

PB 1870 | Factors Associated with Thromboembolic Events Following Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy

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Background: Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS/HIPEC) is an effective treatment for selected patients with peritoneal surface malignancies. Little is known about the prevalence and risk factors associated with thrombosis following CRS/HIPEC.

Aims: To investigate the risk factors, incidence and the role of thromboprophylaxis in the development of thrombosis following CRS/HIPEC.

Methods: We retrospectively reviewed data from a prospectively collected database on patients with CRS/HIPEC.

Results: Overall, 192 patients underwent CRS/HIPEC between 2007-2016. Mechanical (thigh-length pneumatic compression stockings) and pharmacologic thrombo-prophylaxis (40 mg enoxaparin daily, starting 12 hours before surgery until discharge) was provided for all patients. One hundred and sixteen patients (60.4%) also received an extended course of enoxaparin for 2-4 weeks after discharge. Twenty-six patients experienced thrombotic complications (13.5%) including portal-splenic-mesenteric venous thrombosis (n=11, 5.7%), pulmonary embolism (n=10, 5.2%) and deep vein thrombosis (n=5, 2.6%). Most of them (n=21, 80.8%) occurred after hospital discharge. Univariate analysis identified Peritoneal Cancer Index, intraoperative transfusion requirement, operative blood loss, operative time, length of hospital and intensive care unit stay and lack of administration of anticoagulation at discharge as significantly associated with thrombosis. By multivariate analysis only the lack of anticoagulation at discharge remained significantly associated with thrombosis [odds ratio (95% CI): 9.24 (3.03, 28.16), P=0.0001]. Complete resolution of thrombosis was observed in all cases with no recurrent thrombosis during a mean follow up of 21±23 months.

Conclusions: Thromboembolic complications are common following CRS/HIPEC. As significantly lower rates of thrombosis were found in patients who received an extended course of anticoagulation, we support its use for at least two weeks after discharge.

PB 1871 | Clinical Course of Patients Presenting with Acute Superficial Vein Thrombosis: A Post-hoc Analysis of the ICARO Study on 488 Patients

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Background: Superficial vein thrombosis (SVT) is frequently occurring, but only limited follow-up data from adequately sized cohort studies are available.

Aims: To estimate the risk of recurrence, major bleeding, and death in patients presenting with SVT.

Methods: ICARO was designed as a multicenter, retrospective cohort study for the derivation of a clinical score to rule out the concomitance of deep vein thrombosis (DVT) in patients presenting with SVT (N=494). For the present post-hoc analysis, 488 patients with more than 30 days of follow-up were included.

Results: Median age was 56 years (25-75 percentile 42-70) and 309 (63.3%) were women. Concomitant DVT was found in 77 (15.8%) patients at baseline. The most common site of thrombosis was the internal saphenous vein (n=316 [64.8%]), involving the crosse in 43. Anticoagulation was administered to 89.3% of patients with a median duration of 42 days (30 and 182 days in patients without or with concomitant DVT, respectively). Total follow-up was 1768 and 650 patient-years for the outcomes of death and recurrence/bleeding events, respectively. SVT and VTE recurrence occurred in 107 (21.9%) and 64 (13.1%) patients (of whom 15 with pulmonary embolism), major bleeding in 13 (2.7%), and death in 31 (6.4%) patients. Overall annual rates (95% Confidence Intervals) were 16.5% (13.5-19.9) for SVT and 9.8% (7.6-12.5) for VTE recurrence, 2.0% (1.0-3.4) for major bleeding, and 1.7% (1.2-2.5) for all-cause death. In a multivariable Cox regression model fit with age, sex, unprovoked nature of SVT, concomitant DVT, and anticoagulation, only anticoagulant discontinuation was associated with either SVT or VTE recurrence (aHR 4.6 [1.6-13.5]).

Conclusions: SVT and VTE recurrence in this population is not negligible.

PB 1872 | The Assessment of Safe Prescribing and Cost-effectiveness of Low Molecular Weight Heparin (LMWH) Treatment

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Background: In an era of numerous anticoagulants being available, the low molecular weight heparins (LMWHs) retain a significant role in the treatment and prevention of thrombosis. However, LMWH use is not risk averse as demonstrated in the CRUASADE study where 42% of patients receiving weight and renal-based antithrombotics were given doses outside the recommended range. Alternatively, the direct oral anticoagulants (DOACs) are simpler to dose and offer a better patient experience.

Aims: For LMWH treatment:

· Assess dosing according to patients' weight and renal function.

· Economic analysis on the number of patients eligible for DOAC therapy.

Methods: Patients on LMWH (licensed and off-label use) were analyzed across Barts Health NHS Trust, UK. The data collected prospectively were; patient demographics, weight, renal function, co-morbidities, LMWH dosing, indication and duration within a 2-week study period August - September 2016. For DOAC eligibility, the LMWH cost comparison was based on the use of rivaroxaban for non-valvular atrial fibrillation (AF) and edoxaban for venous thromboembolism (VTE).

Results: A total of 162 patients were on treatment dose LMWH, of which 53 (33%) and 6 (4%) patients were dosed inappropriately according to their weight and renal function respectively. Fifty-six (35%) patients were eligible to switch to a DOAC, 40 (71%) with VTE and 16 (29%) with AF. For these eligible patients, the LMWH cost £1,120.06 in comparison to £401.60 if DOACs were prescribed, equating to potential savings of £718.46 over a 10-day period. Extrapolated over a year, this would produce approximately £26,000 savings.

Conclusions: Though adverse events were not observed during this study, approximately a third of patients were at risk due to inappropriate LMWH dosing. Replacement by DOACs can introduce safer anticoagulation therapy by eliminating weight based dosing and produce significant savings.

PB 1873 | Sustained Anticoagulant Effect and High Inter-individual Variability of Treatment with Rivaroxaban. An ex vivo Analysis of Thrombin Generation and Thromboelastometry

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Background: Rivaroxaban, 20 mg orally administered daily, is widely used for secondary prevention of venous thromboembolism (VTE) without the need for routine laboratory monitoring.

Aims: In patients treated for secondary prevention of VTE, we evaluated ex vivo the anticoagulant potency of rivaroxaban treatment at different times after drug intake.

Methods: 97 patients (39 males, 58 females; median age: 59 years) with idiopathic VTE were treated with rivaroxaban (20 mg/day) for a period longer than 6 months and compared to 30 controls age and sex-matched. Rivaroxaban concentrations (STA®-Liquid Anti-Xa), thrombin generation (PPP-reagent 5 pM TF; calibrated automated thrombogram, Stago France) and whole blood thromboelastometry (5 pM tissue factor; ROTEM® Werfen) were assessed. The interval between drug intake and blood sampling was registered.

Results: Treatment with rivaroxaban significantly inhibited thrombin generation (TG) and prolonged clotting time (CT): 93% of patients had TG inferior to the lower normal limit and 77% had CT longer than the upper normal limit. This effect in both assays, was not correlated to its plasma concentrations, it was significant from the 1st hour after drug intake and persisted at similar levels during the daily therapeutic cycle even at low concentrations (30 ng/ml). Rivaroxaban had no effect on clot firmness. The inter-individual coefficient of variation of rivaroxaban concentrations and TG/ROTEM alterations after drug intake ranged from 50% to 100%.

Conclusions: Once daily administration of rivaroxaban induces sustained daily anticoagulant effect, detected by TG and ROTEM assays, with no significant changes related to drug concentration reduction. This profile could explain the wide therapeutic window and the favorable benefit risk ratio of the treatment with rivaroxaban. Due to the high variability the identification of patients with extreme values of TG could be useful strategy for treatment optimization on individual basis.

PB 1874 | Comparison of UFH and Enoxaparin Originated from Bovine, Ovine and Porcine Mucosa with Functional Coagulation Assays

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Background: Pharmaceutical heparin is a linear glycosaminoglycan with an average mass between 10 and 20 kDa extracted from porcine intestine. Other sources such as bovine or ovine intestine are being examined as potential substitutes in order to diversify the heparin supply.

Aims: The present in vitro study examines bovine, ovine and porcine unfractionated heparin (UFH) and respective low molecular weight heparin enoxaparin and compares these to the commercially available heparins.

Methods: Platelet poor plasma (PPP) from 5 healthy volunteers were spiked with increasing concentrations (from 1 to 10 µg/ml) of UFH or enoxaparin of bovine, ovine or porcine origin. The specific anti-Xa activity was measured in PPP (using the STA-Liquid anti-Xa assay) and thrombin generation (TG) was assessed with PPP-reagent 5TF®/Calibrated Automated Thrombogram (CAT®; Stago, France). TG was also triggered in PPP in the presence of pancreatic cancer cells BXPC3 (Rousseau, et al Thromb Res 2015;136:1273-9). The activated partial thromboplastin clotting time (aPTT) was measured with the conventional assays on the STA-R® instrument.

Results: The anti-Xa activity/µg, the concentrations prolonging 2-fold the aPTT, and the IC50% for ETP and MRI from the TG test of the studied preparations of UFH and enoxaparin are shown in Table 1. The studied preparations of UFH and enoxaparin at the concentration of 0.3 anti-Xa IU/ml inhibited 50% TG induced by the BXPC3 cancer cells.

Conclusions: Enoxaparin and UFH prepared from raw material of different species showed similar anti-Xa activity when compared on gravimetrically equivalent units (anti-Xa IU/µg). Enoxaparin and UFH prepared from bovine raw material was slightly less potent on the inhibition of TG as compared to those prepared from ovine or porcine raw material. These data confirm the possibility to use alternative sources of raw material for the preparation of enoxaparin or UFH providing that the purification and depolymerisation methods are not modified.

TABLE 1 Comparison of the UFH and enoxaparin from different origin on the basis of their anti-Xa activity and on their potential to double the aPTT

	Bovine Enoxaparine	Ovine Enoxaparine	Porcine Enoxaparine	Bovine UFH	Ovine UFH	Porcine UFH
anti-Xa IU/µg	0,08	0.12	0.10	0.10	0.21	0.12
aPTT x 2	8 µg/ml	8 µg/ml	8 µg/ml	1.9 µg/ml	1 µg/ml	1.6 µg/ml
IC50 MRI	3 µg/ml	2 µg/ml	2 µg/ml	0.9 µg/ml	0.8 µg/ml	0.8 µg/ml
IC50 ETP	4.1 µg/ml	3 µg/ml	3 µg/ml	1.2 µg/ml	1 µg/ml	1 µg/ml

PB 1875 | Efficacy and Safety of Pharmacological Thromboprophylactic Agents for the Prevention of Venous Thromboembolism after Major Abdominal Surgery: A Systematic Review and Meta-Analysis

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Background: Patients undergoing major abdominal surgery are at high risk for developing venous thromboembolism (VTE) in the post-operative period. However, the ideal agent, dose and duration of thromboprophylaxis remain unclear.

Aims: We conducted a systematic review and meta-analysis to determine the efficacy and safety of pharmacological thromboprophylaxis after major abdominal surgery.

Methods: An electronic search of the following databases was performed: MEDLINE, EMBASE and CENTRAL. Randomized controlled trials (RCT) and prospective cohort studies reporting on the efficacy and safety of different pharmacological thromboprophylactic agents after major abdominal surgery were included. Pooled proportions of VTE and major bleeding complications in the post-operative period were generated for 6 different regimens of pharmacological thromboprophylaxis: fondaparinux; high and low prophylactic doses of low molecular weight (LMWH) and unfractionated heparin (UFH); placebo or observation; and non-pharmacological thromboprophylaxis.

Results: Our literature search study identified 1,569 records. A total of 166 articles were identified as potentially eligible and after review, 87 articles (71 RCTs and 16 prospective cohorts) were included in the pooled analysis. The pooled outcome rates according to each prophylaxis regimen are depicted in table 1.

Conclusions: In this study, there was no difference in the pooled proportion of post-operative VTE between pharmacological thromboprophylaxis and observation or placebo. The pooled proportion of post-operative major bleeding events was higher in patients receiving high dose LMWH.

PB 1876 | Comparative Anticoagulant Effects of Recombinant Thrombomodulin, Antithrombin, and Unfractionated Heparin, Hematological Implications

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Background: Unfractionated heparin (UFH), antithrombin (AT), and recombinant thrombomodulin (RT) are all anticoagulant/antithrombotic agents. Currently, a recombinant and soluble version of human thrombomodulin, ART-123 (Recomodulin ©) is undergoing clinical trials for treatment of sepsis-associated coagulopathy.

Aims: The purpose of this study is to compare the anticoagulant and platelet modulatory effects of RT, UFH, and AT.

Methods: Porcine UFH was obtained from Medefil Inc. (Glendale Heights, IL), AT from Baxter Healthcare Corporation (Deerfield, IL), and RT from Asahi Kasei Pharma (Tokyo, Japan). The effects of RT, AT, and UFH at 0-5 μg/mL on glass ACT and thromboelastography (TEG) were measured. PT, APTT, and TT were measured in citrated whole blood and retrieved plasma. The effect of these drugs on agonist induced platelet aggregation (arachidonic acid, ADP, collagen, thrombin, and epinephrine) was measured in platelet rich plasma from healthy donors.

Results: In contrast to AT and UFH, RT showed no anticoagulant effects in TEG and ACT at 1.25 μg/mL. At higher concentrations of up to 5.0 μg/mL, RT was a much weaker anticoagulant. In the clotting assays, all agents produced anticoagulant effects in the following order: UFH > AT > RT. UFH mildly increased aggregation with some agonists. AT and RT did not produce any effects at concentrations of up to 5 U/ml and 10 μg/ml, respectively, for all of the agonists except thrombin.

Conclusions: This study shows that RT is a much weaker anticoagulant compared to UFH and AT, and at therapeutic concentrations, it does not produce measurable anticoagulant effects. The circulating levels of RT for the management of sepsis-associated coagulopathy range from 0.5-1.5 μg/mL. At supratherapeutic concentrations of

TABLE 1 Pooled outcome rates according to each prophylaxis regimen

Intervention	Fondaparinux	LMWH high dose	UFH high dose	LMWH low dose	UFH low dose	Placebo
Overall VTE(number of studies)	1.23 (4)	4.39 (27)	10.1 (14)	3.36 (19)	7.97 (13)	8.64 (12)
95% CI	0.003-4.64	2.74-6.40	6.60-14.23	1.56-5.82	4.37-12.52	4.09-14.66
DVT(number of studies)	0.92 (3)	3.79 (34)	6.86 (21)	3.55 (27)	6.29 (23)	13.83 (21)
95% CI	0.082-4.78	2.46-5.38	4.423-9.78	1.93-5.65	3.84-9.29	8.15-20.71
PE(number of studies)	0.24 (3)	0.25 (33)	1.04 (18)	0.31 (24)	1.1 (18)	1.39 (13)
95% CI	0.03-0.64	0.15-0.37	0.48-1.82	0.13-0.56	0.59-1.78	0.60-2.49
Major bleeding(number of studies)	1.64 (4)	3.96 (35)	2.44 (16)	2.52 (20)	3.26 (18)	0.98 (17)
95% CI	0.56-3.30	2.70-5.45	0.82-4.87	1.19-4.34	2.01-4.78	0.33-1.96

> 2.5 μg/mL, which may occur in patients with renal dysfunction, RT exhibits weak anticoagulant effects which are unlikely to contribute to any hemostatic deficit resulting in potential bleeding complications.

PB 1877 | Nuts and Bolts of Running a Pulmonary Embolism Response Team: Results from an Organizational Survey of the National PERT™ Consortium Members

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Background: Pulmonary Embolism Response Teams (PERT) are developing rapidly to operationalize multi-disciplinary care for acute pulmonary embolism patients. However, core components of PERT necessary for newly developing programs have not been defined.

Aims: To describe core components of established PERT programs and to understand variation in program structure.

Methods: An online organizational survey of active National PERT™ Consortium members was performed between April and June 2016. Analysis, including descriptive statistics and Kruskal-Wallis tests, was performed on centers self-reporting a fully operational PERT program.

Results: The survey response rate was 80%. Of the 31 institutions that responded (71% academic), 19 had fully functioning PERT programs. These programs were run by steering committees (17, 89%) more often than individual physicians (2, 11%). Most PERT programs involved 3-5 different specialties (14, 74%, Figure), which did not vary based on hospital size or academic affiliation.

Multidisciplinary discussions occurred via phone or conference call (12, 67%) with a minority of these utilizing "virtual meeting" software (2, 17%). Guidelines for appropriate activations were provided at 16 (84%) hospitals. Most PERT programs offered around-the-clock catheter-based or surgical care (17, 89%). Outpatient follow up usually occurred in personal physician clinics (15, 79%) or dedicated PERT clinics (9, 47%), which were only available at academic institutions.

Conclusions: PERT programs can be implemented, with similar structures, at small and large, community and academic medical centers. While all PERT programs incorporate team-based multidisciplinary care into their core structure, several different models exist with varying personnel and resource utilization. Understanding how different PERT programs impact clinical care remains to be investigated.

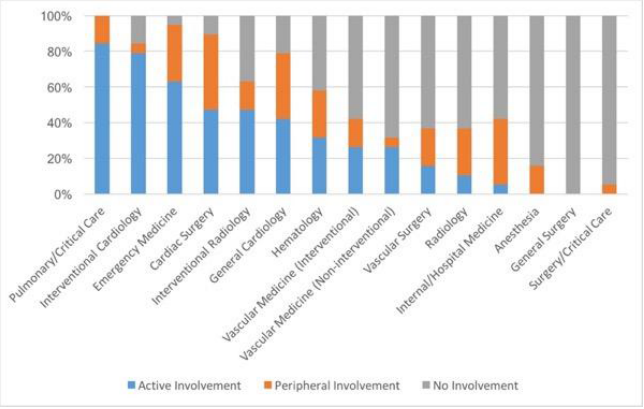

FIGURE 1 Specialty Involvement in Pulmonary Embolism Response Teams

PB 1878 | Long Term Anticoagulant Treatment Management in Patients with Portal Vein Thrombosis at High Bleeding Risk

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Background: Portal vein thrombosis (PVT) is quite common in patients with liver cirrhosis or hepatocellular malignancy. Prospective registries report that up half of the patients with PVT secondary to liver disease do not receive any anticoagulant treatment mainly due to thrombocytopenia and esophageal varices (EV).

Aims: To evaluate the safety and acceptability of long-term anticoagulant treatment with Low Molecular Weight Heparin (LMWH) in patients with PVT secondary to liver disease.

Methods: Patients with liver disease referred to our Center, from January 2011 to June 2016, for concomitant PVT and a high bleeding risk secondary to EV and thrombocytopenia are included in the current analysis. LMWH was tapered to patient's platelet (PLT) count: full dosage for PLT above 50.000/, reduced dosage (75%) for PLT below 50000/μL but above 30000/μL. Prolonged anticoagulant treatment was deemed necessary based on concomitant risk factors or thrombosis recurrences. LMWH discontinuation was patient driven. All subjects underwent a monthly clinical and laboratory follow-up. Compliance to therapy was based on clinical evaluation and answers to a specific questionnaire.

Results: Overall, 20 Caucasian patients with PVT secondary to liver cirrhosis (n=13) or carcinoma (n=7) were included in the current analysis, 11 women, 9 males (mean age: 65.2;range, 56-71 years). All patients had at least grade II esophageal varices associated with moderate to severe thrombocytopenia (mean PLT count: 42 x 10^3/L; range 35-68 x10^3/L). Mean anticoagulant treatment duration was 16 months (range: 12-30 months).Treatment was well tolerated without any major bleeding or premature discontinuation. Four patients complained easy bruising. Compliance to LMWH treatment was good, short-term treatment discontinuation (one day off therapy, every 3 days) was however reported in 5 patients.

Conclusions: In our case cohort, LMWH treatment for PVT was well tolerated in patients with liver disease under regular clinical and laboratory follow-up.

PB 1879 | Antikoagulant Effects of New Vegetable Heparinoid

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Background: Anticoagulant action of some plants is known. Plants of peonies family at which roots there are heparinoid are of special interest.
Aims: To show influence of extracts from roots of peony (P.lactiflora) containing a heparinoid on parameters of coagulant and anticoagulant systems of blood at their single intraperitoneal administration in rats organism.
Methods: Experiments are made on laboratory white rats according to ethical principles of the Helsinki Declaration. Extract from peonies (EP) in a dose of 1 mg/kg was entered intraperitoneal to each experienced rat. A control animal entered in the same way saline. The blood on analyses was taken from a vein jugularis 1 and 5 h after introduction. In a blood plasma determined anticoagulating activity by tests of activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrindepolimerizing activity (FDPA) and activity of the tissue plasminogen activator (t-PA).
Results: The increase of anticoagulating activity of a blood plasma due to elongation of APTT and PT for 80-68% respectively is established 1 h after EP introductions, at this TT practically didn´t change. During this period FDPA and t-PA raised in rats plasma for 110%-45% respectively in comparison with control. Increased anticoagulating activity due to elongation of APTT and PT for 73 and 70% respectively remained 5 h after introduction of the EP. In addition this TT remained within control. At the same time rising of FDPA for 91% and t-PA for 38% was observed.
Conclusions: So, administration of the extract from P.lactiflora causes hypocoagulation due to decrease transformations of a fibrinogen to fibrin. We concluded that the submitted results indicate prospects of study of new heparinoid from peonies as vegetable agent for pharmacological correction of the increased level of blood coagulation.

PB 1880 | Establishing the Heparin Therapeutic Range Using aPTT and Anti-Xa Measurements for Monitoring Unfractionated Heparin Therapy

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Background: The activated partial thromboplastin time (aPTT) test has been used to monitor UFH therapy, but its results can be affected by numerous patient related and laboratory test related variables. The heparin responsiveness of aPTT reagents may change from lot to lot and among different reagents used on different instrument platforms. Each laboratory must set up its own heparin therapeutic range (HTR) to standardized interpretation of aPTT results in the context of heparin therapy.
Aims: To establish an aPTT heparin therapeutic range (HTR) corresponding to therapeutic anti-Xa levels for continuous intravenous UFH administration, and used appropriate monitoring to determine if an adequate dose of UFH was applied.
Methods: A total of 30 ex vivo samples were obtained from 16 patients with a variety of thromboembolisms. All patients received intravenous UFH therapy and were enrolled from June to July 2016 at Aga Khan University Hospital. All laboratory protocols were in accordance with the Clinical and Laboratory Standards Institute guidelines and the College of American Pathologist requirements for aPTT HTR. Ethical exemption was sought from institutional ethical review committee.
Results: An aPTT range of 47 to 65 second corresponded to anti-Xa levels of 0.3 IU/mL to 0.7 IU/mL for HTR under our laboratory conditions. Based on their anti-Xa levels, blood specimen distribution were as follows: less than 0.3 IU/mL, 13%; 0.3–0.7 IU/mL (therapeutic range), 50%; and more than 0.7 IU/mL, 33%. No evidence of recurring thromboembolism was observed.
Conclusions: Although aPTT HTR determination and standardization is laborious but is a good alternative for monitoring of UFH therapy where anti-Xa assay is not available or is not cost-effective.

PB 1881 | Patients Are More Adherent to Treatment with LMWH Nadroparin than Enoxaparin for Cancer Related Venous Thromboembolism

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Background: Current guidelines recommend low-molecular-weight-heparins (LMWH) monotherapy for 3 to 6 months as first-line treatment for cancer-associated venous thromboembolism (VTE). In clinical practice nadroparin and enoxaparin are common agents used. However, differences in therapy adherence between these LMWHs have never been reported.
Aims: To compare adherence to nadroparin and enoxaparin in patients with cancer-associated VTE.
Methods: Consecutive patients with active cancer and objectively confirmed VTE , treated at three Dutch hospitals and one Spanish hospital, were included and followed during LMWH therapy with a maximum of 180 days. Cumulative incidences of discontinuation of both LMWHs were estimated and compared according to the Kaplan-Meier method, applying a competing risk analysis to correct for mortality.

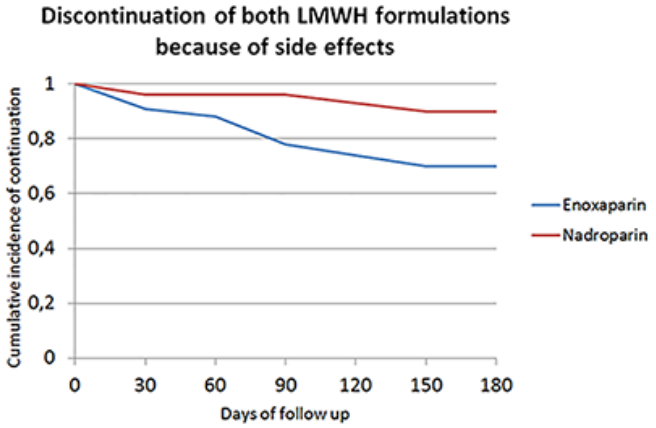

FIGURE 1 Discontinuation of both LMWH formulations because of side effects

Results: 372 patients were analysed during LMWH treatment, of whom 284 patients (76%) were treated with enoxaparin and 88 (24%) with nadroparin. The overall cumulative incidence of discontinuation of enoxaparin and nadroparin treatment because of side effects was 30% (95%CI 24-36) and 10% (95%CI 2.7-17) respectively (*Figure 1*). Competing risk analysis revealed a higher number of patients discontinuing enoxaparin because of side effects (Hazard Ratio: 3.4, 95%CI 2.6-4.2). Unacceptable pain at the injection site was the most common reason of discontinuation in patients using enoxaparin, occurring in 32 patients, while it occurred in one patient using nadroparin.

Conclusions: This analysis reveals that enoxaparin was associated with a higher risk of discontinuation because of side effects compared to nadroparin. Although this was a non-randomised comparison, our findings are relevant for choice of LMWH when treating cancer-associated VTE.

PB 1882 | Prevalence of FOXD1 Mutations in Primigravidae with Pregnancy Loss

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Background: Mutations of the transcription factor gene *FOXD1* impairing the activation of the promotor of the placental growth factor gene *PGF* are associated with recurrent pregnancy loss (Open Biol. 2016 Oct;6(10). pii: 160109) but their prevalence is not documented in other pregnancy loss phenotypes.

Aims: Assess the rate of the 3 non-synonymous *FOXD1* sequence variants [c.1285_1286InsGCCGCG (p.Ins429AlaAla); c .1067 C>G (p.Ala356Gly) ; c.1092C>G (p.Ile364Met)] in primigravida with pregnancy loss (PwPL).

Methods: We tested DNAs from the 6,992 women included in the matched case-control study nested into the NOHA-First cohort (J Thromb Haemost 2005;3(10): 2178-84). *FOXD1* coding region was sequenced as described.

Results: None of the 3 *FOXD1* mutations was evidenced in the 3,496 controls of the NOHA-First study. A total of 53 patients were found positive for the *FOXD1* mutations: 19 of the 412 PwPL before 10 weeks of pregnancy (4.6%; OR 81 [18.7-347], p< 0.0001) and 34 of the 2,705 PwPL from the 10th week (1.26%; OR 22 [5.3-91, p< 0.0001]). In this latter group, all losses in mutated women hapened before 12 weeks (2.9% of the 1,191 PwPL during weeks 10-11). The association with FOXD1 mutations in FV Leiden negative and positive women was 45/6746 (0.67%) *vs.* 8/246 (3.25%, p=0.0007), it was 48/6804(0.7%) *vs.* 5/188 (2.66%, p=0.0115) in *F2* 20210A negative and positive women.

Conclusions: *FOXD1* mutations impairing *PGF* transcription are significantly found in primigravida with pregnancy loss before 12 weeks, mainly in women with embryonic loss, and are significantly associated with the FV Leiden and the *F2* 20210A polymorphisms. The progressive description of causal mutations associated with pregnancy failure could gradually lead to a better understanding of the risk of pregnancy loss associated with thrombophilia.

PB 1883 | Prothrombin Fragment F1+2 (F1F2) in Pregnancy Is Associated with a History of Venous Thromboembolism (VTE) and Thrombophilia

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Background: Since the positive predictive value of factor V Leiden (FVL) and other genetic risk determinants for a pregnancy-associated VTE is low, additional indicators of hypercoagulability are needed to identify women at risk of VTE during pregnancy. An increased level of F1F2 is a potential indicator of hypercoagulation in normal pregnancy.

Aims: We hypothesized that women with previous VTE and FVL or prothrombin G20210A mutation (PTM) are at a higher hypercoagulable state during pregnancy than women without prior thrombotic complications and without genetic thrombophilic risk factors.

Methods: In a prospective study, we determined F1F2 over pregnancy (818 measurements) among 131 women with previous VTE, 109 women with previous fetal loss (1 late or 3 early fetal losses), 51 women with previous severe preeclampsia, and 93 healthy pregnant women. Evaluation of thrombophilia included FVL and PTM. The F1F2 levels were statistically analyzed over the course of pregnancy using a multivariate mixed model.

Results: Among women with a previous history of VTE, F1F2 values were significantly higher during the course of pregnancy than among pregnant women without VTE (p< 0.0001) (p=0.63 for preeclampsia vs. no preeclampsia, p=0.40 for fetal loss vs. no fetal loss). The results were adjusted for the physiological increase of F1F2 over pregnancy (p< 0.0001) and independent of heparin prophylaxis (F1F2 reduced

in women using heparin, p=0.004). In addition, FVL (p=0.004) and PTM (p=0.011) were independently associated with increased levels of F1F2.

Conclusions: Increased F1F2 in pregnancy are associated with thrombophilic risk factors (FVL and PTM) and a history of VTE. Determination of indicators of hypercoagulation such as F1F2 can represent a supplementary approach to identifying women at risk of VTE during pregnancy, independent of known and unknown risk determinants of VTE.

PB 1884 | Recurrent Venous Thromboembolism (VTE) in Pregnancy in Women with a History of First Venous Thromboembolism

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Background: There are insufficient data on the risk of recurrent VTE in pregnany.

Aims: To evaluate the risk of recurrent pregnancy-associated VTE in women with a single previous episode of VTE and a subsequent pregnancy without heparin prophylaxis.

Methods: We included in the study 142 women with at least one pregnancy without heparin prophylaxis after a first VTE. Evaluation of thrombophilia comprised factor V Leiden, prothrombin G20210A mutation, and deficiencies of antithrombin, protein C or protein S.

Results: Nine of 142 women without heparin prophylaxis (6.3%) had an antepartum recurrence of VTE. Among the 66 women with evidence of thrombophilia and a first episode of VTE associated with a temporary risk factor, 4 (6.06%) had an antepartum recurrence of VTE (first trimenon n=1, second trimenon n=2, third trimenon n=1) (first VTE on oral contraceptives (OC) n=3, during surgery n=1). There was no recurrence in the women with thrombophilia and a first episode of idiopathic VTE (n=8). Among the 61 women who had no evidence of thrombophilia and a first episode of VTE associated with a temporary risk factor, 4 women (6.56%) (first trimenon n=2, second trimenon n=1, third trimenon n=1) (first VTE on OC n=1, during immobilization n=1, during surgery n=2), and among 7 women with no evidence of thrombophilia and a first idiopathic VTE, 1 woman (14.3%) had an antepartum recurrence (first trimenon).

In the postpartum period, 11 VTE occurred after live birth in 142 women (7.75%). The first episode of VTE in all these women was associated with a temporary risk factor. 7 of these 11 women showed evidence of thrombophilia.

Conclusions: In addition to guideline-recommended postpartum heparin prophylaxis, our data also support routine antepartum prophylaxis in women whose previous episode of thrombosis was associated with a transient risk factor not related to pregnancy or use of estrogen, regardless of the presence of thrombophilia and starting in the first trimenon.

PB 1885 | Incidence and Risk Factors of Pregnancy-associated Venous Thromboembolism in Singhealth, a Major Healthcare Cluster in Singapore

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Background: Pregnancy-associated venous thromboembolism (VTE), which includes deep venous thrombosis (DVT) and pulmonary embolism (PE), although uncommon, is associated with high maternal mortality and morbidity. Generally thought to be more common in Caucasians than in Asians, recent studies show that the incidence of pregnancy-associated VTE in the Asian population has been on the rise and is approaching that of Western populations (0.71-1.99 per 1000 deliveries).

Aims: To evaluate the incidence and risk factors of pregnancy-associated VTE in the SingHealth cluster.

Methods: Medical records of women from KK Women's and Children's Hospital and Singapore General Hospital with pregnancy-associated VTE that occurred between 2004 and 2015 were retrospectively reviewed. VTE cases diagnosed during pregnancy or within 6 weeks postpartum and confirmed on imaging were analysed for risk factors and timing of thromboembolism.

Results: Among 156,862 deliveries there were 73 cases of VTE, with an overall incidence of 0.465 per 1000 deliveries. 2 cases (2.7%) resulted in maternal death. There were 62 cases of DVT (0.395 per 1000 deliveries) and 13 cases of PE (0.083 per 1000 deliveries), of which 2 were concurrent DVT and PE. 43.5% of DVT cases were diagnosed in the first trimester, and 71.0% were in the left lower limb. Majority of PE cases were diagnosed in the first week postpartum (61.5%) with Malay ethnicity associated with increased risk (OR 3.85, 95% CI 1.00, 14.93; p=0.050). Increased risk of VTE was associated with caesarean section (OR 3.19, 95% CI 1.40, 7.27; p=0.006).

TABLE 1 Timing of pregnancy-associated venous thromboembolism

Stage of pregnancy	DVT	PE	Both	VTE (%)
1st trimester	27	0	0	27 (37.0%)
2nd trimester	13	1	0	14 (19.2%)
3rd trimester	7	2	0	9 (12.3%)
Week 1 postpartum	6	8	0	14 (19.2%)
Week 2 postpartum	3	0	2	5 (6.8%)
Week 3 postpartum	2	0	0	2 (2.7%)
Week 4 postpartum	1	0	0	1 (1.4%)
Week 5 postpartum	1	0	0	1 (1.4%)
Week 6 postpartum	0	0	0	0 (0.0%)

TABLE 2 Sites of pregnancy-associated deep venous thrombosis

Sites of DVT	Number of cases (n=62)	Proportion of cases (%)
Left leg (total/proximal/distal/both)	44/28/11/5	71.0/45.2/17.7/8.1
Right leg (total/proximal/distal/both)	13/10/1/2	21.0/16.1/1.6/3.2
Both legs (total/proximal/distal/both)	3/1/1/1	4.8/1.6/1.6/1.6
Left brachial vein	1	1.6
Mesenteric veins	1	1.6

Conclusions: Incident prevalence of pregnancy-associated VTE in Singapore´s multi-racial Asian population appears lower than what is generally reported in Western populations. Caesarean section and Malay ethnicity were found to be significant risk factors for VTE and PE, respectively. Women in these categories should be considered as candidates for thromboprophylaxis.

PB 1886 | The Features of Pregnancy Management in Paroxysmal Nocturnal Hemoglobinuria

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening disorder with a high risk of thrombosis due to chronic complement-mediated hemolysis. The management of PNH during pregnancy recently has been challenging because of frequent severe maternal and fetal complications. The combination of targeted therapy with eculizumab and anticoagulants radically changed the prognosis in PNH and made it possible not only to increase the survival rate, but also to improve the quality of life.

Aims: to analyze the pregnancy outcomes depending on the therapeutic approach.

Methods: From 1999 to 2016 we have analyzed 29 pregnancies in PNH patients. 15 patients from 2013 exposed to eculizumab during pregnancy with anticoagulants (group 1). Other women (group 2) received only symptomatic therapy (anticoagulation with low molecular weight heparin in intermediate or therapeutic doses. The median age at the start of pregnancy - 25 years (21-34). 21,4% patients registered venous thromboembolism before conception.

Results: Clinical manifestations of hemolysis significantly regressed during eculizumab treatment: normalization of LDH was registered in 71,4% patients. Without eculizumab LDH level increased in all pregnant patients. No thrombotic events during pregnancy and postpartum have been observed. Pregnancy complications were less frequent with eculizumab: abortion threat 40% vs 85,7%, fetal growth retardation syndrome 6,7% vs 21,4%, preeclampsia 6,7% vs 14,3%. Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42,9% on supportive treatment.

Conclusions: Pregnancy in PNH with symptomatic treatment with a high probability ends adversely. The risk of complications during pregnancy and postpartum in PNH may be minimized by applying the management algorithm with eculizumab and anticoagulation treatment. We can conclude that pregnancy outcomes in PNH patients with eculizumab are better than with symptomatic therapy only.

PB 1887 | High Levels of Complement C3 Increases the Risk for Postnatal Venous Thrombosis

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Background: Venous thrombosis (VT) is one of the most serious complications to pregnancy in developed countries. The complement system is part of the innate immune system. It has been suggested that the complement system and the coagulation system cross-talks and high levels of C3 is associated with VT.

Aims: To investigate complement C3 in blood as a risk factor for pregnancy related VT.

Methods: We investigated women in the Norwegian VIP study which is a population based case-control study of VT in pregnancy or within 3 months post partum (cases, n=313) and women without pregnancy related VT (controls, n=353). Informed consent was obtained from all women and the study was approved by the regional medical ethics committee. C3 was analyzed in blood by Behring Nephelometer II (BN II) by immunonephelometry using antisera to Human Complement C3c (Siemens Healthcare Diagnostics, Deerfield, IL, USA). C3 levels above the 90th percentile in the controls was investigated as a risk factor for pregnancy related VT. Antenatal and postnatal VT were analyzed separately. Odds ratios were calculated with logistic regression.

Results: We first investigated determinants of C3 levels in the control women without pregnancy related VT. We found that C3 levels were strongly correlated with body mass index (BMI, r = 0.60) and C-reactive protein (CRP, r = 0.70), but not with smoking.

C3 in blood above the 90th percentile (1.33 g/L) was not associated with antenatal VT, odds ratio 0.87 (95% confidence interval (CI) 0.43-1.7). Adjustment for high CRP and BMI > 25 with multiple logistic regression did not change the odds ratio.

C3 in blood above the 90th percentile increased the risk for postnatal VT, odds ratio 3.0 (95% CI 1.8-5.0). After adjustment for BMI >25, the odds ratio was 2.0 (1.1-4.0). Adjustment for high CRP did not change the odds ratio for postnatal VT.

Conclusions: C3 levels above the 90th percentile increases the risk for postnatal VT, but not for antenatal VT.

PB 1888 | Risk of Venous Thromboembolism in Unsuccessful ART Cycles: An Italian Cohort Study

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Background: Assisted reproductive technologies (ART) have been associated with an increased risk of venous thromboembolism (VTE). In recent studies, it has been reported a 5-fold increased risk in pregnancies after ART as compared to that observed in spontaneous ones (8.5 ‰ vs 1.8 ‰). On the other hand, little is known about the VTE risk in unsuccessful ART cycles.

Aims: To assess

1) the absolute risk of VTE in unsuccessful ART cycles,

2) the weight of all known risk factors,

3) the potential benefit of antithrombotic prophylaxis.

Methods: This observational study was based on a cohort of 998 women approaching ART between April 2002 and July 2011, consecutively referred by local Fertility Clinics to our Unit. All women were investigated for the presence of inherited (Factor V Leiden, prothrombin 20210A mutation, deficiencies in protein S and C and antithrombin) and acquired (antiphospholipid antibodies - aPL) thrombophilias. Clinical information were collected during office visits and/or by phone interviews. Women undergone at least 1 unsuccessful ART cycle (negative pregnancy test) and with a negative personal history for VTE were selected.

Results: Among 671 women undergone ART, 512 experienced at least 1 unsuccessful ART cycle for a total of 1518 failed attempts. VTE was observed in 2 out of 1518 (1.3‰) unsuccessful cycles. Both cases, isolated pulmonary embolism, occurred in absence of antithrombotic prophylaxis: the first one in an overweight (BMI: 26) and aPL-positive woman, the second one in a 45 years old overweight (BMI: 31) woman; both cycles were complicated by OHSS.

Conclusions: In women undergone unsuccessful ART cycles we found a lower VTE incidence than that previously reported in women after successful cycles (1.3‰ vs 8.5 ‰). However, both the recorded events are potentially life-threatening. Antithrombotic prophylaxis could be efficacious in the prevention of ART-related VTE. Further studies on the potential benefit of thromboprophylaxis are needed.

PB 1889 | M2 Haplotype in Annexin A5 Gene and Antiphospholipid Antibodies: Possible Relationship and Clinical Impact in a General Obstetric Population

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Background: M2 haplotype in the Annexin 5 (ANXA5) gene has been associated with the occurrence of gestational vascular complications. More recently, in M2 carriers a higher susceptibility to develop antiphospholipid antibodies (aPL) has been suggested.

Aims: To prospectively evaluate, in general obstetric population, the possible relationship between the M2 haplotype and aPL, and M2 haplotype and/or aPL and obstetric outcomes.

Methods: From an initial cohort of 3097 pregnant women consecutively admitted to 14 obstetric and gynaecology wards of the Campania region (Italy) during a 15-month period (November 2000 - January 2002), 1286 samples were analysed for the presence of both anticardiolipin and anti β2-Glycoprotein I antibodies; samples with available DNA (n= 606) were also investigated for the M2 haplotype.

Results: Overall, 233/1286 (18.1%) women showed the presence of aPL. Among them, 26 (11.1%) experienced a pregnancy loss in the current pregnancy, 201 (86.3%) gave birth to live-born babies (p: ns vs those w/o aPL). M2 haplotype was identified in 140 (23.1%) out of 606 women with DNA available: 20/140 (14.3%) M2 carriers and 97/466 (20.8%) non-carriers tested positive for aPL, respectively (p: ns). 22/227 (9.7%) M2 and/or aPL carriers, and 31/359 (8.6%) non aPL and/or M2 carriers suffered from obstetric complications (p: ns).

Conclusions: In general obstetric population, no relationship between M2 haplotype and presence of aPL was found. Furthermore, neither the M2 haplotype nor aPL were associated with adverse obstetric outcomes. Therefore, in low-risk pregnant women the routine investigation of M2 haplotype and/or aPL is not justified.

PB 1890 | Recurrent Miscarriage Predictive Capacity Using Thrombo inCode - Recurrent Miscarriage

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Background: Recurrent miscarriage (RM) is a clinical problem affecting 1-5% of couples of reproductive age. The contribution of thrombophilia to RM is disputed. This controversy is partly due to low

sensitivity of the genetic variants currently used to evaluate hereditary thrombophilia: the Leiden mutation (identified as rs6025) in the coagulation factor 5 (F5L) gene and mutation G20210A (identified as rs1799963) in the prothrombin (PT) gene.

Aims: Our objective was to determine whether a wider algorithm that includes clinic and genetic variants associated with thrombophilia could be more useful in the prediction for RM than FVL and PT alone.

Methods: A prospective case-control study was carried out. The study included 184 cases of idiopathic early (less than 20 weeks) recurrent miscarriages (not advanced maternal age, normal paternal karyotypes, uterus without disorders, absence of antiphospholipid syndrome or endocrinopathy, obesity or chronic systemic diseases) and 183 healthy women with at least one pregnancy to term and no previous miscarriage. Informed consent was obtained from all of the participants and the studies were approved by the Institutional medical ethics committees. Two algorithms were analysed in each study (see table1).

TABLE 1 Parameters included in 'TiC-recurrent miscarriages' and 'F5L-PT'

TiC-recurrent miscarriages	F5L-PT
F5-Leiden-rs6025	F5-Leiden-rs6025
PT-rs1799963	PT-rs1799963
F5-Hong Kong-rs118203906	
F5-Cambridge-rs118203905	
F12-rs1801020	
F13-rs5985	
Serpin-C1-rs121909548, Serpin-A10-rs2232698	
AB0-rs8176719, rs8176750, rs7853989,rs8176743	
Age, Gender, Body Mass Index, Smoking and Family history of VTE	

The predictive capacity was determined by AUC, sensitivity, specificity, -LHR and +LHR and diagnostic odds ratio (DOR).

Results: F5L-PT showed a non-significant AUC 0.50 [0.449-0.553]. TiC-RM showed a significant AUC 0.77 , 95%CI [0.722-0.818], with a good sensitivity (73%) and specificity (70%), +LHR was 2.43 and -LR was 0.39. The DOR was 6.3.

Conclusions: TiC-RM proved to better predict patients who would suffer thrombophilia related-RM than the classic F5L and PT algorithm.

PB 1891 | Effect of Blood Loss during Caesarean Section on Coagulation

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Background: Venous thrombosis and pulmonary embolism are the leading causes of the maternal morbidity and mortality. The risk of thrombosis is even higher postpartum further increasing following caesarean section and blood loss.

Aims: To study the mechanisms of the prothrombotic state development due to an increased blood loss during caesarean section.

Methods: The endogenous thrombin potential (ETP) and the function of anticoagulant pathways were evaluated via calibrated automated thrombography in the pre- und postoperative plasma samples of 50 pregnant women (age 30,7±4,4 years, 38,5±0,7 weeks of gestation), who underwent a primary caesarean section at the University Clinic of Magdeburg. The blood loss was subjectively und objectively evaluated (weighting of surgical towels and aspirator content and „one-compartmental biometric method") and correlated with the changes in the coagulation system.

Results: Blood loss led to a minor decrease in the ETP: ΔETP -4% (95%CI:-4%;-2%) immediately after a caesarean section and -5% (95%CI:-7%;-2%) in 6 hours postoperatively. In contrast, the ETP in the presence of APC significantly increased immediately after surgery ΔETP_{+APC} 54% (95%CI:28%;80%) and 6 hours postoperatively: 96% (95%CI:48%;144%). The changes both in the ETP and ETP_{+APC} gradually changed in the groups with < 10%; 10-20%, >20% of Hb-decrease. Objective methods of the blood loss evaluation correlated well between each other (Pearson coefficient=0,56), but not with the subjective method.

Conclusions: A mild postoperative ETP decrease reflects a reduction or normalisation of antepartum elevated coagulation factors. The ETP_{+APC} increase indicates an impairment of the anticoagulant pathways' function (protein S, tissue factor pathway inhibitor und APC) that were already decreased in the pregnancy. This results in a simultaneous pronounced deficiency of several anticoagulant proteins, which can explain an increased thrombosis risk following a caesarean section with an increased blood loss.

PB 1892 | Assessment of Peripartum Management in a Population at High Risk for Venous Thromboembolism

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Background: Women with a history of VTE or hereditary risk factors are at an increased risk for venous thromboembolism (VTE) in pregnancy. While risks and benefits of anticoagulant treatment have been well studied in this population, less is known about its impact on peripartum management.

Aims: To assess obstetric and anesthesiological management of delivery in women with a history of thrombosis and/or hereditary risk factors for VTE.

Methods: The medical charts of women who delivered at the University Hospital Bonn were reviewed. Women fulfilling the aforementioned inclusion criteria were matched with women without a history or known hereditary risk factors for VTE, who did not receive anticoagulants. Matching criteria were age, gestational age at delivery, and date of delivery.

Results: 114 women were included. 62 of them had a history of thrombosis, thereof 13 in the current pregnancy. In 72 women, hereditary risk factors for VTE were present, including factor V Leiden (n=45), prothrombin G20210 (n=15), and inhibitor deficiencies (n=15). In 42 women with a history of VTE no hereditary risk factors were identified. 90 women received heparin once daily and 13 twice daily. With 50.9% a significantly lower rate of spinal and epidural anesthesia was observed in comparison to the controls (66.7%, p=0.011%). The rates of caesarean section (41.2% vs 47.4% in the control group), vacuum extraction (6.1% vs 7.9%), and vaginal delivery (52.6% vs 44.7%) did not differ significantly. Median (interquartile range) blood loss at delivery was 300 (225-500) ml in comparison to 400 (250-500) ml in the control group. A statistically significant difference between both groups regarding bleeding complications was not observed.

Conclusions: Our data demonstrate that bleeding complications at birth does not occur frequently in women at high thrombotic risk, despite a high rate of anticoagulant treatment. A higher proportion of women with anticoagulant treatment in pregnancy might be eligible for spinal and epidural anesthesia.

PB 1893 | Role of ADAMTS-13 Activity and Inhibitor Monitoring during Pregnancy in Women with History of Severe Preeclampsia

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Background: The term „thrombotic microangiopathy“ (TMA) brings together clinical conditions of heterogeneous nature characterized by arteriolar and capillary thrombosis with the development of hemolytic anemia, thrombocytopenia and ischemic damages leading to multiple organ failure. Thrombotic thrombocytopenic purpura (TTP) is related to more than 70 mutations of ADAMTS-13. TTP may also be due to decreased activity of ADAMTS 13 under the influence of antibodies (e.g., in patients with antiphospholipid syndrome). Recently it was shown that pregnancy is one of the main initiating agent for development of TTP.

Aims: We studied the role of ADAMTS-13 defects in pathogenesis of severe preeclampsia.

Methods: 128 patients with severe preeclampsia were tested for genetic thrombophilia, antiphospholipid antibodies and elevated homocysteine level. In 60 cases we tested activity of ADAMTS-13 and antibodies titer to ADAMTS-13.

Results: Thrombophilia was detected in 100% women with 2 and more recurrent preeclampsia and in 60% primagravida with preeclampsia. We determined ADAMTS-13 inhibitor in high titer in 11 patients. The basic therapy during pregnancy was low molecular weight heparin guided by D-dimer, serial plasmapheresis in women with low ADAMTS-13 activity, antibodies to ADAMTS-13 and progressive thrombocytopenia. 5 patients with preeclampsia and ADAMTS-13 inhibitor were delivered after 37 weeks, all babies were alive. 4 patients were delivered prematurely with 2 fetal losses.

Conclusions: Severe obstetric complications may be considered as a distinct form of thrombotic microangiopathy, including HELLP-syndrome and severe preeclampsia.

PB 1894 | Assessment of Annexin A5 and Annexin A2 Levels as Biomarkers for Pre-eclampsia: A Pilot Study

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Background: Dysregulated placental function due to fibrin deposition and microthrombi has been implicated in the pathogenesis of pre-eclampsia (PE). Deficient anticoagulant activity of annexin A5 and deficient profibrinolytic activity of annexin A2 have been linked to increased risk for thrombotic events. The identification of biomarkers that can predict incidence and severity of pre-eclampsia would support improved therapies to prevent adverse maternal and fetal outcomes.

Aims: To assess serum levels of annexin A5 and annexin A2 in a cohort of pre-eclampsia patients and investigate their role as biomarkers for the development of the disease.

Methods: We examined a total of 80 women; 40 with healthy pregnancy and 40 with pre-eclampsia of varying severities. Women were subjected to full clinical assessment, ultrasonography, and laboratory testing including complete blood picture, liver and kidney function tests and serum and urine proteins. Serum Annexin A5 and annexin A2 were analyzed using enzyme-linked immunosorbent assay.

Results: Serum annexin A2 but not annexin A5 was significantly reduced (P=0.03) in women with PE (total and severe cases) when compared to those with normal pregnancy. The ROC analysis of annexin A2 level for the prediction of development of PE showed an area under the curve of 0.64 (P=0.03), and the best cut-off value was 0.89 ng/mL with a sensitivity of 70% and a specificity of 70%. Univariate analysis showed higher systolic and diastolic BP, proteinuria, lower platelet count and annexin A2 (< 0.89ng/ml) was associated with significantly higher risk to develop PE.

Conclusions: Based on this pilot study, serum annexin A2 levels may be a useful biomarker for pre-eclampsia. However, a larger study is required to further support this conclusion.

PB 1895 | Attenuated Thrombin Generation and Enhanced Fibrinolytic Activity in Early Onset Preeclampsia

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Background: Preeclampsia is a complication of pregnancy with potentially life-threatening consequences for both mother and baby. Early onset preeclampsia (EOP; onset < 34 gestational weeks) is associated with greater maternal and fetal risks than late onset preeclampsia. The risk of venous thromboembolism is increased, particularly in severe preeclampsia. Consequently, mothers may be considered for thromboprophylaxis. However, risk assessment is complicated by competing bleeding risks such as placental abruption, post-partum haemorrhage, or renal impairment.

Aims: To characterise haemostasis in EOP patients to better understand parameters that may modulate thrombotic and bleeding risk in EOP.

Methods: The study was approved by the relevant ethics committee and written informed consent was obtained. Blood samples were collected from pregnant women with EOP (n=26) and matched pregnant controls (n=20). Patients with multiorgan involvement were characterised as severe cases (n=12). Plasma thrombin generation was measured by calibrated automated thrombography. Coagulation activation, fibrinolytic, and platelet activation biomarkers were measured by ELISA.

Results: Compared to controls, EOP patients have reduced Tissue factor-dependent thrombin generation (Peak thrombin (IIa); 150±80 vs. 205±69nM IIa, p< 0.05), which is more pronounced in severe EOP (136±102nM IIa, p< 0.05). There were increased levels of tissue plasminogen activator in EOP (6919±3116 vs. 1767±733ng/ml, p< 0.0001), particularly in severe EOP (8665±2296ng/ml, p< 0.0001) and reduced Plasminogen Activator Inhibitor-2 levels (122±64 vs. 169±17ng/ml, p< 0.05). Consistent with enhanced fibrinolytic activity, there was a trend towards increased D-Dimer levels in EOP (4.6±2.1 vs. 4.1±1.9µg/ml), particularly in severe EOP (5.6± 2.2µg/ml). There was no significant difference in platelet activation markers (soluble GPVI, platelet factor 4, CXCL7) in EOP and controls.

Conclusions: EOP is characterised by decreased thrombin generation and enhanced fibrinolytic activity.

PB 1896 | Management of Venous Thromboembolism in Women during Pregnancy and Puerperium (SAVE): A cross-sectional registry in Africa, Eurasia, Middle East and South Asia

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Background: The modality of VTE prophylaxis during pregnancy and puerperium is not well documented outside Western countries.

Aims: Assess physician's VTE risk management and its adequacy with ACCP / RCOG guidelines.

Methods: International, observational, cross-sectional multicentre study across 18 countries (Dec2014-Oct2015). Consecutive women visiting for first prenatal consultation or any other consultation during pregnancy or puerperium were enrolled. VTE or antithrombotics led to exclusion. Assessments were made based on Investigator's questionnaires and CRFs. The quality control and validation of data was performed independently from investigators by a contract research organisation.

Results: The majority of women at risk received prophylaxis globally and similarly across regions except South Asia, where 23% women did not receive the prophylaxis as per guidelines. Thromboprophylaxis was prescribed in 47.7% of the patients, rarely in South Asian patients, commonly in African and Middle Eastern women, very often in Eurasian women. Lower rates of thromboprophylaxis during puerperium were observed in Eurasia and South Asia, higher in Africa. This was performed during pregnancy by pharmacological treatments (42.4%: LMWH in 73.5%, aspirin in 46.3%), mechanical treatments (11.5%) or both (46.2%) with heterogeneities across regions. A similar pattern was evidenced during puerperium. Prophylaxis in women at risk was rarer in Blacks and South Asians than in Caucasians. Assisted reproduction technology and Caesarean section were key factors determining prophylaxis, with heterogeneities across regions. Absence of available proof was frequently evoked for not prescribing. More than 80% of physicians declared following the guidelines.

Conclusions: The SAVE registry shows that consulting physicians manage at risk women with appropriate prophylaxis. However, discrepancies observed between the regions warrants further efforts to improve implementation of the guidelines.

PB 1897 | Genetic Variants Associated with Thrombophilia and Gestational Complications in an Argentinian Cohort

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Background: Factor V Leiden (FVL) and Prothrombin 20210A (II20210A) have been recognized as risk factors for recurrent

pregnancy loss (RPL) by impairing normal placental vascular function. Other genetic variants as Fibrinogen-γ´10034T (FGG10034T) and Factor XI 7872C (FXI7872C) have been associated with a higher risk of venous thrombosis but their role in RPL is unknown.

Aims: The aim of this study was to analyze the association of FVL, II20210A, FGG10034T and FXI7872C with: 1) the risk and timing of RPL and 2) the risk of suffering other obstetric complications: intrauterine growth restriction (IUGR), placental abruption and preeclampsia, among Argentine women.

Methods: We performed a case-control study including 247 Argentine women (cases) with idiopathic RPL and 100 fertile controls (women in post-fertile age with ≥2 successful pregnancies and had never experienced any obstetric complication). Cases were stratified in early, n=87 (≥ 2 idiopathic consecutive failed pregnancies < 10th week) and late losses, n=155 (>10th week). FVL, II20210A, FGG10034T and FXI7872C were genotyped both in RPL and control groups.

Results: No differences were found in the distribution of the four genetic variants either among RPL (Figure 1), early or late losses groups vs controls, respectively (p>0.05).

Similarly, no differences were observed in its distributions when we analyzed RPL patients stratified according to obstetric complications (p>0.05), except for the FVL carriage in patients with IUGR vs controls (11.8%, 4/34 vs 2%, 2/100; p=0.017). FVL would be associated, independently from other inherited and acquired thrombophilia, with a higher risk of IUGR [OR=7.11 (1.24-40.93), p=0.028] (Figure 2)

Conclusions: Our results highlight the importance of FVL in obstetric complications, particularly with IUGR, and makes it a suitable candidate to be further evaluated in future investigations. This is the first study that analyzes the potential impact of the genetic variants FGG 10034T and Factor XI 7872C on RPL.

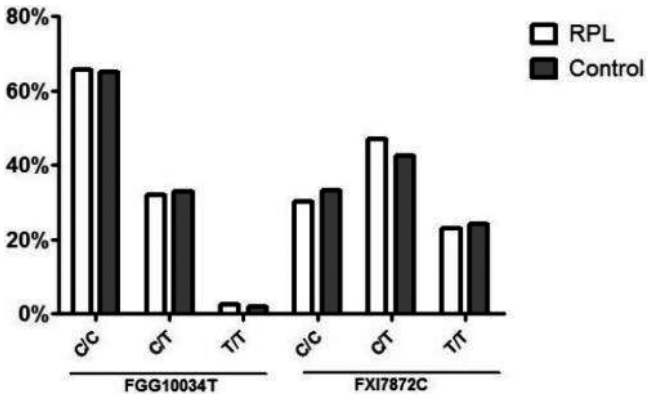

FIGURE 1 Genotype frequencies of FGG10034T and FXI7872C among patients with recurrent pregnancy loss (RPL) and controls

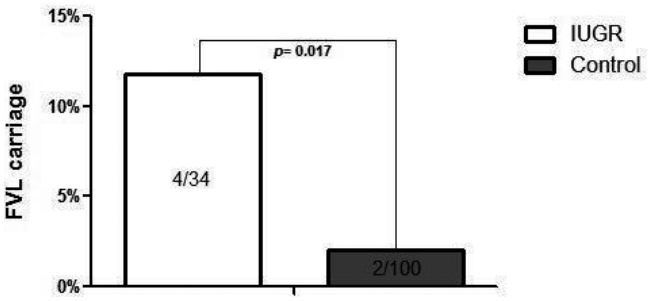

FIGURE 2 Distribution of FVL carriage in patients with intrauterine growth restriction (IUGR) and controls

PB 1898 | Risk Factors for Venous Thromboembolism and Pregnancy Outcomes in Women with Pelvic Vein Thrombosis during Pregnancy - A Retrospective Study from a Stockholm Cohort, Ranging from 2000 to 2014

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Background: Pelvic vein thrombosis is rare outside of pregnancy and pelvic surgery. The recommended anticoagulant treatment is low molecular weight heparin. There is a lack of studies specifically on risk factors for antenatal pelvic vein thrombosis and the safety and efficacy of the administered treatment.

Aims: To document risk factors for antenatal pelvic vein thrombosis, evaluate maternal and foetal outcomes and pregnancy complications.

Methods: The medical records of 39 women who developed antenatal pelvic vein thrombosis and were treated at the Karolinska University Hospital during 2000-2014 were reviewed. The incidence of bleeding complications was used to evaluate safety and the number of recurrent thromboses was used to study efficacy.

Results: The majority of the women were nulliparous (82.1%) and had blood group A (74.4%) and half of them had thrombophilia (51.3%). One third (33.3%) suffered postpartum haemorrhage (PPH, ≥500ml) and 5.1% had severe PPH (≥1000 ml). No patient experienced recurrent thrombosis. One child (2.5%) was born small for gestational age and one patient (2.6%) was diagnosed with preeclampsia.

Conclusions: Nulliparity and blood group A were overrepresented in this cohort, as previously reported for patients with thrombosis. The anticoagulant treatment is effective in preventing recurrent thrombosis, but is appears to entail a somewhat increased risk for non-severe bleeding complications. No increased risk for maternal and foetal complications was observed. Studies on larger cohorts are needed in order to confirm these findings.

PB 1899 | Maternal Carriage of the Annexin A5 M2 Haplotype is Associated with a Higher Risk of Suffering from Gestational Vascular Complications

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Background: Annexin A5 (ANXA5) is a protein abundantly expressed in normal placenta where it was proposed to exert an anticoagulant function. The carriage of the ANXA5 M2 promoter haplotype results in a reduced expression of placental ANXA5 and was associated with an increased risk of Recurrent Pregnancy Loss (RPL) and other placental-mediated pregnancy complications (PMPC), in different European and Asian populations.

Aims: Our aim was to assess the association between maternal carriage of the ANXA5 M2 haplotype and: a) the risk of RPL, b) its influence on the timing of the losses and c) the risk of suffering other PMPC, for the first time in a Latin America population.

Methods: The case-control study included 229 women with RPL and 100 parous controls without pregnancy complications. Moreover, patients were stratified according to: a) the timing of miscarriages: early (< 10 weeks), late (≥10 weeks) and fetal losses (≥20 weeks) and b) having suffered or not these PMPC complications: intra uterine growth restriction (IUGR) and/or pre-eclampsia (PE).

The proximal core promoter region of the *ANXA5* gene was analyzed by amplicon sequencing in all subjects. Differences in the maternal *M2/ANXA5* carriage rate between groups were assessed using the chi-square test. The *odds ratio* (OR) was estimated by binary logistic regression adjusted for potential confounding variables.

Results: An association between maternal carriage of *M2/ANXA5* with predisposition to RPL only, or with the timing of pregnancy losses could not be confirmed. However, the results indicated that it would be associated (OR=2.38; 95%CI 1.04 to 5.45) with a higher risk of suffering IUGR and/or PE, not limited to miscarriage.

Conclusions: The maternal carriage of the ANXA5 M2 haplotype would be partly responsible for the onset of IUGR and/or PE, probably through reducing ANXA5 placental expression. This could be of direct relevance as *M2/ANXA5* might be considered in the diagnostic work-up as a prognostic marker of these gestational vascular complications.

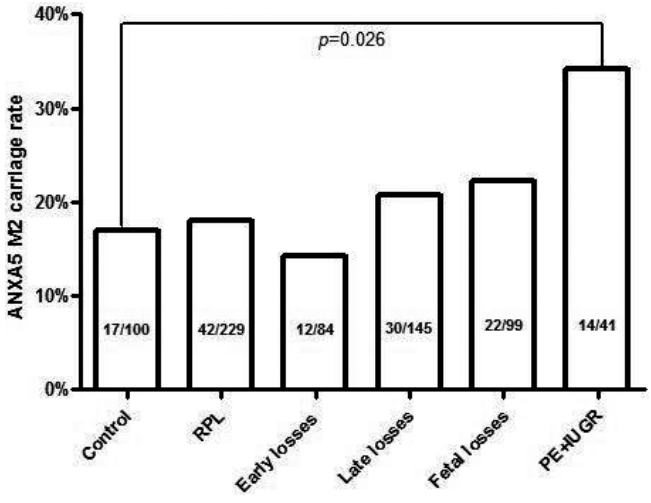

FIGURE 1 Maternal carriage of the ANXA5 M2 haplotype among RPL, PMCP and control groups

PB 1900 | Pregnancy-associated Hemolytic Uremic Syndrome as a Cause of Maternal Mortality

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Background: Pregnancy-associated hemolytic uremic syndrome (P-aHUS) is a severe disorder with a high risk of maternal and fetal morbidity and mortality, defined by the occurrence of comlement-mediated thrombotic microangiopathy (TMA) without ADAMTS13 deficiency. Triggered by pregnancy and another complement-amplifying conditions women develop the syndrome, leading to a disastrous hemolytic disease characterized by diffuse endothelial damage and platelet consumption.

Aims: The evaluation of outcomes depending on the therapeutic approach.

Methods: We present the retrospective analysis of 31 P-aHUS cases.

Results: Pregnancy complications included mild preeclampsia (61%), anthenatal fetal death (32%), 32% of patients were urgency delivered (27-37 weeks). Massive uterine bleeding required hysterectomy occured in 23,2%. Acute kidney injury registered in 100% patients, neurological symptoms had 61%, respiratory distress syndrome- 55%, dilated cardiomyopathy- 16,7%. 61,3% required hemodialysis, 51,8%- respiratory care. All of them underwent plasma exchange and received low molecular weight heparin, 45,1% treated with eculizimab (but a full course was not held anyone). All of patients who died had 2 “waves” of TMA: first wave have damaged 2-5 organs without any proved infections, but treated with combination of antibiotics.Second TMA wave was fatal due to superimposed septic disorders, resistant to antibiotic therapy. Patients on eculizumab treatment had more severe disease at debut with shorter history of aHUS and responded well to eculizumab. 6 other patients died from 2-7 days.

Overall outcomes were poor: died 32%, reached the end stage renal disease by 1 month 19%. Survival on eculizumab treatment was 71,4%, without it - 64,7%.

Conclusions: P-aHUS is a life-threatening disorder associated with a significant maternal morbidity and mortality. Timely diagnosis and effective approaches including early prescribed targeted therapy can improve the survival rate and pregnancy outcomes.

PB 1901 | The Role of Acquired von Willebrand Syndrome in Bleeding during Pregnancy and Cesarean Section in Patients with Hereditary Connective Tissue Diseases (Marfan Syndrome, Ehlers-Danlos Syndrome, Hereditary Hemorrhagic Telangictasia)

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Background: The term of acquired von Willebrand syndrome(AVWS)is now accepted for patients with predominant underlining diseases that lead to defective von Willebrand factor(VWF). In recent years there has been a progressive increase of publications concerning patients with defective VWF in whom the family history and the late onset of bleeding symptoms speak against an inherited forms. The inherited connective tissue diseases is a group of diseases with connective tissue deficiency various localizations. All of this patients have bleeding symptoms due to the connective tissue deficiency in vascular wall. Pregnancy and delivery in such patients are complicated by repeated bleeding and massive hemorrhage. We supposed that AVWS can play an important role in bleeding symptoms in patients with hereditary connective tissue diseases together with deficiency of vascular wall.

Aims: To evaluate the role of AVWS in bleeding symptoms during pregnancy and cesarean section in patients with hereditary connective tissue diseases.

Methods: We describe our experience in diagnosing AVWS in56 pregnant women with confirmed diagnosis of hereditary connective tissue diseases(23with Marfan syndrome, 22 with Ehlers-Danlos syndrome, 11with hereditary hemorrhagic telangictasia (HHT) over more than 8 years.

Results: All pregnant patients had bleeding various localizations. Marfan patients also had cardiovascular complications:18women developed increasing of mitral regurgitation, 10 aortic insufficiency, 13 mitral valve insufficiency. 1 patient with Marfan syndrome was died due to aortic dissection in 45 day after delivery. Cesarean section was performed in 53. AVWS was established in18 patients with hereditary connective tissue diseases.

Conclusions: AVWS can play an important role in bleeding symptoms during pregnancy and cesarean section in patients with hereditary connective tissue diseases together with deficiency of vascular wall. Patients with hereditary connective tissue diseases require the multidisciplinary care and repeated testing for AVWS.

PB 1902 | Impact of Blood Hypercoagulability on in vitro Fertilization Outcomes: A Prospective Longitudinal Observational Study

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Background: Blood coagulation is crucial in the implantation process and its alteration may be related to in vitro fertilization (IVF) failure.

Aims: We conducted a prospective observational longitudinal study in women eligible for IVF to explore the association between coagulation alterations with the IVF outcome and to identify the biomarkers of hypercoagulability which are related with this outcome.

Methods: 38 women eligible for IVF (IVF-group) and 30 healthy, age-matched women (control group). In the IVF-group blood was collected at baseline, 5-8 days after gonadotropin-releasing hormone agonist (GnRH) administration, before human recombinant chorion gonadotrophin administration and two weeks after hCG injection. Pregnancy was monitored by measurement of βHCG performed 15 days after embryo transfer. Thrombin generation (TG), whole blood thromboelastometry (ROTEM®), procoagulant phospholipid clotting time (PPL), thrombomodulin (TMa), Tissue factor activity (TFa), factor VIII (FVIII), factor von Willebrand (FvW), D-Dimers and fibrinogen were assessed at each time point.

Results: Positive IVF occurred in 15 women (40%). At baseline, the IVF-group showed significantly increased TG, TFa and TMa and significantly shorter PPL versus the control group. After initiation of hormone treatment TG was significantly higher in the IVF-positive group as compared to the IVF-negative. At all studied points, the PPL was significantly shorter and the levels of TFa were significantly higher in the IVF-negative group compared to the IVF-positive one. The D-Dimers were higher in the IVF negative as compared to IVF positive group. Multivariate analysis retained the PPL and MRI of TG as predictors for the IVF outcome.

Conclusions: The diagnosis of women with hypercoagulability and their stratification to risk of IVF failure using a model based on the PPL and TG is a feasible strategy for the optimisation of IVF efficiency that needs to be validated in prospective trials.

PB 1903 | Women with Homozygous AT Deficiency Type II Heparin Binding Site (HBS) Are at High Risk of Pregnancy Loss and Pregnancy Complications

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Background: Homozygous antithrombin (AT) deficiency type II HBS is a very rare and severe thrombophilia. Data regarding outcome and therapy of pregnancies in such women are scarce.

Aims: To get a better insight into pregnancy outcome and to identify suitable management procedures.

Methods: After institutional ethical approval a retrospective, descriptive investigation on the obstetric history of women with this thrombophilia was conducted.

Results: Eight women with homozygous AT deficiency type II HBS and at least one pregnancy were identified, each had the mutation c.391C>T p.Leu131Phe in the AT gene (SERPINC1). AT activity levels ranged between 12% and 32%, patients were between 13 and 23 years at first venous thromboembolism (table 1).

TABLE 1 Patient characteristics and first thrombotic events before their first pregnancy

Patient	First thrombotic event	Age at first TE	Age at diagnosis	AT activitiy Level
1	Unprovoked DVT right leg	13	18	12%
2	Provoked DVT right leg and pelvic veins	20	20	13%
3	Provoked DVT left leg	23	23	16%
4	Unprovoked DVT right leg and pelvic veins	16	23	22%
5	Povoked DVT right leg and pelvic veins	18	29	20%
6	No thrombotic events	n/a	35	32 %
7	Unprovoked DVT right leg	23	23	17%
8	Provoked PE	18	22	31%

Overall, the women reported 23 pregnancies between the years 1989 and 2013. One pregnancy was excluded, since it ended by induced abortion. Only seven out of the 22 pregnancies were successful with a live infant, however, all of them were born preterm. Patients suffered seven early pregnancy losses and eight intrauterine fetal deaths. The treatment regimes were widely spread, not least due to the rareness of the disease and thereof resulting lack of experience. The following regimens (figure 1) were used: low molecular weight heparin, intermittent AT concentrates, or a combination of both, or treatment with vitamin K antagonists (VKA). We found no clear association between treatment protocols and outcome. No anticoagulation was given during eight pregnancies and all of those ended in pregnancy loss.

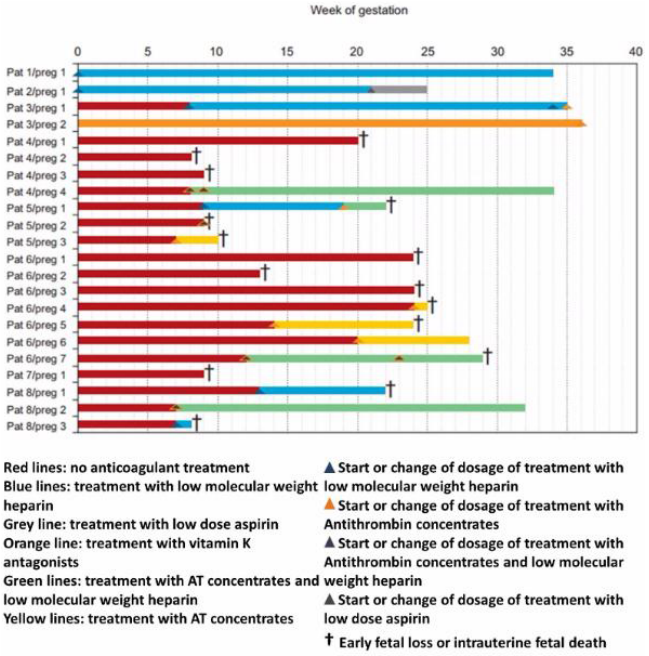

FIGURE 1 Treatment during pregnancy and pregnancy outcome

Conclusions: This rare thrombophilia is associated with a very high risk of pregnancy loss and preterm delivery. Anticoagulation and even AT replacement were only partially successful. VKA might be more effective, but the teratogenic effect has to be kept in mind. We surmise that women with homozygous AT deficiency type II HBS need a rigorous and preferably heparin-independent anticoagulation or intensified AT replacement.

PB 1904 | Use of Intravenous Immunoglobulin in the Antenatal Management of Haemolytic Disease of the Fetus and Newborn (HDFN): A Case Series from the East Midlands, United Kingdom

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TABLE 1 Antibody level, antenatal intervention, gestation at delivery and treatment in the neonate for the twocomparable pregnancies in our cases

Patient	Antibody	Booking and (peak) antibody level non IVIG treated	Booking and (peak) antibody level IVIG treated	Gestation IVIG commenced (weeks) in treated pregnancy	Gestation IUT commenced (weeks) non IVIG treated	Gestation IUT commenced (weeks)IVIG treated	Gestation at delivery (Weeks) non IVIG treated	Gestation at delivery (Weeks) IVIG treated
1	anti D (IU/ml)	5 (468)	18.5 (161)	14	28	31	34+4	34+2
2	anti D (IU/ml)	73 (83.1)	264 (264)	14	18	20	34	IUFD 21+4
3	anti D (IU/ml)	7 (23.6)	25.6 (25.6)	15	33+4	not required	33+5	35
4	anti D (IU/ml)	2.3 (29.3)	15.4 (unable to quantify anti C+G)	18	not required	32+2	35+5	34+4
5	anti D (IU/ml)	8 (24)	35 (35)	14 (1 dose)	not required	22+2	36	34+6
6	anti D (IU/ml)	8 (unable to quantify anti C+G)	554 (554)	16	not required	Not required (IUFD 19+6)	34	NA
7	anti K (titre)	1024 (2000)	8000 (8000)	16	26+6	28+2	IUFD 29	31+1
8	anti K (titre)	none detected	256 (2000)	17	not required	34+1	42	34
9	anti K (titre)	1024 (1024)	2048 (2048)	16	Early miscarriage	18+4	Early miscarriage	33

Background: Red cell alloimmunisation complicates approximately 3% of pregnancies. Standard of care involves fetal monitoring and intervention with intrauterine transfusion (IUT) if signs of anaemia develop; this is associated with significant risks. Administration of maternal intravenous immunoglobulin (IVIG) has been proposed as an alternative treatment option to delay the onset of fetal anaemia and is appealing in its non-invasive nature. There are no randomized controlled trials to support the use of IVIG however case series suggest potential benefit.
Aims: We report a retrospective case series of 9 patients with antenatal HDFN managed with IVIG between 2013 and 2017.
Methods: All women had a history of severe antenatal HDFN and/or significant red cell antibody titres in early pregnancy. Each pregnancy where IVIG was administered was compared to a pregnancy in the same woman without IVIG.
Results:
All women had at least 1 comparable pregnancy. IVIG 1g/kg was given weekly from a median of 16 weeks gestation. IVIG was stopped in 1 case due to asceptic meningitis. 3 women had IUFD; 2 in IVIG treated pregnancies. Of the 8 pregnancies where IVIG was continued; 4 women had IUT in a comparable pregnancy. IUT was commenced at later gestation in the IVIG treated pregnancy in all 4 patients; 1 did not require any IUT at all. 1 woman delivered at a later gestation than the pregnancy without IVIG. 2 women delivered earlier and 4 women had either a miscarriage or IUFD in the comparable pregnancy. There was no difference in treatment required in the neonates.
Conclusions: The aim of giving IVIG was to delay IUT until a later gestation, thus reducing the risk to the fetus. This was achieved in all 4 patients who had required IUT in a comparable pregnancy. This is particularly encouraging as IUT is often required at earlier gestation in subsequent pregnancies. This is a small case series and there is a clear need for further research in this area as randomized controlled trial data to support this management strategy is lacking.

PB 1905 | Genetic Hypofibrinolysis, Fetal Loss Syndrome and Pro-inflammatory Status in Women with Metabolic Syndrome

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Background: Currently the metabolic syndrome (MS) is the most complex medical and social problem. The pregnant patients with MS are at higher risk of developing fetal loss syndrome, intrauterine growth retardation, preeclampsia and other obstetric complications. MS is also associated with at higher risk of thrombosis.
Aims: To determine the possible role of thrombophilic and pro-inflammatory status in the pathogenesis of fetal loss syndrome in women with metabolic syndrome.
Methods: The study involved 90 women aged from 21 to 43 years with the MS and fetal loss syndrome in history. In the history of women missed abortion, fetal death, spontaneous abortions, the failure of IVF were found in 80%, 55.6%,68.9%, 57.8% of cases respectively. The women were examined for pro-inflammatory cytokines genes polymorphisms and hereditary thrombophilia.
Results: The multigenic defects were verified in 100% of cases. The feature of multigenic defects is that the 4G/5G polymorphism of plasminogen activator inhibitor-1gene, the polymorphism in the tissue-type plasminogen activator I/D gene, in the angiotensin-converting

enzyme I/D gene, in the fibrinogen 455G/A gene were found in 85,6%, 58,9%, 45,6%, 47,8%, respectively. The polymorphisms IL-1β -31T/C gene, the polymorphisms IL-6 -174 G/C gene, the polymorphisms tumor necrosis factor-α -308G/A gene were found in 41,1%, 45,6%, 47,8% respectively.

Conclusions: The genetic assay demonstrates the presence of genetic hypofibrinolysis in patient with MS. We suggest the genetic hypofibrinolysis combined with pro-inflammatory status leads to impaired invasion cytotrophoblast and impaired placental development which ultimately leads to the development of obstetric complications, including the fetal loss syndrome.

PB 1906 | Venous Thromboembolism in Women Undergoing Assisted Reproduction: Data from RIETE

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Background: Assisted Reproductive Technologies (ART), increasingly used in western Countries, have been reported to have an increased risk of venous thrombosis. However, the magnitude, duration and the impact of risk factors are poorly known.

Aims: To investigate the incidence, clinical features and risk factors of ART-related events.

Methods: d.Among 63,749 patients so far included in the RIETE registry, we analysed events occurred in the 6,313 fertile-age women: 35 of them were referred as ART-related VTE. The risk factors, localization of thrombosis, the duration of therapy, second recurrent VTE, were assesse.

Results: No difference between the study group and that of 6,278 fertile-age women with VTE outside ART as far as age, BMI, smoking habits, co-morbidities, previous VTE, and thrombophilia (when available) are concerned. Among 35 women (mean age: 35.7+/-5.6) suffering from an ART- related VTE, 21 had a deep vein thrombosis (DVT) at lower limbs, 5 DVT at upper extremities, 15 pulmonary embolism (in 6 cases associated with DVT). In 30 out 35 women an embryo-transfer was performed with 17 following pregnancies. Overall, at least one risk factor (BMI>30, age >39 yrs, known thrombophilia, Ovarian Hyper-Stimulation Syndrome) was present in 19 (54.3%) women. Treatment was performed by low-molecular-weight heparins in 24 cases and vitamin K antagonists in 10 cases, while direct oral anticoagulants were used in 1 case. Overall, the duration of treatment was 224 days (median, range: 12-2119) with a higher duration in isolated PE (n= 273, range 145-1088). 3 (8.6%) women had VTE in spite of thromboprophylaxis No recurrence was observed at 90 days.

Conclusions: These preliminary data indicate that VTE may occur in the context of an ART attempt in unfrequent sites and mostly in the presence of risk factors. Noteworhy, a high number of PE is observed. A possible resistance to prophylactic doses of heparin is documented by the occurrence of VTE in spite of the prevention by heparin.

PB 1907 | Outcomes of Threatened Abortions Following Anticoagulation Treatment to Prevent Recurrent Pregnancy Loss

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Background: Prophylactic dose of low-molecular weight heparin (LMWH) is often prescribed to women with unexplained recurrent pregnancy loss (RPL) despite questionable benefit. Management and outcomes of threatened abortion during treatment with ongoing anticoagulant therapy in these patients has not been established.

Aims: To determine the outcome of threatened abortion in women treated by ongoing LMWH therapy due to RPL.

Methods: A retrospective review of all patients with RPL who experienced threatened abortion while on LMWH therapy between 2000-2016 in two university hospitals.

Results: Data of 114 women with ≥2 consecutive early miscarriages who experienced threatened abortion while on LMWH therapy were analyzed. All patients received enoxaparin at a dose of 40 mg. Fetal cardiac activity was demonstrated in all patients. The median age of the cohort was 33 years. Overall, 74 (64.9%) women had ≥3 previous miscarriages. Thrombophilia was present in 38 (33.3%) women, including 11 (9.6%) with antiphospholipid syndrome (APLS). The overall live birth rate was 58.8% (67/114). Live birth rates were 87.2% (41/47 patients) and 38.8% (26/67 patients) among those who discontinued versus those who continued LMWH therapy, respectively (P< 0.0001). Among the 11 patients with APLS, 10 experienced live birth, 8 of them continued LMWH and 2 discontinued it. In multivariate analysis, discontinuation of LMWH was the only significant predictor of live birth outcome (odds ratio [95% CI]: 10.78 (4.01, 28.93), P< 0.0001). Thrombophilia (P=0.61), presence of subchorionic hematoma (0.69), and severity of bleeding (P=0.33) were not found to be associated with live birth outcomes.

Conclusions: We found the continuation of LMWH therapy following threatened abortion in patients with RPL to be negatively associated with live birth rates. Therefore, we support its discontinuation in this setting. Since LMWH continuation resulted in a relatively high live birth rate among APLS patients, we advocate against its withdrawal in this subset of patients.

PB 1908 | OTTILIA and FIRST: Preliminary Data from Two Ongoing International Registries on Foeto-maternal Prognosis in Women with Recurrent Reproductive Failures after Spontaneous or Assisted Conception

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Background: There is uncertainty about the best management of pregnant women with previous recurrent pregnancy loss or recurrent implantation failures after assisted reproductive technologies (ART).

Aims: To investigate factors affecting reproductive outcomes and assess the best clinical management strategies of women with recurrent otherwise unexplained implantation/pregnancy failures after spontaneous or assisted conception.

Methods: OTTILIA and FIRST are two prospective, observational, multicenter studies.

Inclusion criteria OTTILIA (ClinicalTrials.gov: NCT 02385461): Pregnant women with recurrent pregnancy loss (≥3 or 2 in the presence of at least 1 normal fetal karyotype) or at least 1 intrauterine foetal death (a loss after 20 weeks of a morphologically normal fetus).

Inclusion criteria FIRST (NCT 02685800): ART attempt after ≥2 implantation failures/losses of clinical pregnancies after ART. Baseline characteristics, past and current obstetric history (including antithrombotic prophylaxis) of study women, are obtained during routine clinical follow-up or telephone interviews and recorded into a dedicated database. All women are followed until 4 weeks after the delivery or pregnancy test after ART procedure.

Results: To date, 166 and 57 women (median age: 36 and 37, range: 21-47 and 24-49 years) have been enrolled in OTTILIA and FIRST registers, respectively. Enrolment period, participating centers and management of index- pregnancies/ART cycles are shown in Table 1. Outcome is available in 169 pregnancies (4 were lost to follow-up, 20 are ongoing) and 49 ART cycles (8 are ongoing).

TABLE 1 Panel a. OTTILIA Register (Starting date: February 1, 2012; Participating centers: 10)

Index-pregnancies (n= 193)	No prophylaxis	ASA	LMWH	ASA+LMWH
Pregnancies in FVL heterozygotes, n (%) (n= 36)	1 (2.8)	3 (8.3)	26 (72.2)	6 (16.7)
Pregnancies in PTm heterozygotes, n (%) (n= 29)	1 (3.5)	0	25 (86.2)	3 (10.3)
Pregnancies in carriers of severe thrombophilias, n (%) (n= 13)	0	0	6 (46.2)	7 (53.8)
Pregnancies in non carriers, n (%) (n= 115)	40 (34.8)	19 (16.5)	39 (33.9)	17 (14.8)

TABLE 1 Panel b. FIRST Register (Starting date: October 1, 2015; Participating centers: 4)

Index-ART cycles (n= 57)	No prophylaxis	ASA	LMWH	ASA+LMWH
ART cycles in FVL heterozygotes, n (%) (n= 1)	0	0	1 (100)	0
ART cycles in PTm heterozygotes, n (%) (n= 2)	1 (50)	0	1 (50)	0
ART cycles in carriers of severe thrombophilias, n (%) (n= 1)	0	0	1 (100)	0
ART cycles in non carriers, n (%) (n= 53)	39 (73.6)	3 (5.7)	6 (11.3)	5 (9.4)

ASA: low-dose aspirin; LMWH: low-molecular-weight heparin; FVL: factor V Leiden; PTm: prothrombin 20210A mutation; Severe thrombophilias: homozygosis for FVL or PTm, double mutation, FVL or PTm and/or natural anticoagulants deficiency.

Conclusions: In both the cohorts, antithrombotic prophylaxis is mostly prescribed in women carrying thrombophilias. A larger sample size is required to better evaluate variables affecting outcome and clinical strategies to improve foeto-maternal prognosis.

PB 1909 | Efficacy and Safety of Treatment with High Dose Thromboprophylaxis in Pregnant Women - A Retrospective Study from a Stockholm Cohort, Ranging from 2004 to 2014

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Background: Pregnant women who have a very high risk of developing pregnancy related venous thromboembolism (VTE) should be treated with high-dose thromboprophylaxis (low molecular weight heparin). The efficacy and safety of the treatment have been evaluated mostly in mixed cohorts (high and normal dose thromboprophylaxis).

Aims: To evaluate the safety and efficacy of high dose thromboprophylaxis in pregnant women with high risk for VTE.

Methods: The cohort of the study consisted of 40 women treated with high dose thromboprophylaxis during pregnancy because of previous VTE or thrombophilia. Maternal medical records were reviewed in order to evaluate the efficacy of the treatment through the incidence of recurrent VTE and the safety of the treatment by recording bleeding complications and obstetric outcomes.

Results: The incidence of VTE and bleeding complications during pregnancy was 2,5% (n=1) and 10% (n=4), respectively. Postpartum haemorrhage (PPH) ≥500ml occurred in 30% of the patients (n=12) whereas PPH≥1000ml was recorded in 4,8% (n=3). The incidence of preterm births was 12,5% (n=5) and 12,5% (n=5) of the children were born small for gestational age (SGA).

Conclusions: High-dose low molecular weight heparin is an effective prophylaxis for pregnant patients with high risk of developing VTE, but the treatment may entail a somewhat increased risk of PPH, preterm birth and SGA. Larger studies in this population are required in order to confirm these findings.

PB 1910 | Thromboelastography Analysis of Haemostasis in Pre-eclamptic, Hypertensive and Normotensive Pregnant and Non-Pregnant Women

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Background: Pregnancy is a physiological hypercoagulable state. Hypertensive disorders of pregnancy such as pre-clampsia (PE) are often associated with coagulopathy. Of women who develop severe PE, 20% develop HELLP syndrome and/or DIC with subsequent severe maternal and fetal complications. Standard laboratory tests are useful guides to haemostatic function, however, they are plasma based and thus ignore the dynamic interaction between platelets, coagulation and fibrinolytic factors, as well as their inhibitors, and fail to reflect elements related to the quality of the formed clot. Thromboelastography (TEG) shows the dynamic viscoelastic properties of whole-blood during coagulation and fibrinolysis and is used as a point-of-care monitoring tool.

Aims: To investigate the differences in coagulation status found in non-pregnant state, normotensive pregnancy, gestational hypertension and pre-eclampsia.

Methods: Citrated whole-blood samples were collected from twenty one normotensive women during the third trimester of pregnancy, six pre-eclamptic women prior to delivery and at six weeks and six months post-partum, six women with gestational hypertension as well as fifteen non-pregnant controls. Using thromboelastography and standard laboratory tests, haemostatic functions were evaluated in at delivery and post-partum.

Results: TEG identified increased whole-blood coagulability in PE women at delivery, demonstrating its increased sensitivity over standard laboratory tests at identifying haemostatic alterations associated with PE. Haemostatic alterations were normalized by six weeks post-partum.

Conclusions: These results provide evidence supporting the use of TEG as a sensitive laboratory test in evaluating haemostasis in pregnant and non-pregnant women.

PB 1911 | Pregnancy Loss in APA-positive Women

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Background: As it is known the pregnancy loss is a marker and the most striking manifestation of APS. Pregnant women with APA circulation has a potential risk of placental insufficiency and IUGR development. We evaluated the importance of different antiphospholipid antibodies in patinets with fetal loss syndrome history.

Aims: The evaluation of APA profile in patients with fetal loss syndrome.

Methods: We have investigated anticardiolipin, anti-annexin V, anti-b2-GPI, anti-prothrombin antibodies using ELISA method and LA circulation in 146 women with history of recurrent miscarriage and 60 age matched healthy pregnant women. The study included 74 first trimester pregnant women (I group) and 72 second and third trimesters women (II group) who had a history of unexplained recurrent miscarriage.

Results: 34,2% women were diagnosed APS. LA circulation - 14%, anticardiolipin - 31,5%, anti-annexin V - 31%, anti-b2-GPI - 22,6, anti-prothrombin - 10,3%. Combination of LA, anti-b2-GPI, anticardiolipin was in 12,1%, LA, anti-annexin V and anti-b2-GPI - in 13,7%, anti-prothrombin and anti-b2-GP I - in 8,9%, LA, annexin V and b2-GPI - in 7,9% and was associated with more severe complications. All APS patients received anticoagulant therapy. In women treated before the

pregnancy early miscarriage in the next pregnancy occurred in 1,6%. There was no antenatal death or stillbirth. In II group the frequency of obstetric complications was higher compared with women of I group ($p<0.05$) but still significantly lower compared their history without therapy.

Conclusions: 34% fetal loss cases in our investigattion are associated with antiphospholipid syndrome. The combination of various antibodies at the same time in women with more severe obstetric history demonstrates the diagnostic value of the determination of different groups of APA.

PB 1912 | Obesity and Thromboembolic Risk during Hospitalization in Pregnancy: Preliminary Results

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Background: Worldwide there has been a dramatic increase in the prevalence of overweight (body mass index [BMI, calculated as weight (kg)/[height (m)]2] 25 and higher) and obesity (BMI 30 and higher). BMI 40 and higher (severe obesity) is considered a moderate/high risk for venous thromboembolism (VTE)during hospitalization in pregnancy.

Aims: The goal of this study was to apply a thromboprophylaxis protocol with a VTE risk score for hospitalized pregnant women. The analysis of patients with severe obesity was performed.

Methods: This was a longitudinal, interventional and prospective study of hospitalized pregnant women (n= 4126) in a tertiary hospital. The patients were classified according to the grade of obesity. The patients with severe obesity were compared with the remaining group. Patients were classified as low risk or high risk (score ≥ 3) according to a VTE risk score. The high-risk group received thromboprophylaxis with low-molecular-weight heparin (LMWH) unless the patient had a contraindication for anticoagulation, such as active bleeding or a high bleeding risk. The collected data were descriptively analysed to identify the profile of pregnant women with severe obesity using percentages and absolute values. One patient could have undergone more than one evaluation.

Results: The data of 218 cases with BMI ≥ 40 were descriptively analysed: 26 (12%) were already on anticoagulation. The others 191 (170 patients) were scored: 101(52.9%) were considered high risk. The main associated risk factors in this calculation were: age above 35 y or 40y (respectively 45% and 12.9%) and multiparity (n=43-42%). The type of evaluation was: 157 (82.1%) postpartum (c-section n=95-60.5% of the deliveries). One patient presented postpartum haemorrhage due to uterine atony(0.5%) and no patient presented adverse effects of anticoagulation.

Conclusions: In pregnant women with severe obesity more than half of the patients scored high risk for VTE and the main factors associated were maternal age and multiparity.

PB 1913 | Management of a Pregnant Patient with Antithrombin Deficiency and Acute Venous Thromboembolism

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Background: In women with past unprovoked or estrogen-associated venous thromboembolism (VTE), antepartum and postpartum

TABLE 1 Antithrombin and Anticoagulant Dosing and Trough Levels

Timeline	Antithrombin dosing	Anticoagulant dosing	Trough levels, AT antigen (U/mL)	Trough levels, AT chromogenic (U/mL)
At presentation (5 weeks gestation)		Tinzaparin (175 units/kg)	0.33	0.19
Late 1st trimester	Bolus 4000U then daily 3000-2000U	Unfractionated heparin infusion for 3 days. Enoxaparin 80mg (1mg/kg) Q12H started day 4	0.85	0.76
Early 2nd trimester	3000U MWF	Enoxaparin 80 mg sc Q12H	0.81	0.77
2nd trimester	2000U MWF	Enoxaparin increase to 90 mg sc Q12H based on anti-Xa level	0.63	0.55
3rd trimester	1000U q2d	Enoxaparin 90 mg sc Q12H	0.54	0.45
Admission for induction of labor	Bolus 3000U then 2000U daily	Held for 24h prior to induction. Enoxaparin 40 mg sc given 4h postpartum, and 90mg sc q12h on post-partum day 1	0.83	0.87

low-molecular-weight heparin (LMWH) is recommended to prevent VTE recurrence. Antithrombin (AT) deficiency is a rare, hereditary thrombophilia that increases the risk of VTE during pregnancy. The management of pregnant patients with AT deficiency and acute or past VTE and the role of AT concentrate replacement remains unclear.

Aims: To describe management of a woman with severe AT deficiency and history of recurrent VTE who developed a VTE in early pregnancy despite therapeutic LMWH.

Methods: A 31 year old woman with Type 1 AT deficiency (baseline antigen 0.42U/mL, chromogenic 0.45U/mL) and history of recurrent VTE was switched from warfarin to therapeutic Tinzaparin (175 units/kg) after confirmation of pregnancy. One month later she presented with new left leg symptoms and shortness of breath; Doppler ultrasound confirmed evidence of progressive thrombus involving the femoral vein. She was started on intravenous unfractionated heparin and received AT concentrate (Antithrombin III NF, Baxalta) for 5 consecutive days, followed by 3 times/week for one month to achieve AT trough levels of 80%. Once stable, she was switched to enoxaparin 1 mg/kg twice daily with anti-Xa level monitoring. After the 1st month of treatment the target AT trough level was reduced to 50%. After delivery, the patient remained on therapeutic-dose enoxaparin and AT replacement until transitioned to warfarin.

Results:

Conclusions: This case highlights the potential role of AT concentrate in pregnant women with AT deficiency and acute VTE. While our protocol was effective, it was not without significant medicalization of the pregnancy, patient distress and use of hospital and national blood bank resources. This area would benefit from the development of a prospective registry of pregnant patients with AT deficiency with VTE.

PB 1914 | Prevalence of FOXD1 Mutations in the Obstetric Antiphospholipid Syndrome

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Background: Mutations of the transcription factor gene *FOXD1* impairing the activation of the promotor of the placental growth factor gene *PGF* are associated with recurrent pregnancy loss PL (Open Biol. 2016 Oct;6(10). pii: 160109): their prevalence is not documented in other PL phenotypes.

Aims: Assess the rate of 3 non-synonymous *FOXD1* sequence variants [c.1285_1286InsGCCGCG (p.Ins429AlaAla); c .1067 C>G (p.Ala356Gly) ; c.1092C>G (p.Ile364Met)] in the obstetric antiphopholipid syndrome (oAPS).

Methods: We tested DNAs from the 1,552 women included into the NOH-APS cohort (Blood 2012; 119(11):2624-32). *FOXD1* coding region was sequenced as described.

Results: *FOXD1* mutations were detected in 25 women from the NOH-APS, thrombophilia-negative control group (3.14%): in 20/483 with 3 embryonic PLs before 10 weeks (4.14%) and in 5/313 with one foetal death (1.60%, all before 12 weeks). They were also defected in 10 women positive for the FV Leiden or the *F2* 20210A polymorphisms (3.58%): in 6 /93 with recurrent embryonic PLs (6.45%) and in 4 /186 with one foetal death (2.15%, all before 12 weeks). They were finally detected in 13 of the oAPS women (2.51%): in 10/206 with 3 embryonic PLs (4.85%) and in 3/311 women with one foetal death (0.96%, all before 12 weeks). All foetal losses in mutated women occurred before 12 weeks. The rate of mutated women was not different between the 3 groups with recurrent embryonic PLs (p=0.603) or with one foetal loss (p=0.557). oAPS women positive for FOXD1 mutations were only positive for anticardiolipin IgM.

Conclusions: *FOXD1* mutations impairing *PGF* transcription are mainly found in women with recurrent embryonic losses before 10 weeks, to a lesser extent in case of a foetal loss before 12 weeks. *FOXD1* mutations in oAPS women only positive for anticardiolipin IgM questions the clinical relevance of this traditional APS marker. The progressive description of causal mutations for miscarriages could gradually lead to restricting the perimeter of oAPS.

PB 1915 | Management of Pregnancy in Cobalamin C Defect Causing Homocystinuria and Methylmalonic Aciduria

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Background: Cobalamin C (CblC) defects are rare inherited autosomal recessive disorders of vitamin B12 metabolism with high levels of plasma homcysteine and high methylmalonic urine/plasma levels. Late-onset is rarer than the early-onset disease. We describe a 34 yrs-old Caucasian woman, referred because of a 20th week pregnancy loss of a morphologically normal intrauterine growth restricted foetus.

Aims: To describe management of pregnancy in late-onset cblC defect.

Methods: Patient underwent thrombophilia screening; homocysteine and methylmalonic acid plasma levels were measured by mass spectrometry.DNA was extracted and a Whole Exome Sequencing (WES) performed.

Results: Thrombophilia screening showed 100 microM of plasma homocysteine (tHcy). At that time, she was on calcium folinate, 15 mg/day.She was delivered at term and was in apparently good health until 20 yrs, when she showed a normocitic anemia (Hb: 8.2 g/dL), elevated inflammatory markers (ESR 55), and an impaired renal function (serum creatinine: 1.9 mg/dL). Urinalysis revealed proteinuria (150 mg/L) and micro-hematuria. Neurological examination was normal. A renal biopsy revealed thrombotic microangiopathy with predominant lesions in the glomerulus and minimal lesions in the arterioles. WES showed a

compound heterozygosity for p. Tyr130His and p.Tyr222Stop in the MMACHC gene (Methylmalonic Aciduria type C and Homocystinuria; OMIM *609831). Plasma concentration of methylmalonic acid was 1,09 micromol/L (reference value: 0-0,7). Hydroxocobalamin injection, 2 mg/week i.m., normalized tHcy plasma levels and restored anemia and renal function. Pregnancy was then started and low-molecular weigh heparin at prophylactic doses prescribed in addition to hydroxocobalamin until 4 weeks post-partum. After an uneventful pregnancy, a male baby weighing 2420 gr (Apgar 1' 8, 10': 9) was delivered at 39 weeks.

Conclusions: This is the first case of pregnancy described in a CblC defect. We show that a successful pregnancy is possible in this rare form of homocystinuria.

PB 1916 | Clinical Value of Antiphospholipid Antibodies Assesment in Women Undergoing IVF with History with IVF Failure

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Background: antiphospholipid antibodies have a very multifaced effect on the hemostasis system, harming all its defensive units: endothelial barrier, function of natural anticoagulant, an endogenous fibrinolysis, activating platelet hemostasis and associating with a variety of obstetric complications and infertility. But what is the frequency of circulating aPL in women with IVF failure, and is AFA connected with the worst reproductive outcome?

Aims: To study the effect of AFA on the outcomes of assisted reproductive technologies.

Methods: we examined 387 women between 23 and 45 ages in the IVF program - 238 women with IVF failure (one of the more failed tries of IVF, the number of tries being between 1 and 9) (I group) and 149 women with a pregnancy after the IVF program (II group). The comparison group consisted of 80 women pregnant after IVF (male factor). The control group consisted of 80 pregnant women with physiological pregnancy.

Results: in I group APA circulation (IgG/IgM) was revealed in 42%. Among them antibodies to cardiolipin=8.9%, annexin V=24.7%, b2-glycoprotein I=31%, prothrombin — 13.5%. In II group — APA=19%, cardiolipin=4.5%, annexin V=10%, b2-glycoprotein I=15%, prothrombin — 8%.

Conclusions: we consider a circulation of APA as a temporary contraindication for assisted reproductive technologies program. APA should be considered as an important risk factor of IVF failures. Patients who are involved with ART prior to initiation of an IVF cycle should be tested for the presence of APA. A complete panel of antibodies is necessary for diagnostic of implantation failures associated with APS.

PB 1917 | Antiphospholipid Antibodies in Women with of Severe Preeclampsia

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Background: in the management of pregnant women with APS, its possible to face other obstetric problems, like preterm birth and prematurity,IFGR and hypertensive disorders in the form of pre-eclampsia. Placental dysfunction - is the central mechanism for the development of pre-eclampsia. One of the main targets of AFA during pregnancy is the placenta.

Aims: The evaluation of spectrum of antiphospholipid antibodies (APA) in women with the history of severe preeclampsia (SP).

Methods: ELIZA-method was used to measure IgM/IgA APA screen anti-b2-GP I,antiannexinV, antiprothrombin in 125 women with severe preeclampsia. Control group was consisted of 60 health women.

Results: APA circulation in patients with history of severe preeclampsia was found in 32%. It is interesting that antibodies to b2-GPI were prevailed (31,6%).Antibodies to cardiolipin and antibodies to APA subgroups were respectively 21%and 17%. Lupus anticoagulant circulation was in15,7%. Antiannexin V-in5,2%, antiprothrombin-5,2%. Combination of LA,anti-b2-GPI, anticardiolipin was in 12,1%,LA,anti-annexin V and anti-b2-GPI - in 13,7%,anti-prothrombin and anti-b2-GP I - in 8,9%, LA, annexin V and b2-GPI-in7,9%.The worst clinical picture was observed in women with combination of different APA. Further we managed the next pregnancy in 54of these 125 examined women. Most of them (39) were observed from the fertile cycle. 15 other women admitted already being pregnant. All patients were re-examined for APA circulation. In 3 cases we observed the development of preeclampsia and in 1 case - repeated severe preeclampsia in women who applied in III trimester of pregnancy. All women received anticoagulant therapy. No case of severe preeclampsia development in patients with anticoagulant therapy started from the fertile cycle or I trimester of pregnancy.

Conclusions: The presence of history of preeclampsia is the indication for the APA-testing and the beginning of the anticoagulant therapy from the moment of preparing for pregnancy.

PB 1918 | The Clinical Significance of Determining the Activity and Inhibitor of Adamts-13 in the Management of Pregnant Women with Severe Preeclampsia and Thrombotic Microangiopathy History

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Background: Thrombotic microangiopathy is one of the most difficult occurring thrombotic complications characterized by microvascular lesions of various organs and accompanied by thrombocytopenia and hemolytic anemia. One of the key triggers to the appearance of

thrombotic microangiopathy is pregnancy. ADAMTS-13 deficiency is a hallmark of thrombotic thrombocytopenic purpura, which allows to differentiate the pathology from other thrombotic microangiopathy.

Aims: The evaluation of ADAMTS-13 in women with the history of severe preeclampsia (SP).

Methods: We have examined 68 patients with severe preeclampsia history, including early (before 34 weeks of pregnancy), fetal death. Pregnancy has been interrupted due to multiple organ failure. All women were screened for the presence of lupus anticoagulant, antiphospholipid syndrome, ADAMTS-13 (activity and inhibitor circulation).

Results: On the basis of a high level of antibodies to ADAMTS-13 in 11 patients was suspected acquired form of thrombotic microangiopathy. Almost half of them (6) was found high titer antibodies against b2-glycoprotein I. Subsequently, 7 of them are planned for the next pregnancy. Prevention conducted LMWH, aspirin and in 3 cases dipyridamole also in connection with resistance to aspirin. Dynamic control of activity and ADAMTS-13 inhibitor was conducted. At 34-35 weeks of pregnancy in all the marked growth inhibitor and enzyme activity drop to 10%, which was the reason for a surgical delivery.

Conclusions: The presence of history of preeclampsia and especially with thrombocytopenia is the indication not onle for the APA-testing but for the evaluation of ADAMTS-13 level of activity and the presence of antibodies and the beginning of the anticoagulant therapy from the moment of preparing for pregnancy.

PB 1919 | Different Approaches of Thromboprophylaxis during Pregnancy in the Routine Clinical Practice. Results from the TEAM Project

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Background: Limited data on the magnitude of the thrombosis risk associated with heritable thrombophilias and the interaction of risk factors have impeded the development of evidence-based risk stratification to guide thromboprophylaxis. Consequently, there is a lack of consensus on thromboprophylaxis in national and international guidelines.

Aims: To analyse the clinical management and clinical outcomes of antithrombotic treatment during pregnancy in the TEAM project.

Methods: rom 2010 until 2016 we performed an international observational multicentric study that prospectively included 312 women.

Results: The motive of thromboprophylaxis was 40% previous history of depp venous thrombosis and 23.4% asymptomatic thrombophilia, 14% received no thromboprophylaxis. In 60% od women, they started treatment in the first trimester. Thromboprophylactic doses were used in 79.1 %, intermediate in 15.4 % and 11,4% therapeutic doses. In 60% of cases thrombophilia was positive, most of them had FVL mutation . In terms of safety and efectiviness of treament , minor bleeding was 3 gcases, , 2 of them under theraputic dosages and one under prophylactic dosages and one case of major bleeding not related to treatment with prophylactic dosages. One woman with previous history of thrombosis and a combined deficiency (heterozygous for factor V Leiden and protein S deficiency) under theraupetic dosages of LMWH developed a proximal deep venous thrombosis.

When we analysed the type of treatment depending of the presence of known thrombophilia , the use of LMWH was higher (p=0.04) in women with known thrombophilia. (more than 90%).

Conclusions: Our results shown that more than 90% of woman without thrombophilia received different schemes of thromboprohylaxis. In women with previous thrombosis the tendency was to use therapeutic dosages, and in the majority of cases they started the first trimester. The different approaches of thromboprohylaxis reflect the need of further studies.

PB 1920 | In the TEAM PROJECT, the Management of Placental-mediated Pregnancy Complication (PMC) Reflects a Real Gap between Evidence-based Guidelines and Routine Clinical Practice

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Background: In PMC, evidence-based guidelines are based on studies with controversial results or expert opinion, in this context, the implementation of those guidelines are quite different among physicians.

Aims: To analyze if the management of PMC followed evidence-based guidelines, and also the prevalence of thrombophilia and clinical management of thromboprophylaxis in women with previous PMC in agreement with guidelines or not.

Methods: From 2010 until 2015, we performed an international observational multicentric study that prospectively included 646 women with PMC.

Results: Women had pregnancy loss (50%), foetal death (25%), intrauterine growth restriction (6%) and 20% pre-eclampsia A thrombophilia test was indicated in more than 82.6%. The presence of factor V Leiden and antiphospholipids were the most frequent defect observed. The AAF were performed in less than 70% .In 260 women with previous PMC were referred to establish treatment or not, 10% did not receive any treatment and 46.8% received LMWH +AAS. Most of the women (88.7%) received treatment and recurrence was observed in 14% of women.

Conclusions: Our results reflect that despite guidelines, the thrombophilia test is performed and that most of women with a negative thrombophilia test are treated in subsequents pregnancies. Also that the presence of AAF are not performed in all women against the recommendations of guidelines.It implies an urgent need to perform an international project in order to establish and establish guidelines that cover all the uncertainties in this area.

PB 1921 | Risk Assessment of Venous Thromboembolism in Women Furing Pregnancy and Puerperium (SAVE): A Cross-Sectional Registry in Africa, Eurasia, Middle East and South Asia

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Background: The clinical burden of VTE risk during pregnancy and puerperium is not well documented outside Western countries.

Aims: Assess the rate of women at risk of VTE during pregnancy/puerperium in Africa, Eurasia, Middle-East and South Asia.

Methods: International, observational, cross-sectional multicentre study across 18 countries (December 2014-October 2015). Physicians were randomly contacted from a list of private/public practitioners provided by each country. Consecutive women visiting for first prenatal consultation or any other consultation during pregnancy or puerperium were enrolled (30 patients per site). Prior VTE / ongoing antithrombotic therapy led to exclusion. Assessments were built on the identification of predefined VTE risk factors, based on Case Report Forms. The quality control and validation of data was performed independently from investigators by a contract research organisation.

Results: 181 physicians participated in the study. 4,010 women were included and eligible for the study. Majority of the women were pregnant (90.1%), in their last trimester (38.1%) and were enrolled during a routine visit to the physician (81.9%). Half (51.4%) of the women during pregnancy and post-partum were at risk of VTE. In Eurasia, the majority of the women (90%) were considered at risk while fewer women were at risk in South Asia (19.9%). South Asia had a high percentage of women considered at mild risk of VTE (73.5%), while Africa and MiddleEast followed the trend observed globally, with approximately half of the women being at mild risk of VTE and the other half at moderate and high-risk of VTE. Eurasia had the highest percentage of women at high-risk (20.2% vs. 1.5% [South Asia] to 12.9% [global]).

Conclusions: A high proportion of women consulted during pregnancy and puerperium are assessed for their VTE risk and are considered at risk . Differences and discrepancies observed across regions show that further efforts would be needed to promote and apply a uniform VTE risk evaluation.

PB 1922 | Evaluation of Venous Thromboembolism Risk and Adequacy of Prophylaxis in High Risk Pregnancy in Saudi Arabia (The Saudi Arabian Pregnancy Venous Thromboembolism Registry)

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Background: Pregnant women with inherited and acquired thrombophilias have an increased risk of venous thromboembolism (VTE) and adverse obstetric complications.

For these women, VTE and adverse obstetrical complications may occur during any trimester of pregnancy.

Various pharmacologic interventions have been used in high-risk pregnancies to decrease the likelihood of VTE or adverse obstetrical outcomes.

Aims: To identify high risk conditions in pregnant patients that put the patient at risk of thromboembolism in the outpatient settings. To determine the proportion of at-risk patients who receive effective types of prophylaxis. To determine types of anticoagulant used in the population under study. Drug related side effects: bleeding, local reactions and thrombocytopenia.

Methods: Retrospective analysis of 326 patient had been received anticoagulants during pregnancy from three participating hospitals in Saudi Arabia.

After approval from the research committee in each institute data had been collected and stored electronically.

Results: Recurrent fetal loss is the most frequent induction for anticoagulation followed by previous thromboembolic events. Antiphospholipid antibodys were the most frequent hypercoagulopathy condition.

Enoxaparin was the most frequent used anticoagulant followed by aspirin. Adverse events ranged from Post-partum hemorrhage experienced by 2 subjects (0.61%) DVT, Heparin allergy, thrombocytopenia and other site hemorrhage experienced each by one patient (0.31%).

Neonatal abnormalities (10.12% of the study population) and were mainly underweight and two neonatal death. Protein S deficiency is the most frequent inherited thrombophilia.

Conclusions: Deferent anticoagulations used to prevent recurrent fetal loss with small risk of complication. Inherited thrombophilia are very rare as cause for fetal loss.

PB 1923 | Ischemic-stroke due to an Embolic Thrombus in Carotid Artery in Recent Postpartum: Case Report and Review of the Literature

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Background: The risk of stroke and other postpartum cerebrovascular disease (CVD) occurring after hospital discharge for labor and delivery is uncertain.

Aims: Description of an ischemic stroke that occurred in the puerperium and was successfully treated with thrombolysis.

Methods: Case report.

Results: A 35-year-old postpartum multiparous woman at the 15th day after a c-section was referred with a history of 3 hours of decreased motion, weakness and sensibility on the left side of her body,

preceded by scintillating scotomas. She had a history of pre-eclampsia in her last pregnancy and dyslipidemia. National Institutes of Health Stroke Scale (NIHSS) score at presentation was 12. Physical examination revealed left hemiparesis (brachial and facial predominantly), associated with hemispatial neglect on the same side. CT angiogram of head and neck vessels exhibited a sudden occlusion in the territory of right middle cerebral artery, and an image suggestive of a thrombus at the emergence of the right internal carotid artery, about 2 cm long, resulting in stenosis of approximately 70% of the lumen. Thrombolysis was performed using a recombinant tissue plasminogen activator (rt-PA) roughly 3 hours within the onset of the symptoms. One day after the thrombolysis, a magnetic resonance angiography (MRA) was performed, displaying several areas without diffusion. It was also seen a filling defect on the proximal segment of right carotid artery, extending by approximately 1.7 cm, without significant stenosis, being suggestive of an embolus. A transesophageal echocardiogram was made to look for sources of emboli. After administration of saline water solution microbubbles were detected at the left chambers, compatible with an extra-cardiac shunt. The patient was discharged on low-molecular weight heparin, with no sequelae.

Conclusions: Intravenous thrombolytic therapy improves independent survival in patients with acute ischemic stroke and is perfectly feasible in the puerperium.

PB 1924 | Hypodysfibrinogenemia: AChallenge in Maternity Ward - Course of Pregnancy and a Literature Review

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Background: Reduced fibrinogen level in the presence of a normal fibrinogen antigen is suggestive of hypodysfibrinogenemia. This medical condition is characterized by prolonged Thrombin Clotting Time, a prolonged Reptilase Time and Turbidity tests measuring the rate of polymerization. In the number of over 150 mutations, approximately 25% present hemorrhagic symptoms and 1/5 - are associated with thrombosis. Women suffer from abnormal pregnacy outcome (APO); they have a history of frequent miscarriages and stillbirths. Although the major bleeding risk related to fibrinogen levels below 2.0 g/l is widely recognized by obstetricians, the hazard of thrombosis is underestimated. The possible mechanisms for thrombosis are: excessive levels of free thrombin due to poor thrombin binding, decreased binding of plasminogen and disorganized fibrin structure which impairs fibrinolysis.

Aims: To highlight the need for dual treatment of hypofibrinogenic pregnant patient

Methods: A case report and literature review

Results: A 32 year old patient with hypodysfibrinogenemia. During first pregnacy the fibrinogen level was within the 0.8-1.1 g/l range. There were strong indications for C-section as there was strong suspicion of fetal hypofibrinogenemia. Before the procedure 1g of fibrinogen was administered as supplementation. Further fibrinogen supplementation was mandatory in the puerperium to maintain the recommended fibrinogen level. The anticoagulant prophylaxis was administered for two weeks. No hypofibrinogenemia was observed in the healthy infant (1.48-1.98 g/l).

Conclusions: Rare coagulation disorders require close cooperation between medical specialists in order to avoid complications during pregancy, labour and puerperium. Delivery should take place in special perinatal care units and close surveillance in the neonatal period is highly advisable.

PB 1926 | Perinatal Outcomes in Women whith History of Ischemic Stroke

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Background: Pregnancy-related stroke is a rare event, however, when it occurs, may be life threatening and there may be implications for management of the patient and delivery.

Aims: to evaluate the role of thrombophilia in pathogenesis of ischemic stoke, the effectiveness of pathogenetic prophylactic strategy during pregnancy and outcomes for mother and fetus.

Methods: in our Moscow city maternity hospital N67, specialized in cardiology, we studied 59 women with history of ischemic stroke (32±5,5 years). In 22 pts stroke occurred during current pregnancy. Other 37 pts were included in the prospective study. 20 pts were followed prospectively (group I) in preconception period and during pregnancy (low molecular weight heparin (LMWH) guided by D-dimer, aspirin). In 17 pts (group II) therapy was started in II-III trimester. Control group included 60 pts with normal pregnancy. All women were screened for genetic thrombophilia, antiphospholipid antibodies (APA) and hyperhomocysteinemia.

Results: Stroke was associated with severe medical conditions: hypertension (27,1%), metabolic syndrome (37,3%), rheumatic diseases (16,7%), prosthetic valves (6,8%), past history of thromboembolic complications (21,7%), oral contraceptive use (3,4%). 40,9% pts had a family history of thromboembolic complications ($p < 0,001$ vs 13,3% in the control group). We noted a very high rate of obstetric complications in the past in parous women (n=31) with history of stroke (69,4% vs 11,5% in the control group, $p < 0,001$), including fetal loss syndrome (33,3% vs 0%) and placental obstetric complications (preeclampsia, placental abruption, intrauterine growth restriction [IUGR]) (38,7% vs 11,5%, $p < 0,05$).

Conclusions: Thrombophilia might be the main pathogenic mechanism of obstetrics complications and ischemic stroke in women of childbirth

age. Preconception treatment with LMWH and pathogenetic therapy during pregnancy guided by thrombophilia markers allows preventing pregnancy complications and recurrent thrombosis.

PB 1927 | Pregnancy Complications in Women with Thrombophilia and their Future Pregnancy Outcome after Anti-coagulant Treatment

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Background: Pregnant women with hereditary thrombophilia are prone to pregnancy complications, including early and late abortions. Treatment with prophylactic dose Low Molecular Weight Heparin (LMWH) may have a protective effect in consecutive future pregnancies, on pregnant women with hereditary thrombophilia, and placenta mediated pregnancy complications in previous pregnancies.

Aims: To evaluate the efficacy of prophylactic dose LMWH in preventing pregnancy complications including abortions, in pregnant women with established thrombophilia and pregnancy complications in previous pregnancies.

Methods: A retrospective analysis was performed in Hematology outpatient clinic at Ziv Medical Center in Safed, between the years 2008-2014.Records of 41 pregnant women with established thrombophilia and pregnancy complications in previous pregnancies were analysed. The study was approved by the local ethics committee.

Results: The overall rate of live birth improved with Low dose LMWH (1.1±1.4 to 1.2±1.9), and there was an impressive reduction in early abortions (24/41 to 7/41, P< 0.001) but less effect on late abortions (5/41 to 2/41, P=0.152). The incidence of pre-eclampsia and intra-uterine growth restriction was similar, but there was a trend towards reduction in Intra-uterine fetal death (IUFD) rates (6/41 to 3/41, P=0.358). Figure number 1.

Conclusions: Pregnant women with hereditary thrombophilia associated with pregnancy complications in previous pregnancies, may benefit from low dose LMWH leading to lower rates of early abortions and IUFD in consecutive pregnancies, but a larger cohort of patients is needed in order to strengthen this observation.

PB 1928 | What is the Actual Role of Antiphospholipid Antibodies in Obstetric Morbidity?

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Background: The known association between obstetric morbidity (OM) and antiphospholipid antibodies (aPL) led to the inclusion of OM as clinical criteria in the antiphospholipid syndrome (APS) definition. However, decades and countless studies later, there is still lack of qualified evidence to support this association. Different clinical and / or laboratorial criteria led to inconsistent results.

Previously in 2014, we found 22 (10.6%) out of 208 women with OM, with APS criteria.

Aims: Confirm if the 22 women who presented positivity for aPL accomplished clinical and laboratorial criteria for obstetric APS; surveillance of thrombotic events (TE) and pregnancy outcomes after the initial evaluation; correlate different aPL profiles (single, double or triple positivity) with distinct clinical profiles.

Methods: We reviewed data concerning the 22 women with OM who fulfilled clinical criteria for APS, clinical and laboratorial data.

Results: Only 6/22 women with aPL presented laboratorial criteria of APS (2.9% of 208 women). None of them had recurrent early miscarriages (REM). Table 1 describes aPL profiles and OM. Table 2 describes patients' outcomes. Concerning the remaining 16/22 women, 1 was lost to follow-up, 8 presented weak LA and 7 presented ACA and aß2GPI in low titers, not confirmed in a second sample.

TABLE 1 Characterization of women who accomplished clinical and laboratorial criteria of obstetric APS

Number of women (n=6)	
aPL profile	
5	Triple positivity*
1	LA alone‡
Obstetric morbidity	
0	REM (0/100)
2	fetal death (6.7%; 2/30)
4	pre-eclampsia/ HELLP syndrome (7.3%; 4/55)
0	placental insufficiency (0/23)

*Triple positivity accomplishes positivity for lupus anticoagulant (LA), anticardiolipin antibodies (ACA) and anti-ß2-glicoprotein I (aß2GPI); ‡LA: Lupus Anticoagulant

TABLE 2 Events during follow-up of of women who accomplished clinical and laboratorial criteria of obstetric APS

Number of women (n=6)	
2	successful pregnancies under LMWH#+AAS§
1	3 unsuccessful gestations despite LMWH#+AAS§
3	did not conceive again
0	thrombotic events

§AAS: acetylsalicylic acid; #LMWH: low molecular weight heparin

Conclusions: Despite the assumed association between aPL and REM, our data conflict with it, which is in line with recent reports by others. Triple positivity was previously identified as a risk factor for thrombosis and seems also to be a risk for pregnancy failure. Our results disfavor APS as a major cause of OM.

PB 1929 | Low Molecular Weight Heparin for Women with Pathologic Evidence of Placental Malperfusion

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Background: Placental-mediated pregnancy complications including pre-eclampsia, stillborn, intrauterine growth restriction (IUGR) and placental abruption are related to thrombosis in the placental blood vessels. There are conflicting reports regarding the benefit of heparin or low molecular weight heparin (LMWH) in preventing the development of thrombosis within the placenta in patients at high-risk of placental malperfusion (Dodd JM et al. The Cochrane database of systematic reviews. 2013) (Rodger MA et al. Lancet 2014)(Rodger MA et al. Lancet 2016).

Aims: To determine if using LMWH improves outcomes in subsequent pregnancies in women with prior history of pathologic placental malperfusion.

Methods: We retrospectively identified women referred to our hematology clinic from July 2006 to December 2016 with history of pathologic placental malperfusion that were managed with LMWH in a subsequent pregnancy. We obtained demographics, presence of inherited thrombophilia, complications and outcomes. Patients with antiphospholipid antibodies or other reasons for anticoagulation were excluded. The primary outcome was subsequent live births.

Results: There were 15 women with pathologic evidence of placental malperfusion (Table 1).

TABLE 1 Baseline Characteristics of the women N=11

Age in years (mean and range)	29 (27-39)
Race White	8
Black	1
Hispanic	1
Asian	1
Thrombophilia: Factor II G20210A Heterozygote	5
Factor V Leiden Heterozygote	3
Protein S deficiency type 1	1
Protein S deficiency type 3	1
No thrombophilia	1

Four were excluded as 3 did not become pregnant and one is currently pregnant. Most women were Caucasian and the most common thrombophilia was heterozygosity for factor II G20210A variant. Previous adverse outcomes, treatment and subsequent outcomes are described in Table 2.

TABLE 2 Type of Initial Complication, Treatment and Outcome

Previous Adverse Pregnancy Outcome	Treatment in a Follow up Pregnancy	Outcome
IUGR = 4	LMWH	IUGR = 2
Stillbirth = 3	LMWH	Stillbirth = 0
Preeclampsia = 5	LMWH	Preeclampsia = 0
Abruptio = 3	LMWH	Abruptio = 0

There were 14 subsequent pregnancies (three patients had two successive pregnancies). All received LMWH during pregnancy. Although all pregnancies ended in live births, two women experienced IUGR. One was a heterozygote carrier for the factor IIG20210A and the other had no thrombophilia. There were no venous thromboembolisms or major bleeding events.

Conclusions: The use of LMWH appears to be beneficial in a subgroup of women with pathologic placental malperfusion, most of whom have an underlying inherited thrombophilia.

PB 1930 | Pregnancy and Perinatal Outcomes in Women with History of Venous Thromboembolism

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Background: Despite intensive research, thromboembolism still accounts for significant maternal.

Aims: to determine thrombophilia in patients with thromboembolism during pregnancy and to evaluate the efficiency of antithrombotic prophylaxis in patients with thrombophilia for prevention of recurrent thromboembolism.

Methods: GroupI:n= 87(28,7±4,2 years),subgroup I (n=68)women with history of thromboembolism, subgroup II(n=19) women with thromboembolism during current pregnancy, group II(control) - healthy pregnant women(n=60) were screened for genetic thrombophilia and antiphospholipid antibodies (APA). Subgroup I received prophylaxis with low molecular weight heparin (LMWH)+/- aspirin(50-100 mg/day) in preconception period, during pregnancy and at least 6 weeks postpartum.

Results: In group I 54% had familial history of venous thromboembolism, and 67,8% had personal history of pregnancy complications (fetal loss syndrome, preeclampsia, placental abruption) (p< 0,05 vs. control). In the group I thrombophilia was detected in 92,6%: FV Leiden (22% +/-), prothrombin G20210A (13,2% +/-), multigenic fibrinolytic defects (63,2%); APA (48,5%), hyperhomocysteinemia (44%)
(p< 0,001 vs. control). In 7 of 30 tested pts (23,3%) we found decreased ADAMTS-13 activity with inhibitor. Recurrent thrombosis occurred in 1 woman from subgroup I before the start of LMWH and

in 5 pts from subgroup II (26,3%) (p=0,091). In subgroup I no one had severe obstetrics complications. All pts were delivered at term and all babies were alive. In subgroup II moderate to severe obstetrics complications were noted: preeclampsia, IUGR grade I-III, critical maternal-placental-fetal blood flow disturbances (43,7%). Preterm delivery was required in 43,7% pts from subgroup II.

Conclusions: LMWH was effective for prevention of recurrent thromboembolism and obstetric complications.Women with personal or family history of thromboembolism or with history of obstetric complications should be screened for thrombophilia, including ADAMTS-13 activity with inhibitor.

PB 2138 | Evaluation of a Novel Short-acting Direct Dual FXa/FIIa Inhibitor for its Use in Acute Anticoagulation

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Background: Acute treatment of thrombotic events requires strong anticoagulation with good efficacy/safety profile and control of therapy.

Aims: Our aim was to identify and evaluate small molecules, which directly inhibit FXa and thrombin and ensure controllability by a short half-life and reliable pharmacodynamics.

Methods: BAY-878 was chemically synthesized and tested in various fluorogenic assay formats in buffer and plasma, as well as standard clotting tests in plasma and whole blood. It was evaluated in models of arterial, venous and systemic thrombosis, including an arterio-venous shunt model (vs. bivalirudin) and LPS infusion models (vs. Heparin) in rat.

Results: BAY-878 is a small molecule, which inhibited both, FXa and thrombin ($IC_{50\ 1}$nM/2.2nM) directly and reversibly with a fast binding kinetic, without inhibiting other proteases tested. In plasma it blocked both enzymes equi-potently (IC_{50}30 nM/35 nM) and prolonged the clotting times in plasma (PT/aPTT, EC_{200}0.2 µM) and whole blood without dependency on or consumption of antithrombin. Added after the trigger, BAY-878 reduced increases in thrombin activity, blocked clot-bound FXa and thrombin and improved clot lysis by inhibiting TAFIa generation. When infused in rat in an AV-shunt model BAY-878 was more efficacious in thrombus reduction than bivalirudin (EC_{50} 0.3 vs. 0.8µM) with less blood loss at comparable efficacies, which may be linked to a lower inhibition rate of each of the enzymes compared to the single thrombin inhibition of bivalirudin.

In rat models with LPS infusion, BAY-878 led to a reduction in platelet drop, TAT, IL-6, AST and LDH under prophylactic and treatment conditions, which was not observed with UFH.

Pharmacokinetic experiments in rats revealed a short half-life (< 10 min) with a good pharmacodynamic correlation and a low renal exretion rate.

Conclusions: BAY-878 is a direct dual FXa/thrombin inhibitor for parenteral use, which has the potential to improve the treatment of life-threatening acute coagulation disorders, like ACS and DIC.

PB 2139 | Discovery of ONO-5450598, a Highly Orally Bioavailable Small Molecule Factor XIa Inhibitor: The Pharmacokinetic and Pharmacological Profiles

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Background: In ISTH2015, we reported that ONO-7750512 as a small molecule factor XIa (FXIa) inhibitor has an oral bioavailability (BA) of 22% in rats and has potent antithrombotic efficacy without a prolonged bleeding time in animal models. Recently, we have found a new small molecule FXIa inhibitor ONO-5450598, which is equivalent to ONO-7750512 in terms of the potent antithrombotic efficacy without a prolonged bleeding time and has even higher oral BA.

Aims: To elucidate the *in vitro* pharmacological profile and to investigate the oral BA in various animal species and the *in vivo* antithrombotic and hemorrhagic effects of ONO-5450598 in monkeys.

Methods: Effects of ONO-5450598 against human coagulation and fibrinolytic factors were evaluated by *in vitro* enzyme assays. *In vitro* anticoagulation activities of ONO-5450598 were assessed as PT and APTT in humans and animals. To determine the oral BA, ONO-5450598 was orally or intravenously administered to rats, dogs, and monkeys. The antithrombotic effect of ONO-5450598 was assessed in a monkey arteriovenous (AV) shunt model. The hemorrhagic effect was assessed in a monkey nail-cut bleeding model.

Results: ONO-5450598 inhibited human FXIa activity with a Ki value of 2.0 nmol/L with 5000-fold selectivity over other coagulation and fibrinolytic factors. An *in vitro* plasma assessment revealed that ONO-5450598 doubled the APTT at < 1 µmol/L without PT prolongation even at 33 µmol/L in humans and monkeys. The oral BA of ONO-5450598 was 81% in monkeys, 88% in dogs, and 59% in rats. In a monkey AV shunt model, ONO-5450598 significantly inhibited thrombus formation at a dose higher than 0.0971 mg/kg/h, i.v. (plasma concentration: ≥0.41 µmol/L). In a monkey model of nail-cut bleeding, ONO-5450598 did not prolong the bleeding time even at 30 mg/kg, p.o. (plasma concentration: 13 µmol/L).

Conclusions: We identified ONO-5450598 as a selective human FXIa inhibitor with high oral BA and potent antithrombotic efficacy without the increased risk of bleeding.

PB 2140 | Discovery and Profiling of BAY-224 - A Novel Active Site Inhibitor of Thrombin for Oral Treatment of Thrombosis

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TABLE 1

	Population-based estimates of incidence				VTE as a hospital discharge diagnosis		
Types of Studies	Annual incidences of VTE	Retrospective cohort studies of specific disorders, injuries	Post-surgical VTE rates	Pregnancy-related VTE rates	Prevalence of VTE among all hospital inpatients	Prevalence of VTE in medical inpatients	Prevalence of VTE among surgical inpatients
	Korea 13.8 per 100,000	PE significantly higher in patients with autoimmune and chronic inflammatory disorders	Total Hip Arthroplasty -0.15 - 1.35%,	0.82 per 10 000 deliveries over the five year period from Korea	wide range	High risk medical and stroke -- 0.2 - 0.9%	Major trauma- 0.39%
	Taiwan 15.9 per 100,000	Higher DVT/VTE incidence in spinal cord injury	Total Knee Arthroplasty-0.22 - 1.2%,		11 to 65 per 10,000 for DVT,	Chronic liver disease - 1.6%	General, gastric and bowel surgery - 0.23 - 0.24% Colorectal surgery - 0.85%
	Hong Kong 19.9 per 100,000	Higher DVT/VTE incidence in organophosphate toxicity, carbon monoxide poisoning	Hip fracture Surgery-1.60%		2.5 to 23 per 10,000 for PE		Hong Kong - 0.17% (1998), 0.76%(1999) and 0.69%(2000)
		Higher DVT/VTE incidence in schizophrenia, sleep disorders, hormone replacement therapy and type 2 diabetes mellitus	Cancer surgery-0.67%		11 to 88 per 10,000 for VTE.		Mainland China - HFS -0.66% THR and TKR - 1.0 - 1.85%

Background: Thrombosis is a major underlying pathologic mechanism of severe diseases with thrombin as a key player.
Aims: Our aims were to identify and evaluate novel thrombin Inhibitors with an improved profile either alone or in combinations
Methods: A novel assay detecting fibrinogen turnover was used to screen for chemical lead structures. The optimized compound BAY-224 was characterized in fluorogenic assays and standard clotting tests. Clot lysis was measured turbidimetrically. Thrombin generated in situ in plasma was used as trigger for cytokine mRNA expression in HUVEC measured by RT-PCR.
BAY-224 was tested in various thrombosis models, including a venous reperfusion model (V. jugularis) in rabbit. The Pharmacokinetic (PK) profile was determined after administration in rat.
Results: BAY-224 is a direct, reversible inhibitor (IC_{50}0.71nM) with a different kon-/koff-profile compared to dabigatran and a high potency in plasma (IC_{50} 2nM). The clotting times in human plasma were prolonged (aPTT, EC_{200}0.7µM). BAY-224 had a stronger impact on peak height (IC_{50}0.43 µM) than lag time in the thrombin generation assay without rebound signs. BAY-224 inhibited clot-bound thrombin (IC_{50}100nM), accelerated clot lysis and reduced the induction of inflammatory cytokine mRNA expression in HUVEC.
BAY-224 showed antithrombotic effects in arterial, venous and systemic thrombosis models with an improved bleeding profile vs. dabigatran alone or in combination with FXa Inhibitors. In a rabbit venous reperfusion model, BAY-224 accelerated strongly the reperfusion of the V. jugularis.
The PK profile is compatible with oral administration without the need of a prodrug approach. BAY-224 has a low renal excretion rate.
Conclusions: BAY-224 is an active site inhibitor of thrombin with a slower, but longer binding to the target, strong adjuvant effects on clot lysis and an improved bleeding profile. Without prodrug approach it offers the potential for oral treatment and low risk for accumulation in renal insufficiency.

PB 2141 | Incidence of Venous Thromboembolism in Asian Populations: A Systematic Review

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Background: Despite the marked recent increase in the number of publications describing the incidence of venous thromboembolism (VTE) in Asia, there remains a lack of consensus on the true risks, and trends over time, to inform appropriate clinical practice.
Aims: To examine the evidence through systematic literature review on the burden of disease from symptomatic VTE in Asia.
Methods: Databases were searched for publications from Asia on VTE, deep vein thrombosis (DVT) or pulmonary embolism (PE) between January 1995 and February 2016. Review of eligible studies was conducted independently by two reviewers. Publications were excluded for the following reasons: small sample size; letter to the editor or literature review without new data; lack of denominator value therefore no incidence reported or calculable; survey based studies with poor response rates; duplicated data; described mainly non-symptomatic/sub-clinical disease diagnosed on screening; incidence

estimate extrapolated from < 90% of the population of interest; the report concerned predisposition for PE in people with a DVT; or VTE was not a main study outcome of interest.

Results: 1,095 studies were identified of which 72 were eligible for full text review and data extraction. Data were grouped into categories including post-surgical, non-surgical, chronic conditions, medical subgroups and cancer. Notable findings include the strong associations of increasing VTE risk with advancing age, strong emphasis on cancer associated VTE and the rising trend of VTE rates across Asia. The abbreviated findings are summarised in the table.

Conclusions: Population-wide incidence estimates were about 15-20% of the levels recorded in Western countries but have clearly increased over time. It is anticipated that this synthesis of evidence on the incidence of VTE and predisposing factors will assist with refining and updating Asian VTE-related guidelines and increase awareness about the importance of VTE in Asian populations.

PB 2142 | Thrombin Exosite 1 Inhibition with JNJ-375 Causes Anti-coagulation and Inhibits Thrombus Formation in a Human Translational Model of Thrombosis

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Background: JNJ-375 is a novel first-in-class anticoagulant that binds to the exosite 1 region of thrombin, whilst leaving the catalytic activity of the protease unaffected. It is derived from an anti-thrombin IgA paraprotein identified in a patient with elevated coagulation tests but without a history of bleeding episodes. Results from animal models of thrombosis suggest that selective exosite 1 inhibition may have advantages over current anticoagulants in widening the therapeutic window between anti-thrombotic and bleeding effects.

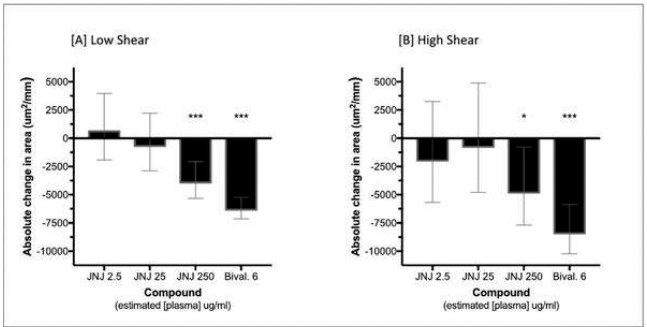

Figure 1.
The effect of study compound on *ex vivo* total thrombus formation at [A] low shear stress (212 s^{-1}) and [B] high shear stress (1690 s^{-1}) as compared to placebo. Data shown are adjusted mean absolute difference (um^2/mm) compared to placebo ± 95% confidence intervals; *p ≤0.05, **p ≤0.01, ***p ≤0.001. Abbreviations used: JNJ, JNJ-375.

FIGURE 1 The effect of study compound on ex vivo thrombus formation

Aims: To assess the anticoagulant and antithrombotic effects of JNJ-375 in an *ex vivo* human translational model of thrombosis.

Methods: Fifteen healthy volunteers participated in a double-blind randomized crossover. The effect of JNJ-64179375 (2.5, 25 and 250 µg/mL), bivalirudin (6 µg/mL; positive control) and matched placebo on coagulation assays and thrombus formation were determined using an *ex vivo* perfusion model of thrombosis.

Results: JNJ-375 produced concentration-dependent prolongation of all blood coagulation tests (correlations: r=0.98 for prothrombin time; r=0.87 for activated partial thromboplastin time; r=0.91 for thrombin time (TT), p< 0.001 for all), with TT the most sensitive to the anticoagulant effect. Compared to placebo, JNJ-375 (2.5, 25 and 250 mg/mL) reduced mean total thrombus area by -6.4% (95% CI: -41.3. 20.0; p=0.67), 7.1% (-23.0, 30.0; p=0.60) and 40.8% (21.5, 55.4; p< 0.001) at low shear and by 13.5% (-22.2, 38.8; p=0.40), 5.2% (-33.4, 32.7; p=0.75) and 32.9% (5.4, 52.5; p=0.02) at high shear stress respectively (Fig. 1).

Conclusions: In a human translational model of thrombosis, JNJ-375 produced concentration-dependent prolongation of coagulation time, and reduced *ex vivo* thrombus formation under conditions of both venous and arterial flow. Our results suggest that JNJ-375 may have a favourable anticoagulant and antithrombotic profile, and that further investigation in clinical trials is warranted.

PB 2143 | Vena Cava Filter Retrieval Rates and Factors Associated with Retrieval in the United States

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Background: The United States uses 20-40 times more inferior vena cava filters (IVCFs) than European countries. Retrieval of IVCFs is important for the safety of these devices as complications increase with longer dwell times, but is low in the U.S.

Aims: To assess IVCF retrieval rates and patient demographic and clinical factors associated with retrieval in a national cohort.

Methods: A retrospective cohort of patient receiving IVCFs was identified from a healthcare claims database. The indication for IVCF placement was identified as pulmonary embolism (PE) with or without deep vein thrombosis (DVT), DVT only, or prophylactic). Patient demographic and clinical characteristics were included in proportional hazard regression models to find associations with early (90-day) and one-year IVCF retrieval. Initiation of anticoagulation and the correlation between time-to-retrieval and time-to-initiation of anticoagulation was observed.

Results: Of 54,766 patients receiving an IVCF, 36.9% had PE, 43.9% had DVT only, and 19.2% had no apparent VTE present. Over the one-year of follow-up, the cumulative incidence of VCF retrieval was 18.4%, which differed based on indication, age, and several other key patient factors. Retrieval increased over time from a low of 14.0% in 2010 up to approximately 24% in 2014.

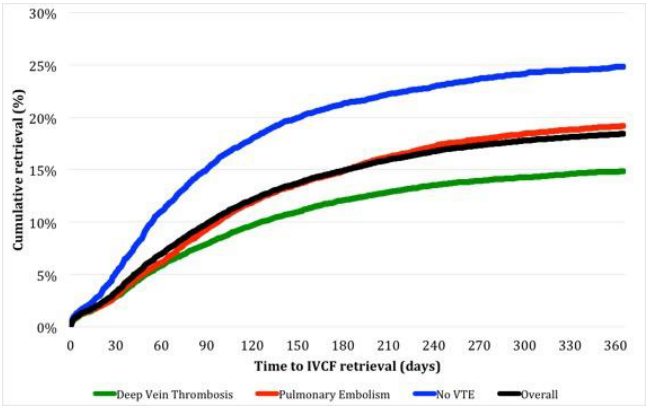

FIGURE 1 Cumulative incidence of vena cava filter retrieval

In adjusted time-to-event models, increasing age, differing regions, and some comorbidities were associated with poorer retrieval rates. Initiation of anticoagulation was poorly correlated with retrieval, with anticoagulation preceding retrieval by a median of 51 days while those without retrieval had a median of 278 days of exposure to anticoagulation.

Conclusions: IVCF retrieval increased over the study period but remained suboptimal. Improving retrieval rates can improve patient outcomes, prevent complications, and supplement clinic revenue. IVCF retrieval should be a priority for quality improvement initiatives at the institutional and national level.

PB 2144 | Dabigatran Neutralizing Antibody, Idarucizumab, Exhibits Procoagulant and Platelet Activation Responses in Whole Blood

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Background: Idarucizumab is a humanized monoclonal antibody fragment that is clinically available to neutralize Dabigatran. There is currently limited data on its effects on the blood coagulation and platelet activation profiles.

Aims: The purpose of this study is to determine its effect on blood coagulation and platelet activation.

Methods: Idarucizumab was purchased as a 50 mg/mL solution from Loyola University Hospital Pharmacy. Synthetic dabigatran was obtained from Sellec Chemical (Houston, TX). Whole blood from healthy volunteers was collected for TEG and ACT analysis. Citrated whole blood was used for the preparation of platelet rich plasma (PRP) which was used in platelet aggregation studies. Arachidonic acid, ADP, epinephrine, thrombin, and collagen were used as agonists.

Whole blood was supplemented with Idarucizumab at 0-10 mg/mL and studied in a Haemoscope 500 TEG instrument. The ACT studies were carried out in celite tubes in a concentration range of 0-5 mg/mL. The agonist-induced platelet aggregation and HIT antibody mediated platelet aggregation profiles were studied after pre incubating PRP with Idarucizumab at a concentration of 1.0 mg/mL.

Results: Idarucizumab produced a dose-dependent hypercoagulable effect in the TEG profile, reducing R time by 35% and max amplitude by 45%. At high concentrations, Idarucizumab significantly affected clot retraction. Idarucizumab at 5 mg/mL reduced the ACT by 6%. Idarucizumab variably augmented the different agonist mediated platelet aggregation profiles. Idarucizumab at 1.0 mg/mL increased HIT antibody mediated platelet aggregation by almost 20%.

Conclusions: These studies show that Idarucizumab produces mild procoagulant effects as studied by TEG and ACT. It also augments the platelet aggregation profile by various agonists including Anti-heparin platelet factor IV antibodies. These procoagulant effects of Idarucizumab may contribute to potential hypercoagulable/prothrombotic events associated with its use and warrants further investigations.

PB 2145 | ONO-5450598, an Orally Available Small-molecule Inhibitor of Activated Blood Coagulation Factor XI, Inhibits Arterial Thrombus Formation without Increasing Bleeding when Used in Combination with Clopidogrel in Rabbits

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ONO PHARMACEUTICAL CO., LTD, Osaka, Japan

Background: Both platelet aggregation and blood coagulation are involved in arterial thrombus formation. Therefore, a combination of antiplatelet and anticoagulant drugs is desired for prevention of arterial thrombosis. However, this combination therapy is known to increase the risk of bleeding. Our nonclinical studies have revealed that ONO-5450598, an orally available inhibitor of activated blood coagulation factor XI (FXIa), is lower in bleeding risk than existing anticoagulant drugs.

Aims: Combined effects of ONO-5450598 and clopidogrel were evaluated in rabbit arterial thrombosis and bleeding models to explore the possibility of whether ONO-5450598 can be a novel anticoagulant without increasing the risk of bleeding even when used in combination with antiplatelet drugs.

Methods: Clopidogrel was orally administered to rabbits at a dose of 3 mg/kg once daily for 3 days. On Day 3 of clopidogrel treatment, ONO-5450598 or rivaroxaban (an inhibitor of activated blood coagulation factor X) was orally administered. Time to occlusion (TTO) of the vessel was measured in a ferric chloride-induced carotid artery thrombosis model. Blood loss volume (BL) was measured in an abdominal incision bleeding model. In addition, activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured.

Results: When administered in combination with clopidogrel, 10 mg/kg ONO-5450598 prolonged TTO without increasing BL compared with clopidogrel alone, whereas 3 mg/kg rivaroxaban prolonged TTO and increased BL. ONO-5450598 prolonged APTT by approximately 2-fold from baseline without PT prolongation while rivaroxaban prolonged both APTT and PT by approximately 1.4- and 1.3-fold, respectively.

Conclusions: ONO-5450598 is expected to be a novel oral anticoagulant without increasing the risk of bleeding even when used in combination with antiplatelet drugs.

PB 2147 | Inter-individual Variation in Plasma Levels of Direct Oral Anticoagulants in Patients with Non-valvular Atrial Fibrillation

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Background: Inter-individual variation in plasma drug levels of direct oral anticoagulants (DOAC) among patients raised the question of whether some patients may be at increased risk of bleeding events or treatment failure.

Aims: To evaluate inter-individual variability in patients with non-valvular atrial fibrillation (NVAF) on dabigatran, rivaroxaban or apixaban in the anticoagulation unit.

Methods: A total of 65 patients were enrolled, of which 30 were on dabigatran (taking 150 mg or 110 mg twice-daily, respectively), 20 on apixaban (taking 5mg or 2.5 mg twice-daily) and 15 on rivaroxaban (taking 20 mg once-daily). Blood was taken at trough level for dabigatran or apixaban and at peak level for rivaroxaban within the first 3 month of treatment. Diluted-thrombin time (DTI) was performed with Hemoclot (Hyphen BioMed) for dabigatran. Anti-FXa measurments for apixaban and for rivaroxaban were calibrated with Hyphen DiXal (Hyphen BioMed).

Results: The mean value for dabigatran 150mg 2x/daily was 86 ng/ml (30 - 182) with CV 55% and for 110mg 2x/daily was 107 ng/ml (43 - 162) with CV 44 % at trough . For apixaban 5 mg twice-daily the mean value was 77 ng/ml (36 - 122) with CV 44 % and for 2.5 mg twice-daily 51 ng/ml (20 - 75) with CV 43% at trough. The mean peak value for 20 mg rivaroxaban was 195 ng/ml (96 - 346) with CV 40% . Two patients with overweight had dabigatran levels below the lower limit (< 30 ng/ml) and one with obesity on apixabane also had levels below 20 ng/ml. The measurements of rivaroxaban at trough level was difficult because most of them are below the lower limit of detection.

Conclusions: The drug concentration levels varied in the range of six times among the patients for dabigatran on 150mg 2x/daily and about four times for apixaban on 5 mg 2 x/daily at trough. We found below on-therapy levels of DOAC in patients with overweight.

PB 2148 | Idarucizumab, a Specific Antidote for Dabigatran, Cross-reacts with Melagatran and May also Interact with other Benzamidine-containing Compounds

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Background: Dabigatran etexilate is used to prevent embolic stroke is patients with atrial fibrillation. Idarucizumab is an anti-dabigatran Fab fragment that binds to the benzamidine group on dabigatran and inhibits its anti-thrombin activity.

Aims: To determine the relative specificity antidote for benzamidine as a sole antidote for dabigatran.

Methods: Several of the anti-thrombin agents (argatroban, melagatran, hirudin, and bivalirudin, human antithrombin, thrombomodulin, heparin cofactor II, and heparin-AT complex and anti-factor Xa (rivaroxaban, apixaban and DX-9065a) agents were supplemented to citrated plasma at concentrations ranging from 0.1 to 100 µg/mL. Idarucizumab was added to each mixture at a concentration of 1 mg/mL and anticoagulant activity was assessed using PT, aPTT, thrombin time and chromogenic anti-IIa/Xa and flurometric thrombin generation assays.

Results: Idarucizumab itself did not produce any effect on whole blood or plasma clotting profile at concentrations of < 1.0mg/ml. The antibody showed strong specificity for the inhibition of dabigatran and did not affect the anticoagulant and other effects of the other synthetic and natural thrombin and FXa inhibitors with the exception of melagatran. The prolongation of the PT, APTT and thrombin time by melagatran was completely inhibited by idarucizumab. Idarucizumab more effectively inhibited the prolongation of thrombin time by dabigatran than the prolongation induced by melagatran.

Conclusions: The cross-reactivity of idarucizumab with melagatran may result from the presence of a common benzamidine. Since the benzamidine is present in a number of serine protease inhibitors as well as drugs such as pentamidine, propamidine and dibromopropamidine. These observations suggest that simultaneous administration of idarucizumab may compromise the pharmacodynamics profile of benzamidine derived drugs such as the anti-malarials, anti-psychotic, anti-fungal and other compounds.

PB 2149 | Influence of Dabigatran on Rotational Thromboelastometry

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Background: Rotational thromboelastometry (ROTEM®), that provides a point-of-care analysis of the viscoelastic properties of clot formation and dissolution, is widely implemented in the management of bleeding and coagulopathy. It is affected by anticoagulant drugs, such as warfarin and heparin.

Aims: We aimed to assess the effect of dabigatran on different ROTEM® parameters.

Methods: Ninety seven patients, 42 women and 55 men, on average 72±10 years old were included in the study after signing informed consent. From each patient blood was collected and time of last drug ingestion was carefully recorded. In whole blood ROTEM® assays EXTEM, INTEM, FIBTEM and APTEM were performed on ROTEM® delta analyzer (all Tem International GmbH, Germany) within one hour of blood collection and the following parameters were recorded: clotting time (CT), clot formation time (CFT), alpha angle, amplitude at 10 minutes (A10) and maximum clot firmness (MCF). Plasma dabigatran levels were measured with dilute thrombin time (dTT) with Test Thrombin Reagent on CS2100i analyzer (both Siemens, Germany) and ranged from 8 to 466 ug/L 10 minutes and up to 16 hours after the last drug intake covering both trough and peak levels.

Results: Dabigatran concentration significantly correlated with the CT of EXTEM, INTEM, FIBTEM and APTEM assay (correlation coefficients 0.79, 0.64, 0.80 and 0.75, respectively, all $p \leq 0.001$). APTEM was the most sensitive ROTEM® assay with 81/97 (83 %) CT results above the upper reference value compared to EXTEM (68 %), INTEM (65 %) or FIBTEM (63 %). A statistically significant but weaker correlation was observed between dabigatran and A10 and MCF within all the assays, but the majority of the MCF results were within the reference range. CFT and alpha angle were not affected by dabigatran of any ROTEM® assay.

Conclusions: Dabigatran prolonged CT of all ROTEM® assays: EXTEM, INTEM, FIBTEM and APTEM in a dose-dependent manner and could, therefore, be useful in emergency situations when high dabigatran levels are suspected.

PB 2150 | Direct Oral Anticoagulants for Thromboprophylaxis in Orthopedic Surgery in the Real World: A Systematic Review of Population Based Studies

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Background: Safety and efficacy of direct oral anticoagulants (DOACS) has been established in randomized trials but an increasing number of „real world" data outside clinical trials has become available after approval.

Aims: To assess the efficacy and safety of DOACS in population-based studies in the context of thromboprophylaxis in orthopedic surgery (OS).

Methods: We conducted a systematic review of observational studies assessing DOACs (dabigatran, rivaroxaban and apixaban) in patients with OS. Efficacy outcomes included VTE, and safety outcomes included major bleeding (MB) (as defined by investigators), gastrointestinal (GI), central nervous system (CNS) bleeding, and bleeding-related deaths. We conducted a meta-analysis of pooled incidence rates (IR) proportions using both fixed and random effects models. Given the non-randomized nature of the studies we did not conduct a meta-analysis of effect sizes.

Results: Ten studies were included: 9 assessed rivaroxaban and 1 dabigatran. 6 studies were prospective and 4 retrospective cohorts. Most studies used low molecular weight heparin (LMWH) as comparator and included patients with total hip and knee arthroplasty. The meta-analysis included rivaroxaban studies with available information. The Results of the meta-analysis of proportions is shown in table 1.

Bleeding related mortality was low in both rivaroxaban and LMWH patients. In a sensitivity analysis by funding source, no difference in estimated pooled estimates of VTE and MB were observed between industry and non-industry funded studies.

Conclusions: Rivaroxaban is effective and safe in orthopedic surgery in "real-world" observational studies, similar to RCTs. Observational data for other agents is currently limited.

TABLE 1 Evaluable patients and pooled proportions of VTE and MB

Outcome	Drug	n/N	Fixed Effects Model		Random Effects Model	
			Pooled proportion (%)	95% CI	Pooled proportion (%)	95% CI
VTE	Rivaroxaban	227/27,158	0.8	0.73-0.95	1.6	1.39-1.79
	LMWH	435/25,409	1.7	1.55-1.87	2.9	2.72-3.16
MB	Rivaroxaban	192/27,130	0.7	0.61-0.81	2.2	2.04-2.46
	LMWH	333/25,409	1.3	1.17-1.45	2.6	0.38-2.82

PB 2151 | Heparin Calibrated Anti-Xa Assays for the Measurement of Low Levels of Direct Factor Xa Inhibitors

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Background: Apixaban, edoxaban and rivaroxaban do not require frequent monitoring but an assessment of the intensity of anticoagulation may be required in emergent or elective surgery. Some experts reported that anti-Xa activity below 0.1 IU/mL using heparin calibrated chromogenic assays may assert the absence of clinically relevant (i.e. < 30 or < 50 ng/mL depending on the clinical situation) direct factor Xa levels. However, it is not clear if difference in response will depend on the anti-Xa agent and also on the chromogenic anti-Xa kit used to assess the anti-Xa activity.

Aims: To assess if a cut-off of 0.1 UI anti-Xa/mL is able to exclude apixaban, rivaroxaban or edoxaban concentration < 30 ng/mL or < 50 ng/mL using different heparin calibrated chromogenic anti-Xa kits.

Methods: Apixaban, edoxaban and rivaroxaban were added to normal pooled plasma at increasing concentrations ranging from 0 to 500 ng/mL. Anti-Xa activities were measured using

(1) STA®-Liquid Anti-Xa (STA®LAX) on a STA-R Evolution Coagulometer,

(2) Biophen®Heparin LRT (BP®LRT) on a STA-R Evolution coagulometer and

(3) HemosIL®-Liquid Anti-Xa (IL®LAX) on a ACL-TOP 700 according to manufacturer recommendations.

Results: At 30 ng/mL of rivaroxaban, BP®LRT, STA®LAX and IL®LAX provided anti-Xa results >0.1 IU/mL. At 30 ng/mL of apixaban or edoxaban, BP®LRT and IL®LAX were below the cut-off but the STA®LAX was not. At a concentration of 50 ng/mL, only edoxaban with the BP®LRT kit showed an anti-Xa activity < 0.1 UI/mL.

Conclusions: Low (< 0.1 IU/mL) anti-Xa activity is not safe to exclude clinically relevant direct factor Xa levels and should be avoided. It can only inform if the drug is present or not. Chromogenic anti-Xa assays calibrated against the appropriate agent and using the appropriate procedure remains the more accurate method to assess accurately low levels of direct FXa inhibitors.

TABLE 1 Anti-Xa activities using STA®LAX on a STA-R Evolution Coagulometer, BP®LRT on a STA-R Evolution coagulometer and (3) IL®LAX on a ACL-TOP 700

concentration (ng/mL)	anticoagulant	STA®LAX (UI/mL)	BP®LRT (UI/mL)	IL®LAX(UI/mL)
30	Rivavoxaban	0,19	0,12	0,08
30	Apixaban	0,09	0,03	0,04
30	Endoxaban	0,07	0,02	0,02
50	Rivavoxaban	0,45	0,34	0,16
50	Apixaban	0,17	0,10	0,07
50	Endoxaban	0,13	0,06	0,04

PB 2152 | Efficacy of a Novel Contact Pathway Inhibitor, Ir-CPI, on in vitro Clotting Induced by PCI Catheter Segment

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Background: Ir-CPI, a protein derived from the tick *Ixodes ricinus* salivary, is a serine protease inhibitor of both factor XIa (FXIa) and FXIIa. In patients undergoing percutaneous coronary intervention (PCI), catheter thrombosis may occur as catheters trigger activation of FXII/FXI.

Aims: The aim of this study was to evaluate the effect of Ir-CPI on in vitro clotting induced by PCI catheter segment.

Methods: Catheter segments were pressed flat, shaped into rings and placed around the perimeter of wells (96-well plate), leaving the center of the well unobstructed. To the wells were added serial dilution of Ir-CPI (until 10 µM) with normal pooled plasma (NPP) or plasmas deficient in FXI or FXII. After incubation at 37°C and addition of a $CaCl_2$ solution, clot formation was assessed by monitoring absorbance at 340nm. Time to reach one-half maximal absorbance (IC50) was defined as the clotting time. Thrombin generation test (TGT) was also assessed using catheter segment as trigger of the process. Positive inhibitory controls were used (fondaparinux, enoxaparin).

Results: Presence of the catheter reduced the clotting time of NPP; an effect reversed by the addition of Ir-CPI. At high concentrations (≥ 5µM), Ir-CPI allowed to overpass the clotting time without catheter. On TGT (Fig 1), catheter segments decreased lag time and time

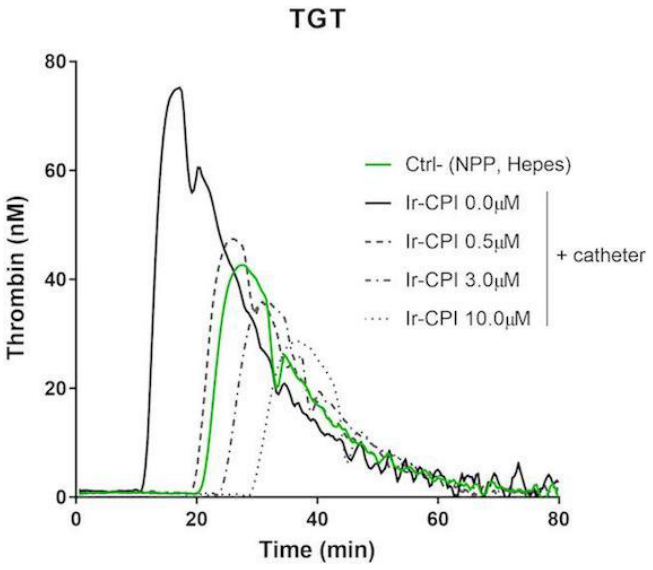

FIGURE 1 Effect of Ir-CPI on Thrombin Generation Time (TGT) in NPP exposed to PCI catheter segments

to peak while the endogenous thrombin potential (ETP) and the peak were increased. The presence of Ir-CPI allowed the restoration of baseline value, i.e. value of the NPP without exposition to catheter segments, in a concentration-dependent manner.

When clotting was triggered with FXII deficient plasma, we confirmed that catheter thrombosis is linked to FXI activation and that clotting can be abrogated with 3µM of Ir-CPI.

Conclusions: Ir-CPI can be used to inhibit the clotting induced by catheter segments and achieve antithrombotic effect. Ir-CPI is a promising agent with a better safety profile than heparins to face the problem of catheter thrombosis during PCI procedures.

PB 2153 | New Parenteral Poly (2-(acrylamide)-2-methylpropanesulfonic Acid) - Based Anticoagulants (NPACs): Efficacy and Safety Studies in Rats

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Background: Unfractionated heparin (UFH) remains an indispensable parenteral drug inhibiting blood coagulation. The biologic variability, immunogenicity, unpredictable anticoagulation, and narrow therapeutic range limit its utility. Other parenteral anticoagulants lack of an efficient antidote. We recently presented a novel agent neutralizing all parental anticoagulants (Trans Res 2016). There is still a need for synthetic UFH alternative with the available safe antidote and without unacceptable adverse effects.

Aims: The aim of the present study was to develop a novel, safe, and easily synthesized poly (2-(acrylamide)-2-methylpropanesulfonic acid) (PAMPS)-based parenteral anticoagulants.

Methods: We synthesized, purified and characterized 4 novel PAMPS-based polymers named by us as new parenteral anticoagulants (NPAC1, NPAC2, NPAC3, and NPAC4) and PAMPS. Then, we screened polymers for potential anticoagulant and antiplatelet activity using the *in vitro* assays. Finally, we examined efficacy and safety of the most active polymers in male Wistar rats. We assessed aPTT, PT, anti-factor Xa activity, calcium concentration, and platelet aggregation as efficacy endpoints and cardiorespiratory and hematological parameters as safety measures.

Results: We found that all synthesized PAMPS-based polymers and PAMPS dose-dependently prolonged aPTT and PT and decreased platelet aggregation *in vitro*. The effect of NPAC1 on aPTT and PT was the weakest. Thus, we discontinued studying of this polymer. NPAC2 and NPAC4 significantly increased the anti-fXa activity. *In vivo*, all polymers significantly prolonged aPTT and PT. NPAC4 and PAMPS decreased, NPAC3 increased, whereas NPAC2 did not alter platelet aggregation. Unlike NPAC2 and NPAC3, NPAC4 and PAMPS caused unacceptable cardiorespiratory and/or hematological complications in rats (Table 1).

Conclusions: Documented efficacy and safety of NPAC2 in rats makes this polymer a promising candidate for a novel parenteral anticoagulant.

Funding: National Science Centre, 2016/21/B/ST5/00837.

PB 2154 | INR Might Be a Useful Predictor for Upcoming Minor to Major Bleedings in Patients Treated with NOAC

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Background: Patients treated with Novel Oral Anticoagulants (NOAC) are not required to be monitored laboratory, as no qualitative tests or therapeutic intervals are recommended today. Despite favorable pharmacokinetic properties, bleeding events are some of the few complications to the treatment. As there are no recommended global anticoagulation tests, prediction of these complications is today not possible.

TABLE 1 In vivo efficacy and safety of new parenteral anticoagulants (NPACs)

	Vehicle	NPAC2	NPAC3	NPAC4	PAMPS
aPTT (seconds)	25.7±4.9	94.1±21.9***	82.9±27.8***	113.3±24.8***	109.9±23.4***
PT (seconds)	10.8±0.5	13.5±0.9***	12.6±1.0***	114.5±1.2***	14.6±1.5***
Platelet aggregation - maximal extension (Ω)	12 (8.5-13)	11 (5.5-13.5)	14 (13-19)**	4 (2.5-6)**	4 (0.5-6.5)**
Platelet aggregation - slope	6 (4.5-7)	6 (3-8)	8 (6-10)*	3 (2-4)***	3 (2-4.5)**
Platelet aggregation - lag time (seconds)	72 (64-89)	71 (69-89)	67 (35-74)	119 (74-196)**	106 (79-297)**
Platelet aggregation - area under the curve	39 (26-41)	34 (19-43)	49 (41-63)**	11 (5-20)**	12 (1-19)**
Blood platelets (10^3/mm^3)	666±65	654±68	666±46	562±103*	497±79***
Cardiorespiratory complications	-	-	-	Cardiac and respiratory arrest	Cardiac and respiratory arrest

Results are shown as mean±SD or as median with lower and upper limits.*P<0.05, **P<0.01, ***P<0.001 vs. vehicle, unpaired Student t test or Mann-Whitney test, n=6-11.

Aims: To explore if International Normalized Ratio (INR) assay could be used as a predictor for bleeding events in patients treated with NOAC.

Methods: Medical records of 243 patients were systematically reviewed retrospectively up to 30 months, with focus on minor and major bleedings defined using International Society on Thrombosis and Haemostasis (ISTH) criteria. Furthermore, INR elevation and fall in hemoglobin were assessed.

Patients treated with either Apixaban, Dabigatran or Rivaroxaban in recommended doses, due to either Non-Valvular Atrial Fibrillation (NVAF) or Venous Thromboembolism (VTE), were randomly selected.

Results: A peak of INR ≥ 1.5 was statistically significant associated with an increased risk of minor and major bleeding events.

There was a significant statistic correlation between INR elevations and bleeding events in the Apixaban treated patients (P-value: 0.025), and the same pattern was seen in the Dabigatran treated patients (P-value: 0.038), and also in the Rivaroxaban treated patients (P-value: 0.045). (**Table 1**)

TABLE 1 Minor to major bleeding incidents and INR correlation at peak INR > 1.5

	Bleeding and INR elevation	No bleeding but INR elevation	P-value
Apixaban	27.3%	9.7%	0.025
Dabigatran	36%	12.5%	0.038
Rivaroxaban	48.6%	25.7%	0.045

Conclusions: Patients with INR elevation ≥1.5 had a statistically significant higher risk of bleeding and/or development of anemia compared to patients with lower elevation or normal INR. This study therefore suggests that the low cost assay INR could be a predictor for upcoming bleeding events in patients treated with NOAC, but further prospective exploration will be needed.

PB 2155 | Correlation between Thromboelastometry and HPLC-MS/MS for Rivaroxaban Monitoring

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Background: Rivaroxaban acts as a direct factor Xa inhibitor. Although the absence of drug monitoring requirement, it is important to have a clotting assay that correlates plasma drug levels and hemorrhagic risk.

Aims: Evaluate the impact of rivaroxaban dosage and their correlation with plasma concentration quantified by chromatographic assay with mass spectrometric and Rotational thrombelastometry (ROTEM®).

Methods: We evaluated 46 patients treated with rivaroxaban: 13 with 10mg daily (OD), 16 with 15mg OD, nine with 20mg OD, and eight with 15mg twice a day (BID). Blood was collected 2h before (trough) and 2h after drug intake (peak). Non activated TEM was employed for: clotting time (CT), Clot Formation Time (CFT), Alpha angle, Amplitude after 5 minutes (A5), Amplitude after 10 minutes (A10) and Maximum Clot Firmness (MCF).

Results: There were significant difference between trough and peak response to CT, CFT and alpha angle parameters for 15 and 20 mg OD. For 10 mg OD it was possible to differentiate trough from peak in CT parameter. The A5 trough and peak were different only for 15 mg OD. For A10 parameter it was possible to differentiate trough and peak only in 20 mg OD. The comparison of HPLC-MS/MS and TEM, shows: a significant correlation at peak time between plasma concentration of rivaroxaban and CT (r=0.7879; p< 0.0001), CFT (r=0.7843; p< 0.0001), alpha angle (r= -0.7713; p< 0.0001), A5 (r= -0.7630; p< 0.0001) and A10 (r= -0.6800; p< 0.0001). No correlation was observed between the MCF and the plasma concentration of the drug in trough and peak times.

Conclusions: CT, CFT and alpha angle parameters show a statistically significant measured after 2h when compared to trough time, in a dose-dependent way. The ROTEM limitations in response to rivaroxaban in trough time present some advantages that can detect rivaroxaban effects quickly.

PB 2156 | Comparative Studies on the Anticoagulant Actions of Recombinant Thrombomodulin and Heparin and their Neutralization by FEIBA as Measured by Thromboelastography

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Background: rTM (recombinant Thrombomodulin) is currently developed for various clinical indications and reduces thrombogenesis without increasing bleeding risk.

Aims: The purpose of this study is to compare the anticoagulant effects related to bleeding risk of rTM and Unfractionated Heparin (UH) at various levels and their neutralization by FEIBA.

Methods: Citrated Whole Blood samples were supplemented with rTM or UH in a concentration range of 0-10µg/ml (n=10). TEG analysis was performed on a Haemoscope 5000 instrument and the R time, K Time, MA and Angle were measured. The relative neutralization profiles of UH and rTM by FEIBA at 1 and 0.1 U/ml were also studied.

Results: rTM exhibited much weaker anticoagulant effects compared to UH. At 1µg/ml rTM did not produce any anticoagulant effects. At levels greater than 2.5µg/ml and up to 10µg/ml, rTM produced a concentration dependent and relatively weak anticoagulant effect.

UH was a much stronger anticoagulant and completely inhibited clot formation for the duration of TEG analysis at concentrations greater than 2.5µg/ml. FEIBA at 1U/ml completely neutralized the anticoagulant effect of rTM at 10µg/ml and UH at 0.5µg/ml. FEIBA at 0.1U/ml neutralized the anticoagulant effects of rTM at 10µg/ml completely, but only partially neutralized the anticoagulant effects of Heparin at 0.5µg/ml.

Conclusions: These studies suggest that rTM is a relatively weaker anticoagulant in comparison to UH. The expected concentration of rTM in its given indications is between 0.5-1.5µg/ml. At these concentrations, this agent is not expected to produce any anticoagulant effects. In contrast UH, at therapeutic concentrations of 1-2µg/ml produces strong anticoagulant effect. These TEG studies indicate that rTM is an antithrombotic agent mediating its effects via multiple mechanisms whereas heparins primarily produce anticoagulant effects. Supratherapeutic levels of thrombomodulin and heparin are neutralizable by FEIBA.

PB 2157 | Diclofenac for the Treatment of Acute Symptomatic Intermediate-risk Pulmonary Embolism

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Background: The inflammatory response induced by acute pulmonary embolism (**PE**) might contribute to the development of right ventricular (**RV**) dysfunction.

Aims: We conducted a double-blind, randomized, placebo-controlled pilot trial to investigate whether diclofenac improves RV dysfunction in intermediate-risk PE patients.

Methods: This single center study only enrolled patients who had acute symptomatic PE, echocardiographic RV dysfunction, and a lack of systemic hypotension. Patients were randomized 1:1 to receive intravenous diclofenac (two doses of 75 mg in the first 24 hours after PE diagnosis) or matching placebo in addition to standard anticoagulant therapy. The primary and secondary efficacy outcomes were persistence of RV dysfunction at 48 hours and at 7 days after randomization, respectively. The primary safety outcome was major bleeding within 7 days after randomization.

Results: The trial was stopped prematurely due to low recruitment. We randomly assigned 34 patients who had intermediate-risk PE to receive diclofenac (17) or placebo (17). In intention-to-treat analyses, rates of persistent RV dysfunction at 48 hours were 59% (95% confidence interval [**CI**], 33-82%) in the diclofenac group and 76% (95% CI, 50-93%) in the placebo group (absolute risk difference (**ARD**), -17 percentage points; 95% CI, -47 to +17). By day 7, diclofenac and placebo groups had similar RV dysfunction rates (35% in both arms). Major bleeding occurred in none of patients in the diclofenac group and in 5.9% of patient in the heparin group (ARD, -5.9 percentage points; 95% CI, -31 to +18). The randomized groups has similar rates of other adverse events.

Conclusions: This trial did not detect a significantly lower rate of RV dysfunction at 48 hours and at 7 days due to the addition of diclofenac to anticoagulation in patients who had acute symptomatic intermediate-risk PE. The diclofenac and placebo treatment groups had similar rates of adverse outcomes.

PB 2158 | Anticoagulant and Antiaggregation Effect of New Aminoestrogen

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Background: The estrogens are involved in the regulation of many physiological functions, through estrogen receptor. Selective estrogen ligands are highly promising targets for different treatments. However, synthetic estrogens have side effects (e.g. increasing the risk of thrombosis). We are interested in developing drugs called aminoestrogens, with antithrombotic activity. We designed and synthesized two original compounds: N-[3-hydroxy-1,3,5(10)-estratrien-17beta-il]-4-metoxibencilamine and N-[3-hydroxy-1,3,5(10)-estratrien-17beta-il]-3,4,5-trimetoxifeniletilamine, Moame and Treoame respectively. Obtained by reaction of estrone with 3,4,5-trimethoxyphenylethylamine and 4-methoxybenzylamine followed by reduction with sodium borohydride.

Aims: Determining the anticoagulant and antiaggregant effect of two new aminoestrogens and compared with the aminoestrogen Tyrame.

Methods: The compounds were synthesized at UNAM. Male adult CD1 mice were kept under controlled conditions. After 24h of pretreatment (0.3-10mg/100g body weight compounds or DMSO (control), sc), we measured the blood clotting time with 25uL of a whole blood sample, obtained of the dorsal vein of the mice. The blood was made alternately flow by gravity between two marks in a capillary tube. We record the time until the blood is clot and stops to flow in the capillary tube. For platelet aggregation, a sample of blood (anticoagulated) was obtained under anesthesia and centrifuged. We added 10uM ADP or 1ug/mL collagen and PRP to a well of half-area 96-well plates that was at 37°C for 5min shaking and the absorbance was measured at 595nm. The result obtained with mice treated with DMSO was consider 100% of aggregation, using PPP wells as reference.

The protocol followed all the ethical and legal regulations outlined for animal experiments and was approved by the ethical institutional committee.

Results: The results are shown on table 1.

TABLE 1 IC50 of amynoestrogens

	IC 50 Moame (μM)	IC 50 Treoame (μM)	IC 50 Tyrame (μM)
Cotting time (seconds)	0.29	0.7	0.045
ADP induced platelet aggregation (%)	3.26	25.83	0.009
Collagen induced platelet aggregation (%)	9.59	5.77	7.66

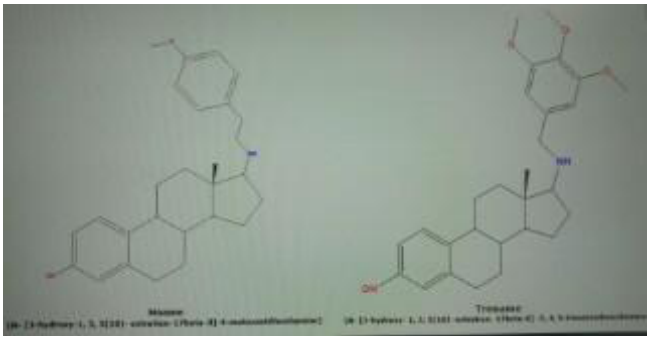

FIGURE 1 Molecular estructure of new amynoestrogens

Conclusions: The three aminoestrogens have anticoagulant and antiaggregant effect. Tyrame is the most potent.

PB 2159 | Anticoagulant Actions of Recombinant Thrombomodulin and Heparin and their Neutralization by FEIBA

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Background: Thromboelastographic (TEG) analysis represents a global approach to monitor the clotability of the native whole blood. This method provides an integrated hemostatic profile including both the cellular and humoral phases. rTM (recombinant Thrombomodulin) is currently developed for various clinical indications and exerts multiple actions on blood and its components to reduce thrombogenesis without increasing a bleeding risk. This is in contrast to heparins which mediate their actions accompanied by some bleeding risk. FEIBA (Factor VII Inhibitor Bypass Activity) contains non activated factors II, IX and X and activated factor VII and has been used in the management of bleeding in hemophilia patients.

Aims: The purpose of this study is to compare the anticoagulant effects related to bleeding risk of rTM and Unfractionated Heparin (UH) at various levels and their neutralization by FEIBA.

Methods: Citrated Whole Blood samples were supplemented with rTM or UH in a concentration range of 0-10ug/ml (n=10). TEG analysis was performed on a TEG 5000 system in which the clotting was initiated by reclacification of the whole blood and set parameters as R time, K time, MA and Angle were measured. The relative neutralization profiles of the UH and rTM by FEIBA at 1 and 0.1 U/ml were also investigated. All results were analyzed in terms of Means Values Standard Deviation.

Results: At 1ug/ml rTM did not produce any anticoagulant effects as evident by TEG profile. At > 2.5ug/ml, rTM produced a concentration dependent anticoagulant effect which altered the TEG profile. UH produced relatively stronger anticoagulant effects and at a concentration > 2.5ug/ml, totally inhibited the clot formation. At higher levels, UH produced strong effects, rTM at 10ug/ml produced weaker effects. FEIBA at 1U/ml neutralized the anticoagulant effect of rTM at 10ug/ml and UH at 0.5ug/ml.

Conclusions: These studies suggest that rTM is a relatively weaker anticoagulant in comparison to UH which are neutralized by relatively lower dosages of FEIBA.

PB 2160 | Sulodexide Efficiently Inhibits Thrombin Generation and Clot Formation via the Anti-Xa and Anti-IIa Activity. An in vitro Comparison with the LMWH Enoxaparin and UFH

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Background: Sulodexide is an orally active natural glycosaminoglycan composed of two entities, a fast-moving heparin fraction and a dermatan sulfate (20%). Recent phase III clinical trial data showed that oral treatment with sulodexide has a favorable risk/benefit profile when administered for long-term secondary prevention of VTE. However, the mechanism of the antithrombotic action of sulodexide remains unclear.

Aims: The effect of sulodexide, enoxaparin and unfractionated heparin (UFH) on thrombin generation (TG), clot formation kinetics and quality were compared.

Methods: Platelet poor plasma (PPP) from 5 healthy volunteers was spiked with increasing concentrations (up 10 µg/ml) of sulodexide, enoxaparin or UFH. The anti-Xa activity was measured in PPP (using the STA-Liquid anti-Xa assay) and TG was assessed with PPP-reagent 5TF®/Calibrated Automated Thrombogram (CAT®; Stago, France). For the TG test, ETP (Endogenous Thrombin Potential) and MRI were recorded. Whole blood thromboelastometry, triggered by low tissue factor concentrations (5 pM) was assessed with the ROTEM®; Clotting Time (CT), Clot Formation Time (CFT), α-angle, Maximum Clot Firmness (MCF) were analyzed. The activated partial thromboplastin clotting time (aPTT) and the thrombin time (TT) were measured.
Results: Sulodexide is a potent inhibitor of TG, delays clot formation and decreases clot firmness. The anti-Xa activity/µg, the concentrations of sulodexide, enoxaparin and UFH prolonging 2-fold the aPTT, TT, CT, CFT, and the IC50% for α-angle, MCF, ETP, MRI are shown in Table 1.

TABLE 1 In vitro comparison of the effect of sulodexide, enoxaparin and UFH on apt, TT, thrombin generation and clot formation kinetics and quality

n=5	Sulodexide	Enoxaparin	UFH
anti-Xa activity (IU/µg)	0.08	0.12	0.12
aPTT x 2 (µg/ml)	3.3	8.0	1.6
TT x 2 (µg/ml)	1.20	3.1	0.6
IC50 MRI (µg/ml)	0.80	2.0	0.8
IC50 ETP (µg/ml)	1.0	3.1	1.0
CTx2 (µg/ml)	1.8	5.8	1.0
CFTx2 (µg/ml)	1.0	4.1	1.0
IC50 α-angle (µg/ml)	3	>6	1.5
IC50 MCF (µg/ml)	3.5	-	3.3

Conclusions: Sulodexide as compared to enoxaparin and UFH, possesses 40% less anti-Xa activity per µg. The effect on aPTT and TT reveals that sulodexide has more anti-IIa activity than LMWH and about 50% less than the UFH. The inhibitory potency of sulodexide on TG and clot formation, which is the result of the synergistic action of the anti-Xa and anti-IIa activities, is in a range between UFH and enoxaparin.

PB 2161 | Porcine and Ovine Mucosal Heparins and Their Depolymerized Derivatives are Comparable in Contrast to Their Bovine Equivalents

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Background: The currently used unfractionated heparin (UFH) and low molecular weight heparins (LMWH) are mostly derived from Porcine mucosal tissue. Recently bovine and ovine heparins have also become available.
Aims: The purpose of this study is to compare multiple individual batches of UFH obtained from Bovine, Ovine, and Porcine origin and their depolymerized product obtained by benzylation followed by alkaline hydrolysis representing enoxaparins.
Methods: The molecular profile of the heparins and enoxaparins from various sources were determined using the size exclusion method. The anticoagulant potency was measured using clot based methods such as aPTT and Thrombin Time whereas the USP potency in terms of anti-Xa and anti-IIa activities were measured using a commercial kit (Hyphen Biomedical, Ohio, USA). The relative interaction of the heparins and enoxaparins with heparin induced thrombocytopenia (HIT) antibody induced aggregation of platelets were investigated using aggregometry.
Results: The molecular profiles of the Bovine, ovine, and porcine heparins and enoxaparins were almost comparable and ranged from 15-18 kDa. The global anticoagulant and amidolytic protease assays for the bovine heparin were consistently lower than porcine and ovine samples. The USP potency of 28 batches of porcine ranged from 170-200 U/mg, whereas the 21 batches of ovine heparins exhibited comparable potencies in the range of 160-210 U/mL and 24 batches of bovine heparin ranged from 110-140 U/mg. The ovine and porcine enoxaparin exhibited comparable potencies which ranged from 94-110 U/mg whereas bovine enoxaparin was slightly lower, ranging from 80-87 U/mg.
Conclusions: These studies show that UFHs from various origin exhibit comparable molecular profiles. While the porcine and ovine heparins exhibit similar biological potencies, the bovine heparin was weaker. The enoxaparins derived from these species exhibit similar molecular profiles, however, in functional assays, they showed weaker potency.

NURSES AND ALLIED HEALTH

PB 1051 | Remote ‚Bridging Clinics' Maximise Efficiency and Patient Satisfaction in the Perioperative Management of Anticoagulation

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Background: King's Thrombosis Centre receives approximately 130 referrals per annum for perioperative management of anticoagulation. The workload is largely nurse-led using local guidelines supported by Consultant Haematologists. In 2015 we changed our guidance to incorporate recommendations from the ‚BRIDGE' study, which results in fewer patients requiring bridging but referrals continued to increase. Patients were previously booked into a dedicated outpatient clinic to

formulate perioperative management plans. A virtual ‚bridging clinic' was introduced to reduce the need for patients to attend in person, with allocated telephone slots offering the opportunity to have a discussion and formulate a plan. Patients identified as needing face-to-face input were seen in the dedicated outpatient clinic.

Aims: The virtual clinic was created to reduce the number of hospital visits for patients, improve patient experience and promote efficiency in the anticoagulation clinic.

Methods: A virtual clinic was introduced in 2015 where a telephone appointment at a specified time and date was arranged with an anticoagulation clinical nurse specialist (CNS). During the consultation, discussion and blood result review took place as per the outpatient clinic and a perioperative management plan was devised. Data were collected prospectively on 61 patients who were reviewed in the virtual clinic from June 2015 to October 2016. Patient feedback was sought at the end of the consultation.

Results: By allocating virtual clinic slots instead of actual clinic slots, 82% of outpatient visits were avoided. This improved workload efficiency by increasing productivity. 100% of patients confirmed they were satisfied with the process and preferred a virtual clinic.

Conclusions: Perioperative anticoagulation advice is an integral part of the CNS workload and demand for this service increasing. It is important for the correct advice to be given in a timely manner and can be achieved via a virtual telephone clinic, with improved patient satisfaction.

PB 1052 | Expanding the Role of the Anticoagulation Nurse

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Background: As the number of patients on DOACs (Direct Oral Anticoagulants) continues to increase, anticoagulation clinics face increased pressure to cope with this demand. The roles of all members of the multidisciplinary team including nurses should be enhanced to run the DOAC clinics effectively.

Aims: To help develop a new care model for anticoagulation by expanding the role of the anticoagulation nurse within nurse-led DOAC follow up clinics.

Methods: The training process comprises several stages. Nurses are trained by working closely with an anticoagulation specialist pharmacist. Training included familiarization with the relevant clinical trials and summaries of product characteristics. Experience and knowledge is gained by screening referrals and identifying if the DOAC is prescribed appropriately according to its license. Nurses then observe the pharmacist or doctor in clinic. They then undertake clinics under supervision of the pharmacist until adjudged competent to run the clinic independently.

The patient pathway was established and communicated to all stakeholders. A referral to the DOAC clinic initiates this pathway. Referrals get screened and discussed in the multidisciplinary team meeting to triage the patient to see a doctor, pharmacist, or nurse according to the complexity of the case. The plan for the patient is devised including blood tests, referrals to other specialist teams, or further review if needed.

Results: By undergoing structured training from a pharmacist in addition to supervision by a haematologist, the nurse-led DOAC follow up clinic successfully delivers a high quality service compliant with local and national guidelines.

Conclusions: Structured training on DOACs and coordinated patient pathways will help expand the role of the anticoagulation nurse and enable provision of an efficient, safe and cost effective service. Patients are seen by skilled and competent clinicians not limited to the doctor or pharmacist thereby reducing waiting times as well.

PB 1053 | Nurse-led Clinic Initiative for Patients Commencing Direct Oral Anticoagulants (DOACs) in the National Coagulation Centre, Dublin, Ireland

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Background: An increasing number of patients are commenced on DOACs, as an alternative to warfarin. Inorder to optimise patient safety, it is necessary to provide standardised, comprehensive followup and education for these patients. A nurse-led clinic was set-up as part of the existing anticoagulation service. The governance of the clinic is overseen by a Coagulation Consultant Haematologist.

Aims:

· To standardise care for patients on DOACs in accordance with the local SOP.

. To provide comprehensive education for patients on DOAC.

· To monitor renal prolife and dose adjust if needed.

Methods: An electronic referral system was set-up for referrals from both outpatient and inpatients commencing on DOAC within the hospital. The patients are booked for a group education session within two weeks followed by a review in the nurse-led clinic in 3 months. During the group education sessions patients receive information on the medication, indications, duration, side-effects and complications and follow-up care. The review clinic includes a detailed history on bleeding history, missed doses, concomitant medication along with a brief discussion reminding patients of the risks associated with anticoagulation. The patients creatinine clearance is monitored to ensure appropriate dosage of anticoagulation.

Results: To date, a total of 135 patients have attended the DOAC education session and 90 patients have been reviewed in the nurse-led clinic. Early contact between patients and the anti-coagulation nurse has enabled identification of early complications and clarification of treatment plans.

Conclusions: A nurse-led DOAC clinic is a new initiative which has been operational since May 2016. Considering the limited safety network for patients on DOAC in comparison with VKA-anticoagulants, a nurse-led clinic plays a oivitol role in improving anticoagulation care.

Patient education, monitoring and followup has optimised patient safety for those on DOAC.

PB 1054 | Haemorrheologic and Fibrinolytic Activities of Sickle Cell Subjects in Steady and Crisis States in Abuja Metropolis, Nigeria

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Background: Sickle cell disease (SCD) is a common term for a group of haemoglobinopathies characterized by sickle cell anaemia, sickle beta thalassemia syndromes and other haemoglobinopathies in which haemoglobin S (HbS) is in association with abnormal haemoglobin.
Aims: This study was designed to evaluate the haemoglobin, platelet count, plasma viscosity, fibrinogen and euglobulin lysis time test values of Sickle Cell subjects in steady and crisis states that can be used in monitory the progress of sickle cell anaemia (SCA) patients treatment.
Methods: The study included 50 sickle cell (HbSS) subjects attending Federal Staff Hospital in Abuja metropolis, 40 were Sickle cell patients (HbSS) in steady state while 10 were sickle cell patients (HbSS) in crisis state. Haemoglobin electrophoresis was performed on cellulose acetate strip at alkaline pH 8.6 on all subjects. Euglobulin lysis time was determined using Haugie's method; fibrinogen level was estimated based on Clauss technique; plasma viscosity was determined using Reid and Ugwu method, while haemoglobin and platelet count were determined using Abacus Junior Haematology Analyzer. It was analysed statistically by using student's t-test, analysis of variance and Chi Square tests by statistical software SPSS version 16.0.
Results: There was a significant increase ($P < 0.05$) in the mean value of FIB 6.5±0.37 and significant decrease in PLT count 199±61.4 while no significant difference was seen in the mean values of ELT, RPV and Hb levels in sickle cell anaemia subjects in crisis state when compared to those in steady state. The mean ages in years of all subjects studied were 8.23±1.24 and 8.37±1.22 for HbSS in steady state and 7.68±1.18 HbSS in crisis. No significant difference was observed based on age
Conclusions: Fibrinogen concentration is shown in this study to be higher in crisis state than in steady state. Therefore, fibrinogen assay can be use as a tool to assess and monitor the progress of sickle cell anaemia patient especially those in crisis state.

PB 1055 | Social Work Research and Funding Opportunities: The Canadian Experience Claude Bartholomew, St. Paul´s Hospital, Vancouver, Canada Neale Smith, University of British Columbia

C. Bartholomew[1], N. Smith[2]

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Background: Practice-based evidence is a component of social workers' knowledge base and is essential for providing best care to patients in both community and hospital settings. According to the Society for Social Work and Research, from 1994-2014, social work produced the fewest researchers and doctoral scholars among peer disciplines. We need additional ways to encourage knowledge development. Our aim is to understand the current capacity of social workers in practice contexts throughout Canada - i.e., providing primary patient care services and supports -- to initiate, participate in, and publish research.
Aims: We will describe the extent to which Canadian social workers currently direct or participate in research. Individual-level barriers to this include comfort with subjects like research design or data analysis. Organizational barriers include the scarcity of psychosocial grants, and lack of protected time to engage in research. To address such challenges, better understanding of funding options for research, evaluation and training purposes is needed. In Canada, sources for social work-led research funding can include foundation-based research, private-public partnerships, industry grant requests, academic grants, and individual hospital research. Relevance, availability, advantages and limitations of these different opportunities is discussed.
Methods: To be presented
Results: NA
Conclusions: An absence of social work perspectives eliminates important forms of practice-based evidence, to the detriment of patient care and frustration of professionals' career aims. By utilizing one or more forms of possible funding, we can elevate the participation of social workers in the research realm. For instance, having social work organizations lead in initiating projects and opportunities for skill development. Our aim is to give individuals and organizations a blueprint to increase the body of social work research and enabling potential participants to be successful, through identifying and accessing funding sources.

PB 1056 | Scapular Injection of Low Molecular Weight Heparin in Neonates Receiving Treatment for Thormobosis

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Background: Low molecular weight heparin (LMWH) is used for treatment of various thromboembolic events such as deep vein thrombosis, cerebral sinovenous thrombosis and renal vein thrombosis in neonates. To be quickly absorbed, LMWH must be injected into fat below the skin, typically, into the arm or leg. One of the primary concerns regarding administration of subcutaneous injections is the small amount of subcutaneous fat on the body of a premature neonate, especially the arm and leg. Therefore other sites of administering subcutaneous injections, such as the scapular region, need to be investigated.
Aims: The aim of the trial is to assess parent satisfaction, efficacy and bleeding following the use of scapular injection of LMWH in neonates.

Methods: This is a non randomized, prospective, pilot, cohort study of 5-10 neonates with diagnosed thrombosis who require LMWH for treatment. All patients will be treated using the current standard of LMWH administration (1.5-2.0 mg/kg of LMWH) to achieve a therapeutic anti-Xa level between 0.5-1.0 U/ml. Premature and term neonates aged < 28 days corrected age, with non cerebral thrombosis diagnosed by imaging will be included. Neonates on ventilators or those with physical deformities/anomalies at the injection site will be excluded. Parent satisfaction will be ranked using a Likert scale and will range from 1 (unsatisfied) to 5 (extremely satisfied). Efficacy will be determined by the achievement and maintenance of a therapeutic anti Xa level (0.5-1.0 U/ml) throughout the course of injections. Adverse events such as bleeding or bruising at the injections sites, or palpable areas of firmness at the injection site will be closely monitored and recorded for all study participants. The trial has been approved by the Hamilton Integrated Research Ethics Board.

Results: The study is ready to be implemented pending nursing education.

Conclusions: Conclusions will be made pending results of the trial.

PB 1057 | Empowering a Link Practitioner Network to Enhance Venous Thromboembolism (VTE) Prevention and Anti-coagulation Care

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Background: A link practitioner;

· Acts as a role model and clinical leader for VTE prevention and anti-coagulation.

· Updates their multidisciplinary team on any new evidence and changes to guidelines.

· Conducts audit and quality improvement cycles and sharing best practice.

Aims: The aim of the link practitioner network is to increase the availability of expert knowledge at a clinical level and proactively drive quality improvement. A nurse, midwife or healthcare assistant can act as a link.

Methods: Link practitioners receive extra training in VTE prevention and anti-coagulation in their clinical area so that their role is suited to their context. Bi-monthly update meetings bring links together to share best practices and challenges.

Being motivated to extend their role and promote patient safety is a key requirement for the recruitment of a link. In return their involvement in audit and quality improvement places them in a good position when it comes to career progression. Learning gained can be demonstrated for purposes of re-validation with their relevant professional body.

Support from managers is also vital in the success of the network. Most managers have allocated two staff members to the role so that continuity of expertise is available across shifts.

The network is facilitated by the VTE prevention nurse specialist. Time required is approximately two days a month which includes organizing meetings, feeding back audit results, meeting links in their clinical area and facilitating improvement projects.

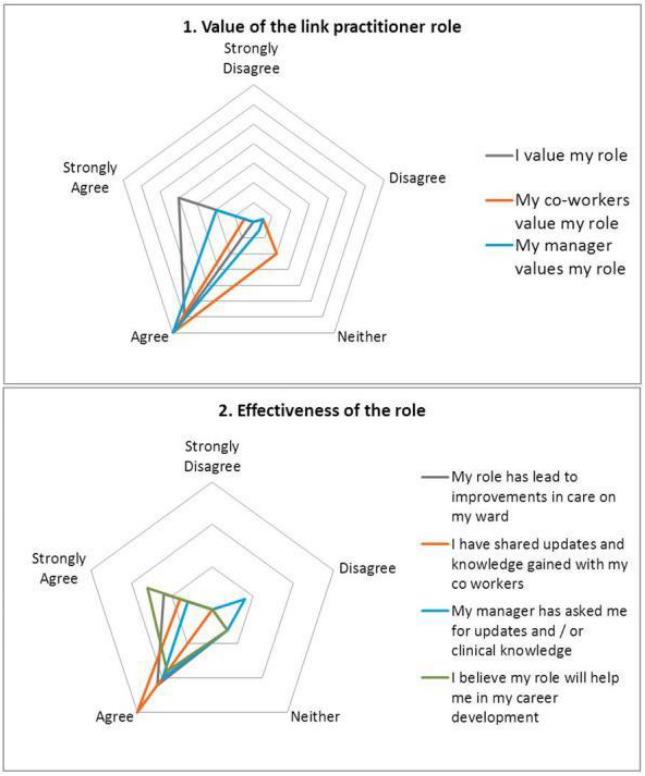

FIGURE 1 Graphs 1 and 2

Results: Graphs 1 and 2 show responses from the link network on the value they perceive it to provide.

Conclusions: There are many factors involved in VTE prevention and anti-coagulation care. These self-reported results show that the link practitioner network has a valuable contribution to improving care.

PB 1059 | Has the Introduction of the Direct Oral Anticoagulants (DOACs) Made Any Difference to Telephone Calls to A Secondary Care Led Anticoagulant Clinic?

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Background: The local secondary care led Anticoagulant Clinic manages all patients within the surrounding community receiving any oral anticoagulant. In 2010 we audited telephone calls to our clinic ansaphone over a 4 week period and we received 747 messages. This was an average of 37.4 messages per day and we had 3,900 registered patients (all taking warfarin).

Aims: The clinic now has 4,700 patients registered and 41% of these are now on a DOAC. The aim of re-auditing telephone calls was to determine whether the content of the phone calls has been influenced by the change in prescribing of oral anticoagulants.

Methods: We audited calls to the clinic ansaphone by retrospectively categorising messages received and logged by the clinic messaging service over a 4 week period in October 2016.

Results: In total we had 631 calls over the 4 week period. This equated to 31.55 calls per day.

We analysed the reason that patients were contacting us. In 2010 the most common reason to contact the clinic was patients leaving a home test INR results (18% of all calls). In 2017 this was still the most common reason to call us and now represented 46% of all calls to the clinic. The next most common reasons to contact us were calls from the hospital wards regarding planned discharges or for inpatient advice (6%) and for queries regarding peri-operative advice (6%). Calls relating to warfarin dose query dramatically reduced from 105 to 26 and change in INR retest date from 37 to 19.

Queries regarding DOACs were low, with only 21 calls in total and represented just 3.3% of calls to our clinic.

Messages left relating to bleeding were low in 2010 (1.3%) and was again low in 2016 with 8 patients (1.3%) contacted the clinic regarding bleeding on warfarin and 3 (0.48%) on a DOAC (1.75% in total)

Conclusions: It is clear that the implementation of DOACs has influenced the type of calls received by the Anticoagulant Clinic. There were far fewer calls relating to queries such as: change in INR retest date and query re dose of warfarin.

PB 1061 | When the Absence of Prophylaxis in Patients with Hemophilia, Surgery is Inevitable

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Background: People with hemophilia due to the deficit of coagulation factors often bleed into joints, and the most common companion in their lives is hemophilic arthropathy. Lifetime propensity to, leads to passivity and different levels of disability, depending on the degree of factor deficiency which is is a major social problem. Abundant bleeding in males occur as a result of blood coagulation disorders and it can be: spontaneous and in minor injuries or interventions. As a standard for surgical procedures, compensation of the missing factor is 100% necessary. In the care and treatment of patients, where the complex surgical interventions are planned, nurse participation and a multidisciplinary approach are indispensable.

Aims: Emphasize the importance of a multidisciplinary approach, with special attention to the work of nurses, when complex procedure of knee joint replacement in patients with hemophilia is planned.

Methods: Case report.

Results: The use of prophylactic treatment has improved chances of reaching adulthood without the development of arthropathies. Patients who did not received prophylaxis in childhood are now adults with problems such as different levels of disability, or most commonly Haemophilus arthopathies.

Conclusions: In order to prevent potential bleeding, compensation of the missing factor is necessary before every minor or major surgical intervention.

Together with hemostatic screening and observation of hematologist, participation of trained hematology nurse, along with other members of the multidisciplinary team is essential.

Skill for specific application of coagulation factors as well as for specific care and health education of patients, nurses acquire through continuous education and work in the hematology team.

PATHOGENESIS OF THROMBOEMBOLISM

PB 614 | Efficacy of Dabigatran Etexilate in a Cancer Associated Thrombosis Murine Model

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Background: Recurrent venous thromboembolism (VTE) is a common complication of cancer. Bleeding is particularly problematic due to the use of anticoagulants and the frequent need for surgical interventions. The treatment of cancer-associated thrombosis (CAT) consists primarily of low molecular weight heparin (LMWH) given parentally. Recently, direct oral anticoagulants (DOACs) have become available. DOACs require less dose adjustment and are given orally. A recent metanalysis of cancer patients treated with DOACs for VTE demonstrate that DOACs were as effective and safe as warfarin for use in cancer patients. However, no studies have directly studied DOACs in cancer patients with VTE nor compared DOACs with LMWH with selective inclusion of cancer patients only.

Aims: Compare the efficacy of dabigatran and LMWH in CAT and VTE murine models.

Methods: Thrombus formation is induced in the inferior vena cava (IVC) by partial flow restriction in wild type mice. For the CAT model, mice are injected with murine B lymphoma cells (Eμ-Myc cells). After 6 days, thrombosis is then induced as described above. Treatment with either dalteparin (200 IU/kg/day subcutaneously) or with dabigatran (45 mg/kg) by oral gavage is started 24h after thrombosis induction.

Results: Both dalteparin and dabigatran are effective in reducing the size of the thrombus in our VTE and CAT murine models. In our VTE model, dalteparin reduces thrombus size by 32% as compared to 18% for dabigatran, whereas, in our CAT model, dalteparin reduces thrombus size only 11% as compared to 24.9% with dabigatran.

Conclusions: Our data suggest that dalteparin is more effective when compared to dabigatran in VTE whereas dabigatran is superior in CAT. As a proof of principle, the above described models can be further employed to study the pathophysiology of VTE and CAT and provide biologic plausibility for pursuing further studies for using NOACs, specifically, dabigatran, in CAT.

PB 615 | Incidence of Venous Thromboembolism in Cancer Survivors: The Scandinavian Thrombosis and Cancer (STAC) Cohort

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Background: Cancer patients have increased risk of venous thromboembolism (VTE). The risk is highest shortly after diagnosis and decreases afterwards. Few studies have investigated whether the incidence of VTE reaches that of the general population in long-term cancer survivors.

Aims: To compare the incidence of VTE in long-term cancer survivors with a matched control group in a population based cohort study.

Methods: The STAC cohort includes person-time data of 144,952 Danes and Norwegians. Informed consent at study enrollment between 1993 and 1997 permitted linkage to medical databases by the unique personal civil registration number. The Danish and Norwegian National Cancer Registers provide data of cancer diagnoses coded with the ICD-10 system. Incident symptomatic VTEs during follow-up until 2012 were objectively confirmed. All cancer-exposed subjects (excluding non-melanoma skin cancers), and five age-, sex- and nationality-matched controls, free of cancer at index date, were included in the study. Study entry was date of cancer diagnosis, and time since cancer diagnosis/index date was the time axis. We calculated incidence rates (IR) of VTE occurring later than five years after cancer diagnosis and compared the incidence of VTE in cancer-survivors to controls by incidence rate ratios (IRR).

Results: Of 14.272 diagnosed with overt cancer, 3.298 were alive five years after cancer diagnosis. Among these, 63 VTEs were observed later than five years since cancer diagnosis (IR 4.7 pr. 1000 p-y, 95% CI 3.7 - 6.1), which was higher than in the control group (IRR 1.4, 95% CI 1.1 - 1.9). Relative risks of VTE later than five years after cancer diagnosis varied with cancer type; from IRR 3.0 (95% CI 1.3 to 6.7) in hematologic malignancies to IRR 0.8 (95% CI 0.4 to 1.8) in colorectal cancer.

Conclusions: The incidence of VTEs more than five years after cancer diagnosis remains slightly elevated relative to the general population, but varies by cancer type.

PB 616 | Incidence of Occult Cancer in the Year Following a Second Unprovoked Venous Thromboembolism Event

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Background: Between 3.2% and 10.0% of patients presenting with unprovoked venous thromboembolism (VTE) will be subsequently diagnosed with cancer, with the highest risk in the first year after the diagnosis of VTE. Several subgroups of patients with unprovoked VTE might benefit from systematic cancer screening, such as those with a previous unprovoked VTE event.

Aims: The aim of this study was to determine the one-year incidence of cancer following a second unprovoked VTE event.

Methods: The EDITH cohort is a cohort of patients followed at the Brest University Hospital for an objectively proven, symptomatic, deep vein thrombosis of the lower limbs and/or pulmonary embolism. For this analysis we used data from patients who experienced two unprovoked symptomatic VTE events. We selected only patients for whom time spanned between the first and recurrent VTE event was less than 2 years.

Results: Between May 1st, 2000 and December 31st, 2013, 201 patients with two documented symptomatic unprovoked VTE events that occurred in a 2-year's time were included in the EDITH cohort. Patients' mean age was 66.5±16.3 years, 101 (50.2%) were men. Of the 201 VTE recurrences, 21 (10.4%) occurred on therapeutic anticoagulation. In total, 15 cancers were diagnosed in the year following the second unprovoked VTE event (7.46% of the study sample): one cancer was patent at the time of the second VTE event, 8 were diagnosed to the 21 patients on therapeutic anticoagulation at the time of VTE recurrence (cumulative incidence: 30.77% (95% CI 15.06-56.36)), and 6 were diagnosed to the 180 patients off anticoagulation at the time of VTE recurrence (cumulative incidence: 4.69% (95% CI 2.43-9.35)) (p< 0.001 by log-rank test).

Conclusions: In this hospital-based cohort, the 1-year incidence of occult cancer in patients with a second unprovoked VTE event was low suggesting systematic cancer screening should not be performed. However, this incidence was high when VTE recurs on full anticoagulation.

PB 617 | Risk Factors of Occult Malignancy in Patients with Unprovoked Venous Thromboembolism: Results from the MVTEP Study

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Background: Venous thromboembolism (VTE) can occur as the first manifestation of an underlying occult malignancy. It remains unclear whether or not a better selection of high risk patients might lead to more efficient occult cancer screening strategies.

Aims: To assess the predictors of occult malignancy diagnosis in a population of patients with unprovoked VTE.

Methods: Univariate analyses were performed to assess the effect of candidate predictors on occult cancer detection in patients enrolled in a prospective, multicenter, randomized, controlled study (MVTEP study) whose primary aim was to compare a limited screening strategy with a strategy combining limited screening and FDG PET/CT in patients with unprovoked VTE.

Results: Between March 3, 2009, and August 18, 2012, 399 patients were included. Five patients withdrew consent and refused the use of their data, and no VTE was confirmed in 2 patients who were excluded from this analysis. A total of 25 (6.4%) out of the 392 analysed patients received a new diagnosis of malignancy during the 2-years follow-up. Age > 50 years (p=0.01), male gender (p=0.04), leukocytes count (p=0.01), and platelets count (p=0.03) were associated with occult cancer detection. Previous VTE and current smoker status were not associated with occult cancer diagnosis (p>0.05).

Conclusions: Demographic characteristics (age and sex), and laboratory tests (leukocytes and platelets) may be predictors of occult cancer detection in patients with unprovoked VTE.

PB 618 | Impact of Tumor Grade on the Risk of Venous Thromboembolism in Cancer; the Scandinavian Thrombosis and Cancer (STAC) Cohort

I.L. Gade[1], S.K. Brakkan[2,3], I.A. Naess[4], J.-B. Hansen[2,3], F.R. Rosendaal[5], S.C. Cannegieter[5,6], K. Overvad[7,8], H. Jensvoll[3], J. Hammarstrom[9], O.V. Gran[2], H.H. Eriksen[10], S.R. Kristensen[1,11], M.T. Severinsen[1,12]

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Background: Cancer is associated with an increased risk of venous thromboembolism (VTE). The risk differs between cancer types, possibly partly with tumor grade. Tumor grade is associated with aggressiveness, and therefore the risk of VTE should be investigated in frameworks that treat death as a competing event.

Aims: To investigate the association between tumor grade and risk of VTE in a population based cohort; the Scandinavian Thrombosis and Cancer (STAC) Cohort.

Methods: The STAC cohort consists of data from three large population based cohort studies. Enrolment ran from 1993 through 1997. 144,952 participants free of previous cancer and VTE were followed prospectively; date of last follow-up for VTE was December 31, 2012. Information on tumor grade was obtained from the Norwegian Cancer Registry and the Danish National Pathology Registry, respectively providing the sixth digit of the ICD-O-3 code and SNOMED codes. Cancers were divided into low grade or high grade. Cumulative incidence (CI) of patients with VTE, treating death as competing event expressed risk of VTE. Cancer stage, age and sex were included as possible confounders in the Fine-Gray regression model, low grade was reference. Time since cancer diagnosis was used as time axis.

Results: Excluding subjects with VTE before cancer and haematological malignancies left 13,140 with a diagnosis of cancer, 29% had low grade tumors, 18% had high grade tumors. In 54% information on grade was not available from the registers. In total, 416 VTEs were observed during follow-up. The risk was highest in high grade tumors, 5-year CI 4.2 (3.4-5.1) versus 2.5 (2.0-3.1) in low grade tumors (figure 1).

The crude sub-distributional hazard ratio (SHR) for VTE in high grade was 1.5 (1.2 to 2.0), adjusted SHR in high grade tumors was 1.3 (1.0 to 1.8), this association was, however, not uniform in analysis stratified by cancer types.

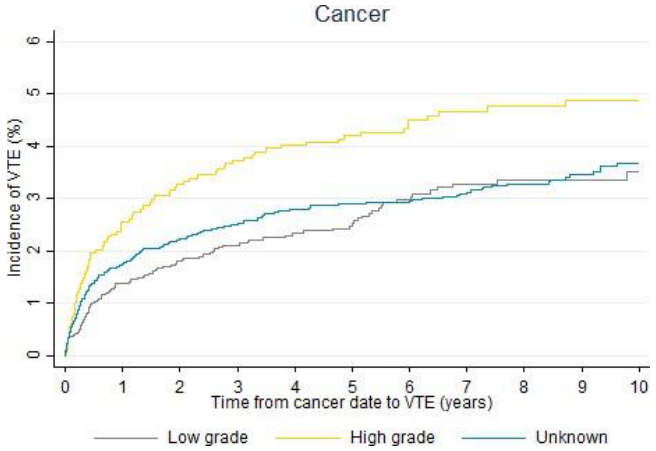

FIGURE 1 Cumulative Incidence of VTE in cancer

Conclusions: High grade tumors are in general associated with a higher risk of VTE than low grade tumors.

PB 619 | Thromboembolic Events in Cancer Patients on Active Treatment with Cisplatin-based Chemotherapy; Another Look!

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Background: The risk of thromboembolic events (TEE) is higher among cancer patients; especially so in patients undergoing chemotherapy. Cisplatin-based regimens has been widely used and claimed to be associated with high TEE, beyond what is usually seen in clinical practice.

Aims: In this study we report on the incidence of TEE in patients on active cisplatin-based chemotherapy and its relation to variables like primary tumor, disease stage and Khorana risk score.

Methods: Medical records and hospital database were searched for all adult patients treated with any cisplatin-based regimen for any kind of cancer. Thrombosis was considered cisplatin-related if diagnosed any time after the first dose and up to 4 weeks after the last. Khorana risk assessment model was performed on all.

Results: Between 2007 and 2015, a total of 1677 patients (65.5% males, median age: 50 years) treated with cisplatin-based regimens were identified. Head & neck (22.9%), lung (22.2%), lymphoma and gastric (11.4% each) were the commonest primary tumors. Thromboembolic events were reported in 110 (6.6%); highest in patients with gastric cancer (20.9%) and lowest in head & neck cancers (2.3%). Thrombosis included DVT in 69 (62.7%), PE in 18 (16.9%) and arterial thrombosis in 17 (15.6%). Majority (69.1%) of the patients had stage IV disease and only 10% had stage I or II.

High Khorana risk score, stage IV disease and gastric as the primary tumor but not age, sex or type of cisplatin combination regimen were associated with significantly higher rates of TEE.

Further analysis highlighting the role of mean cumulative cisplatin dose, presence of central venous catheters and other risk factors will be presented.

Conclusions: TEE events among cancer patients on active cisplatin-based chemotherapy are not as common as previously reported. However, higher TEE rates were encountered in patients with gastric cancer, stage IV disease and in those with high Khorana risk score which may justify a recommendation to offer such patients thrombosis prophylaxis.

PB 620 | Low Discriminating Power of the Modified Ottawa VTE Risk Score in a Cohort of Patients with Cancer from The RIETE Registry

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Background: Treatment of patients with cancer-associated venous thromboembolism (VTE) remains a major challenge. The modified Ottawa score is a clinical prediction rule evaluating the risk of VTE recurrences during the first six months of anticoagulant treatment in patients with cancer-related VTE.

Aims: We aimed to validate the Ottawa score using data from the RIETE (Registro Informatizado Enfermedad TromboEmbolica) registry.

Methods: We assessed the prognostic value of the modified Ottawa score in predicting the likelihood of VTE recurrences and overall mortality during the first six months of anticoagulant treatment.

Results: A total of 11,123 cancer patients with VTE were included in the analysis. According to modified Ottawa score, 2,343 (21%) were categorized at low risk for VTE recurrences, 4,525 (41%) at intermediate risk, and 4,255 (38%) at high risk. Overall, 477 episodes of VTE recurrences were recorded during the course of anticoagulant therapy, with an incidence rate for low, intermediate, and high risk groups of 6.88% (95% CI 5.31-8.77), 11.8% (95% CI 10.1-13.6), and 21.3% (95% CI 18.8-24.1) patient-years, respectively. Overall mortality had an incidence rate of 21.1% (95% CI 18.2-24.3), 79.4% (95% CI: 74.9-84.1), and 134.7% (95% CI: 128.3-141.4) patient-years, respectively. The accuracy and discriminating power of the modified Ottawa score for VTE recurrence was modest, with low sensitivity and specificity and a C-statistics of 0.58 (95% CI: 0.56-0.61). Similar results were obtained for overall mortality with low sensitivity and specificity and a C-statistic slightly better (0.65; 95%CI: 0.64-0.66).

Conclusions: Our analysis showed that the modified Ottawa score is not able to accurately predict VTE recurrence and overall mortality among patients with cancer-associated thrombosis, thus hindering its use in clinical practice. It is time to define a new score including other clinical predictors.

PB 621 | End of Life Management of Venous Thromboembolism in Metastatic Cancer: Current Practice, Place of Death and Complications

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Background: Guidelines recommend cancer associated thrombosis (CAT) is treated with low molecular weight heparin (LMWH) for three to six months. Indefinite anticoagulation should be considered for patents with ongoing active/ metastatic cancer. There are no data on the management of CAT at the end of life and no guidance when to stop anticoagulation as death approaches.

Aims: To review current practice at the end of life care for advanced cancer patients who have been anticoagulated on LMWH for venous thromboembolism (VTE).

Methods: Prospective data was collected on patients attending a regional CAT service and cross-referenced with death notifications. Hospital, hospice and community patient's healthcare records were reviewed to evaluate end of life care within the context of VTE management.

Results: 214 patients with metastatic cancer and a history of VTE died over a 2-year period. 98 (46%) died at home, 59 (27%) in a hospice, 53 (25%) in acute hospital and 4 (2%) in a community hospital.

Of these 108 (50%) continued LMWH until death, 23 (11%) up to 7 days prior to death, 23 (11%), 1 week to 1 month 29 (13.5%), over one month 40 (18%).

Clinically relevant non-major (CRNM) bleeding occurred in 9/ 131 (7%) of the patients who continued LMWH to death or 7 days up to death.

Conclusions: The majority of CAT patients with metastatic disease remain anticoagulated up to or within days of death. Whilst symptoms of pulmonary emboli are controlled as death approaches, this comes with a 7% incidence of CRNM bleeding.

PB 622 | Outcomes of Extended Anticoagulant Treatment of Venous Thromboembolism in Patients with Cancer

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Background: Venous thromboembolism (VTE) is a common complication in patients with cancer. The optimal duration of anticoagulant therapy in cancer-associated thrombosis is not known, but anticoagulation beyond 3 months should be considered for those with active cancer and/or those receiving anticancer treatment. Data on extended anticoagulant therapy in cancer patients with VTE is scarce.

Aims: To establish safety and efficacy of extended outpatient treatment of cancer patients with VTE.

Methods: Anticoagulant treatment was assessed in 290 consecutive ambulatory treated cancer patients (age 68 ± 12 years, 134 (46%) women) with objectively confirmed VTE one month, six months and two years after VTE diagnosis. Bleeding events (major and minor), recurrent VTE events and death were identified by reviewing patients´ medical charts and the data from the anticoagulant therapy management computer program.

Results: Low molecular weight heparin (LMWH) was used for long-term treatment (6 months) in 243 (83%) patients. Vitamin K antagonists (VKA) were used in 130 (64%) and LMWH in 44 (22%) patients for extended treatment. Proportion of all complications during anticoagulant therapy is shown in figure 1. The majority of bleeding and recurrent VTE events occurred during the first 3 months of therapy with LMWH and in the first year of therapy with VKA. Stage of cancer was the only independent prognostic factor for developing a recurrent VTE (p< 0,01). In the two-year period 55% of patients died. In 87% the cause of death was cancer. Bleeding event was fatal for 4 (3 on LMWH, 1 on VKA) patients and pulmonary embolism was fatal for 4 (2 on LMWH, 2 on VKA) patients while receiving anticoagulant therapy. Survival rate was lower in patients who suffered recurrent VTE on VKA.

Conclusions: The risk of bleeding and VTE recurrence is the highest in the first months of anticoagulant therapy in cancer patients. In patients without complications, carefully monitored extended outpatient therapy seems to be beneficial.

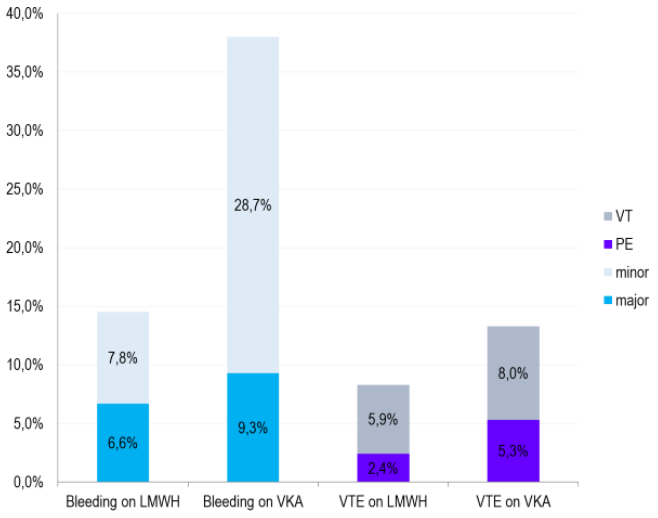

FIGURE 1 Proportion of complications during the two-year follow up (VT-vein thrombosis, PE-pulmonary embolism)

PB 623 | Joint Effects of Body Height and Cancer on the Risk of Incident Venous Thromboembolism: The Tromsø Study

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Background: Tall stature is associated with risk of both venous thromboembolism (VTE) and cancer, and VTE is a common complication in patients with malignancy. No previous study has investigated the joint effects of body height and cancer on the risk of VTE.

Aims: To study the joint effects of body height and cancer on the risk of incident VTE using a large population-based cohort.

Methods: Subjects (n=30 405) were recruited from the forth (1994-95) fifth (2001-02) and sixth (2007-08) surveys of the Tromsø Study. Validated VTE events and cancer diagnoses were registered until December 31, 2012. VTEs occurring 6 months before to 2 years after a cancer diagnosis were defined as cancer-related. Cox regression was used to determine age- and sex adjusted hazard ratios (HR) for VTE by cancer status and quartiles (Q) of height (Q1: ≤163cm, Q2: 163.1-170cm, Q3: 170.1-177cm, Q4: 177.1-207cm).

Results: There were 2981 incident cancer diagnoses and 658 VTE events, of which 175 were cancer-related, during a median follow-up of 17.5 years. The risk of VTE increased across quartiles of body height (p for trend < 0.001) with a 2-fold higher risk (HR 2.1, 95% CI 1.4-3.0) in Q4 compared to Q1. A similar trend was observed for VTE in cancer (HR Q4 vs. Q1: 1.6 95% CI 0.9-2.9). In subjects with cancer, the VTE risk increased from 17-fold (HR 17.7, 95% CI 13.0-24.0) in Q1 to 26-fold (HR 26.0, 95% CI 17.1-39.5) in Q4, when compared to non-cancer subjects in Q1.

Conclusions: Our findings demonstrated a dose-response relationship between body height and VTE in the general population and in cancer patients, suggesting a joint effect of malignancy and tall stature on the risk of VTE.

PB 624 | Patients with Cancer-associated Thrombosis Benefit from Anticoagulant Treatment Beyond 6 Months

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Background: Guidelines recommend treatment with low-molecular-weight heparin (LMWH) for 3-6 months in patients with cancer-associated thrombosis (CAT), and prolonged treatment during active cancer. Data on safety and efficacy of anticoagulant therapy beyond 6 months in CAT is scarce.

Aims: To compare the incidence of recurrent VTE, bleeding and death after 6 months between patients with CAT treated for 180±30 days and those treated >210 days.

Methods: We included patients with active cancer enrolled in the RIETE registry between January 2009 and January 2016. RIETE is an international prospective registry of patients with symptomatic acute VTE, approved by a medical ethics committee. All patients provided informed consent. Patients treated with anticoagulants for 180±30 days were compared to those treated >210 days using propensity score matching. Follow-up started after cessation of treatment in the group treated for 180±30 days, and after 180 days of treatment in the other group.

Results: A total of 3,240 patients were included, of which 598 (18.5%) treated for 180±30 days. The distribution of cancer types differed, with higher numbers of breast and prostatic cancer in the prolonged treatment group. Metastatic disease was equally present (37% vs. 39%).

Initial treatment consisted mostly of LMWH (92-97%). LMWH was also preferred for longterm treatment (54-66%).

During follow-up (median 174 and 130 days, respectively) , the rates of recurrent VTE and death were higher in the patients treated for 180±30 days. The rate of bleeding was higher in the patients with prolonged treatment. After propensity score matching (including 2,727 patients in a 4:1 ratio), the hazard ratios for recurrent VTE and death favoured prolonged treatment (HR for recurrent VTE 0.20 (0.11-0.35) and death 0.68 (0.53-0.89)). The HR for major bleeding was 1.93 (0.64-5.82).

Conclusions: After 180 days of anticoagulant therapy, patients with prolonged treatment had lower risk of recurrent VTE and death as compared to patients treated for 180±30 days.

PB 625 | Impact of Time Since Diagnosis and Mortality Rate on Cancer-associated Venous Thromboembolism in a General Population - The Scandinavian Thrombosis and Cancer (STAC) Cohort

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Background: Venous thromboembolism (VTE) is a common complication in cancer patients, and it has been suggested that aggressive cancers such as pancreatic, lung and brain cancers harbor the highest risk of VTE. However, competing risk by death may result in overestimation of VTE risk in cancers with high mortality.

Aims: To estimate the risk of VTE by cancer sites, accounting for the differential mortality between cancers.

Methods: The Scandinavian Thrombosis and Cancer (STAC) cohort is a merging of three Scandinavian population-based cohorts (n=144,952). Subjects were included in 1993-1997 and followed up to 2007-2012. We assessed incidence rates, cause-specific hazard ratios (HR) and sub-distribution hazard ratios (SHR, i.e. accounting for competing risk by death) for all cancers combined and by cancer sites according to time-intervals since cancer diagnosis.

Results: During follow-up, 14,272 subjects developed cancer and 567 of these had a cancer-related VTE. In cause-specific analyses, the risk of VTE was highest in the first 6 months after cancer diagnosis (HR 17.5, 95% CI 15.1-20.3), and declined rapidly thereafter. However, when mortality was taken into account, the risk were similar in the period 6 months before (SHR 4.8, 95% CI 3.6-6.4) and 6 months after (SHR 4.6, 95% CI 3.9-5.4) cancer diagnosis. Cancers with a high mortality rate had a high cause-specific HR of VTE, but in competing risk analyses the range of the 2-year cumulative incidences of VTE for all cancer sites was substantially narrowed (from 1-10% in conventional analyses to 1-4% in competing risk analyses).

Conclusions: The risk of VTE by different cancer sites was substantially influenced by the mortality rate and the time since cancer diagnosis. Our findings may suggest that the cancer itself is a major contributor to VTE risk and that competing risk by death should be taken into account when predicting VTE risk in cancer.

PB 626 | Quality of Life Related to Health in Cancer Associated Thrombosis. Pilot of Qca Study

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Background: Venous thromboembolism (VTE) is a disease with high morbidity and mortality, but there is little evidence about health-related quality of life (HRQL) in oncological patients.

Aims: Our objective was to evaluate the HRQL in cancer associated thrombosis (CAT) and compare with controls (cancer without thrombosis).

Methods: Prospective, multicenter, national, case-control study. Cases: patient with symptomatic acute CAT. Controls: patient with cancer without VTE. All patients were given EQ-5D-3L questionnaire and an integrated system for assessing the health-related quality of life of cancer patients (EORTC QLQ-C30 questionnaire). In CAT, questionnaires were passed 1 month after VTE.

Results: From January 2014 to January 2017, 261 patients were evaluated in 13 hospitals, of which 209 (80%) completed the questionnaires. The mean age was 60 +/- 14 years, with 59% of men. We analysed 161 controls and 99 cases, and 63% deep vein thrombosis, 25% pulmonary embolism and 12% both. The most frequent tumour locations were: lung (30%), lymphoma (20%), gynaecological (14%), pancreas (8%) and gastric (6%). Sixty per cent had metastases and 87% presented ECOG 0-1. Except for the Anxiety/Depression subscale, cases (CAT patients) had substantially lower QoL than controls. In the EORTC QLQ-C30 questionnaire, the cases presented, statistically significant, worse Global Health Status (median: 50 vs. 67), Physical Function (median: 67 vs. 87), Role Function (58 vs. 83), Fatigue (50 vs. 33) and Pain (33 vs. 17) than controls.

Conclusions: In oncological patients, the presence of VTE had a negative impact in HRQL, even 1 month after thromboembolic event.

PB 627 | Differential Impact of Triggers on the Risk of Venous Thromboembolism in Subjects with and without Cancer - Results from a Population-based Case-crossover Study

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Background: Cancer is a strong risk factor for venous thromboembolism (VTE). Recent work has demonstrated that the risk of VTE immediately before and after cancer diagnosis is equal when competing risk by death is taken into account. This suggests that cancer itself, rather than it´s treatment (e.g. surgery) and complications (e.g. immobilization), plays a greater role on VTE risk.

Aims: To investigate the impact of various VTE-triggers in subjects without and with cancer.

Methods: We conducted a large case-crossover study of VTE patients (n=707) recruited from the general population (The Tromsø study). All hospitalizations and triggers were registered within each subject during the last 90-day period before the VTE diagnosis and in four preceding 90-day comparison periods. A 90-day washout period between the comparison- and VTE-periods was used to avoid potential carry-over effects. Conditional logistic regression was used to obtain odds ratios (ORs) for each trigger by cancer status. Each patient served as their own control allowing for within-subject adjustment for fixed covariates.

Results: There were 172 (24.3%) patients with cancer. When comparing the VTE-period to the control periods, the impact of various triggers was greater in non-cancer than in cancer. Triggers such as major surgery (OR 12.3, 95% CI 7.9-19.1 vs. OR 0.8, 95% CI 0.4-1.6), immobilization (OR 69.4, 95% CI 41.9-114.8 vs. OR 22.1, 95% CI 10.0-48.8), infection (OR 25.4, 95% CI 17.3-37.3 vs. OR 10.9, 95% CI 5.9-20.1), central venous catheters (OR 22.4, 95% CI 7.7-64.9 vs. OR 11.0, 95% CI 4.0-30.1) and transfusions (OR 16.4, 95% CI 7.6-35.4 vs. OR 9.9, 95% CI 4.4-22.3) had greater impact on the VTE risk in non-cancer compared to cancer patients, respectively.

Conclusions: We found that established triggers for VTE had a stronger impact on the VTE risk in non-cancer than in cancer patients. Our findings reinforce the concept that cancer itself outweighs the impact of traditional triggers on the VTE risk in cancer.

PB 628 | Venous Thromboembolism in Patients with Lymphoma

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Background: There is limited data regarding the prevalence and risk factors of VTE in Asian patients with lymphoma.

Aims: To determine the prevalence, the predictive factors, and the clinical outcomes of VTE among Thai patients with lymphoma.

Methods: We reviewed a cohort of 1,769 lymphoma patients diagnosed according to the World Health Organization classification and treated at Ramathibodi Hospital, Bangkok, Thailand between October 2005 and September 2015.

Results: The prevalence of VTE was 5.1% (91 patients). The most common site of VTE was deep vein thrombosis of leg (53.9%). In 41 patients (45.1%), thrombosis occurred during treatment or up to 3 months after completion of therapy whereas in 35 patients (38.4%) thrombosis was diagnosed prior to therapy. Median time from lymphoma diagnosis to VTE occurrence was 38 days. Of patients with VTE vs those without VTE, 51.6% vs 50.2%, respectively, were female (p=0.83) and the mean age was 61±15 vs 55±19 years (p=0.01), respectively. Type of lymphoma in corresponding groups included B-cell Non-Hodgkin lymphoma (NHL) (93.4% vs 76.6%), T-cell NHL (5.5% vs 9.6%) and Hodgkin disease (HD) (1.1% vs 9.0%), p≤0.01. In the multivariate analysis, relapsed disease, poor performance status, bulky mass ≥10 cm and pre-chemotherapy platelet count ≥350 x 10^9/L were independently predictive factors of VTE (Table)

TABLE 1 Logistic regression analysis of predictive factors of VTE in lymphoma patients

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age >60 years	1.6 (1.0-2.4)	0.03	1.6 (0.9-3.0)	0.11
B-cell type NHL	4.3 (1.9-10.0)	<0.01	2.7 (0.9-7.3)	0.06
Relapsed disease	2.6 (1.7-4.0)	<0.01	3.4 (1.9-6.2)	<0.01
B-symptoms	1.8 (1.1-2.8)	<0.01	1.5 (0.8-2.9)	0.22
Poor performance status (ECOG ≥2)	1.9 (1.1-3.2)	0.01	2.1 (1.0-4.1)	0.04
Extranodal involvement >1 sites	1.9 (1.2-3.0)	<0.01	1.1 (0.5-2.0)	0.87
Bulky disease (Maximum diameter ≥10 cm)	2.9 (1.8-4.8)	<0.01	2.8 (1.5-5.2)	<0.01
Prechemotherapy platelet count ≥350 x 10^9/L	1.8 (1.1-3.0)	0.03	2.2 (1.1-4.1)	0.02

whereas high international prognostic index (IPI) score >3 (OR 1.5) and Khorana score ≥3 (OR 1.2) were not. The mortality rate in the patients with VTE and without VTE was 25.3% and 11.2%, respectively and the two-year overall survival was 65.9% vs 85.5%, respectively (p< 0.01).

Conclusions: The prevalence of VTE in Thai patients with lymphoma is not low. Relapsed disease, poor performance status, bulky mass, and elevated pre-chemotherapy platelet count are independent predictive factors of VTE. Khorana score is not predictive for VTE in Thai

patients. Predictive risk score for Thai patients with lymphoma will be further developed.

PB 629 | Cancer Associated Thrombosis in Cancer Patients Receiving Chemotherapy or Hormonal Therapy in an Ambulatory Setting

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Background: Venous thromboembolism (VTE) represents one of the most important causes of morbidity and mortality in cancer patients. Patients with the highest 1-year incidence rate are those with advanced disease of the brain, lung, uterus, bladder, pancreas, stomach and kidney.

While receiving chemotherapy, cancer patients have a 7-fold risk of developing VTE as compared with other patients without cancer. Chemotherapy can increase the risk of VTE by four mechanisms: acute damage to vessel walls, non-acute damage of the epithelium, a decrease in natural anticoagulant inhibitors (reduced levels of protein C, S and anti-thrombin 111) and platelet activation.

Aims: To identify if a specific cancer type or particular chemotherapy drug or regime predisposed patients to developing VTE.

Methods: We observed patients presenting with a confirmed VTE and a diagnosis of cancer between February 2013 and December 2016 who received either chemotherapy or hormonal therapy in an ambulatory setting.

Results: In total there were 111 VTE events (1.2%) out of total of 9332 patients being treated for cancer.The type of cancer with the highest number of VTE events was colorectal (27/111 VTE events) but in terms of the % incidence relating to total numbers of cancer patients receiving treatment the highest incidence of VTE was seen in patients with brain tumours (3/55 - 5.45%).

Of the 233 chemotherapy agents administered, Alkylating agents represented 36.5% of the drugs administered which had an association with VTE.

Diagnosis of VTE varied with Pulmonary Embolism (PE) representing 69/111 (62%), Proximal DVT 18/111 (16.2%) and distal DVT 23/111 (20.7%).

Mortality rate was 66/111 patients (59.4%) with 18/66 deceased within 1 month of diagnosis of VTE.

Conclusions: In our experience incidence of cancer associated VTE was low. Brain tumour and the use of alkylating agents had the highest associated risk of VTE. Poor prognosis of VTE and cancer may reflect both a combination of fatal complications (PE) and higher disease aggressiveness.

TABLE 1 VTE events per cancer diagnosis from February 2013 to December 2016

Cancer Type	Number of VTE events	Total number of patients treated	% incidence of VTE
Colorectal	27	853	3.16
Breast	18	1220	1.48
Haematological	16	550	2.91
Lung	14	542	2.58
Urological	11	1289	0.85
Gynaecological	11	358	3.07
Upper GI	11	492	2.23
Brain	3	55	5.45
Total	111	9332	1.19

PB 630 | Long-term Impact on Mortality of Isolated Superficial Vein Thrombosis in Patients with Active Cancer

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Background: Cancer patients who develop venous thromboembolism (VTE) are at higher risk of death than cancer patients without VTE. However, the impact of isolated superficial venous thrombosis (iSVT) (i.e. without concomitant deep vein thrombosis or pulmonary embolism) on the prognosis of cancer patients is unknown.

Aims: To determine the impact on mortality at 3 years of iSVT in patients with active cancer.

Methods: Using data from the OPTIMEV prospective, multicentre, observational study, we compared at 3 years the incidence of death in 34 patients with cancer-related iSVT to 68 cancer patients with

TABLE 1 Comparison of incidence of death during 3-years follow-up between different study populations

	Incidence %PY [95%CI], (n)	Crude Hazard Ratio HR [95% CI]	Adjusted Hazard Ratio aHR [95% CI]
Cancer-related iSVT	23.2 [14.2 - 37.9] (16)	Ref	Ref
iSVT without cancer	1.3 [0.5 - 3.6] (4)	0.1 [0.0 - 0.2]	0.1 [0.0 - 0.2]
Cancer without VTE	27.1 [20.6 - 35.8] (50)	1.1 [0.6 - 2.0]	1.0 [0.6 - 1.8]
Cancer-related proximal DVT	42.2 [31.2 - 57.1] (42)	1.7 [0.9 - 3.0]	1.5 [0.8 - 2.6]
Varicose veins status	-	-	0.6 [0.4 - 1.0]

isolated proximal DVT (matched 1:2 on age and sex), to 102 patients with iSVT without cancer (matched 1:3 on age and sex), and to 102 patients with cancer but without VTE (matched 1:3 on age and sex). All VTE events were confirmed by objective tests and all iSVT patients underwent a systematic whole leg compression ultrasound to exclude concomitant DVT. Survival analysis was conducted using Cox proportional hazards model with populations and varicose veins status as covariates.

Results: Patients with cancer-related iSVT had a significantly higher risk of death than patients with iSVT without cancer (23.2% per patient-year (PY) vs. 1.3% PY, p< 0.001), a lower risk of death than patients with proximal DVT (42.2%PY, p=0.2) and a similar risk of death than patients with cancer but without VTE (27.1%PY, p=0.9) (Table 1). Presence of varicose veins on physical exam was associated with a statistically significant lower risk of death (p=0.05). When restricting our analysis to patients without a history of VTE, results remained similar in magnitude.

Conclusions: Unlike for DVT or PE, occurrence of iSVT does not seem to strongly influence the prognosis of patients with active cancer in terms of death. However, this reassuring result needs to be confirmed by larger studies and does not apply to cancer patients whose iSVT occurred in non-varicose veins. In this latter case iSVT can constitute a marker of cancer severity.

PB 631 | Prediction of Venous Thromboembolism in Newly Diagnosed Patients Treated for Lymphoid Malignancies - Validation of the Khorana Risk Score among Patients with Diffuse Large B-cell Lymphoma and Hodgkin Lymphoma

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Background: The utility of the venous thromboembolism (VTE)-risk assessment model developed by Khorana known as the Khorana Risk Score (KRS) in patients with the most frequent subtypes of lymphoma receiving outpatient chemotherapy is unknown.

Aims: We evaluated the association of KRS with VTE in patients treated for diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL).

Methods: Retrospective analyses were performed on 428 adult patients (median age 50, 51% females), of whom 241 were newly diagnosed diffuse large B-cell lymphoma (DLBCL) and 187 had Hodgkin lymphoma (HL). No patients received thromboprophylaxis. There was no routine screening for VTE.

TABLE 1 Comparison of patients' characteristics with/or without VTE

	Overall population n=428	VTE group during follow-up n=64	Non-VTE group during follow-up n=364	p value
Median age, range years	50 (18-98)	49 (22-81)	50 (18-98)	0.9698
Gender, male n (%)	209 (49%)	34 (53%)	175 (48%)	0.4562
Type of lymphoma: DLBCL	241 (56%)	45 (70%)	196 (54%)	0.0143
Stage IV of the disease	218 (51%)	38 (59%)	180 (49%)	0.1430
Presence of constitutional symptoms	258 (60%)	23 (36%)	41 (64%)	0.5025
Poor Prognostic Group (score ≥ 3 IPI/IPS)	178 (42%)	34 (53%)	144 (40%)	0.0423
High Khorana Risk Score	64 (15%)	11 (17%)	53 (15%)	0.5868
Death	56 (13%)	17 (27%)	39 (11%)	0.0005

TABLE 2 Comparison of patients' characteristics in the high or intermediate groups of the Khorana Risk Score

	Overall population n=428	High risk group n=64	Intermediate risk group n=364	p value
Median age, range years	50 (18-98)	40 (19-79)	52 (18-98)	0.0706
Gender, male n (%)	209 (49%)	28 (44%)	181 (50%)	0.3778
Type of lymphoma: DLBCL	241 (56%)	25 (39%)	216 (59%)	0.0026
Stage IV of the disease	218 (51%)	34 (53%)	184 (51%)	0.7039
Presence of constitutional symptoms	258 (60%)	49 (77%)	209 (57%)	0.0039
Poor Prognostic Group (score ≥ 3 IPI/IPS)	178 (42%)	41 (64%)	137 (38%)	<0.0001
Presence of VTE	64 (15%)	11 (17%)	53% (15%)	0.5868
VTE within 6 months after diagnosis	35 (8%)	5 (45%)	30 (57%)	0.4490
Death	56 (13%)	11 (17%)	45 (12)	0.2912

Results: During a median follow-up of 37 months (25th-75th percentile 16-57), 64 (15%) patients developed VTE and 56 (13%) died. Most of the thrombotic events (55%) occurred within 6 months after diagnosis. In comparison to the HL group, patients with DLBCL were older and more patients were in advanced stage (p< 0.001).

45 patients of the 241 (19%) treated for DLBCL developed VTE compared to 19 patients of the 187 HL patients (10%, p=0.0143), Table 1. Analysis of the risk of thrombosis, according to the Khorana model, showed an intermediate risk in 364 patients (85%, 1-2 points) and a high risk in 64 patients (15%, ≥3 points), Table 2.

More patients with HL were in the high-risk group of KRS (score ≥3), in comparison to the DLBCL group (39 vs. 25, p=0.0026). Eleven patients of the 64 (17%) from the high KRS group developed VTE compared to fifty-three patients of the 364 patients (57%) from the intermediate group (p=0.5868). In our patients, the KRS did not adequately stratify or predict VTE (PPV 15%, NPV 82% and C statistic 0.51).

In Kaplan-Meier analysis of VTE-free survival rates, no difference was found between patients in the high KRS and in the intermediate KRS group (log rank test 0.12, p=0.9079).

Conclusions: In this cohort of patients with lymphoid malignancies, the KRS did not adequately stratify or predict VTE events in patients at higher risk of VTE.

PB 632 | High Real-life Incidence of Venous Thromboembolism in Multiple Myeloma: A Need for More Effective Thromboprophylaxis at a Lower Thrombosis Risk Threshold

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Background: Thromboprophylaxis is frequently used during the treatment of multiple myeloma (MM), dependent on the regimen applied. Despite prophylaxis, MM is often complicated by venous thromboembolism (VTE). However, the rate in published studies does not exceed 5-10%.

Aims: To determine real-life incidence of VTE in newly diagnosed MM patients treated with different anti-myeloma regimes.

Methods: We used two databases that prospectively registered consecutive patients with newly diagnosed MM: one covered all four hospitals in the province Friesland, the second the University Medical Centre Groningen, the reference centre for the northern part of the Netherlands. Data on treatment regimens, thromboprophylaxis and VTE were retrospectively collected. Dutch law does not require ethical review for chart review studies.

Results: Between 2002 and 2012, 521 patients were included. 57% were male, median age was 66 yrs (range 31-92). Initial treatment was immunomodulatory drugs (IMID)- (49%), proteasome inhibitors (PI)- (13%), vincristine, adriamycine and dexamethasone (VAD)-based (11%) or other, including expectative policy (26%). 37% underwent autologous stem cell transplantation. Thromboprophylaxis was prescribed to 63% in IMiD, 23% in PI and 15% in VAD-based group. VTE was diagnosed in 15% at a median of 91 days (range 0-2026) after diagnosis.

VTE risk was not associated with initial treatment: 16% in IMID, 13% in PI and 18% in VAD-based group. Similarly, VTE risk was equal for no thromboprophylaxis (13%), aspirin (19%) and low molecular weight heparin (21%). Data were similar for both databases.

Conclusions: VTE risk in real-life MM treatment is unacceptably high at 15%, with similar rates in all first line treatment regimens and for both aspirin and LMWH. This could be explained by physicians tailoring prophylaxis to VTE risk. A possible option to reduce VTE risk is to lower the threshold for higher intensity thromboprophylaxis.

PB 633 | The Procoagulant Fingerprint of Breast Cancer Cells Varies in Function of Their Aggressiveness and the Tissue Factor Expression

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Background: Blood hypercoagulability is a common systemic alteration in patients with cancer and varies according to the histological type of cancer. Tissue factor (TF) is a key actor in cancer-induced hypercoagulability. We previously showed that contact of breast cancer cells line MCF7 enhances thrombin generation (TG).

Aims: To identify the procoagulant „fingerprint" of breast cancer cells of various degrees of aggressiveness.

Methods: Adhesive cell cultures of MCF7, MCF7-ShWisp2, BT-20 cancer cells lines and HUVEC (control) were analyzed for TF expression by flow cytometry and western blot assay using anti human TF murine IgG1 monoclonal antibody (ref 4503; American Diagnostics; USA). Studied cells were put in contact with normal platelet poor plasma (PPP ; Diagnostica Stago, Asnières, France) and TG was triggered with CaCl2 addition and measured with the Calibrated Automated Thrombogram assay (Diagnostica Stago, Asnieres France) as previously described (1).

Results: *MCF7-ShWisp2* and BT-20 cells expressed higher amounts of TF as compared to MCF7 and HUVEC. The MCF7-ShWisp2 and BT-20 cells significantly accelerated the initiation and propagation phases of TG and slightly increased the Peak and the ETP as compared to MCF7. The MCF7 cells enhanced TG as compared to HUVEC profile. Analytical data are depicted in Table 1.

Conclusions: The procoagulant „fingerprint" of breast cancer cells varies in function of their histological phenotype. The more aggressive

TABLE 1 Effect of breast cancer cells on TG of normal human plasma. Values are means±sd. (*<0.05 versus HUVEC; +<0.05 versus MCF7 or MDA-MB-231)

	MCF7-ShWisp2	BT-20	MCF7	HUVEC
Lag-time (min)	2.64±0.61*+	2.26±0.38*+	5.37±1.34	8.6±0.51
Peack (nM)	117.87±11.55*+	129.04±6.17*+	75.3±21.97	80.63±5.48
ttPeack (min)	6.92± 1.53*+	6.03±1.13*+	11.98±1.01	14.67±.0.60
ETP (nMxmin)	1144.72±53.79	1174.55±9.14	940.33±103.56	974.99±36.63
MRI (nM/min)	28.92±8.02*+	35.35±8.22*+	12.59±6.28	13.39±1.35
TF (MIF)	60.80±3.22*+	44.59±1.53*+	8.44±0.92	0.9±0.33

cells MCF7-ShWisp2 and BT-20 present the higher procoagulant potential which is correlated with TF expression on the cell membrane. The identification of cancer-type specific procoagulant fingerprint could improve the efficiency of the antithrombotic strategies.

1. Rousseau A, Van Dreden P, Mbemba E, Elalamy I, Larsen A, Gerotziafas GT. Cancer cells BXPC3 and MCF7 differentially reverse the inhibition of thrombin generation by apixaban, fondaparinux and enoxaparin. Thromb Res. 2015;136:1273-9.

PB 634 | Joint Effect between Smoking Status and Cancer on the Risk of Venous Thromboembolism: The Scandinavian Thrombosis and Cancer Cohort

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Background: Smoking is a well-established risk factor for cancer. Conflicting results have been reported on the association between smoking and risk of venous thromboembolism (VTE). In studies reporting a relationship between smoking and VTE, the association appeared to be mediated by smoking-related diseases (i.e. cancer). No study has investigated the joint effects of smoking and cancer on the risk of VTE.

Aims: To investigate the joint effect of smoking status and cancer on risk of VTE using a large population-based cohort.

Methods: The Scandinavian Thrombosis and Cancer (STAC) cohort is a merging of three population-based cohorts (Tromsø study, Danish Diet Cancer and Health Study and Nord-Trøndelag Health Study). Subjects (n=144 952) were included in 1993-97 and followed up to 2007-12. Former smokers were excluded (n=35 890). VTEs occurring from 6 months before to 2 years after a cancer diagnosis were defined as cancer-related. Smoking habits were registered by self-administrated questionnaires at baseline. Age- and sex- adjusted Cox regression was used to obtain hazard ratios (HR) for VTE across cancer- and smoking status. Rothmans synergy index ($S= (RR_{A+B+}-1)/(RR_{A+B-}-1)+(RR_{A-B+}-1)$) was used to assess the presence of synergism between smoking and cancer.

Results: There were 10 815 incident cancer diagnoses and 1 611 VTEs, of which 275 were cancer-related, during a median of 11 years of follow-up. There were 48 341 current smokers, of which 5948 developed cancer. In cancer-free subjects, smoking was not associated with VTE (HR 1.0, 95% CI 0.9-1.1). In cancer patients, however, the risk increased from 6-fold (HR 6.6, 95%, CI 5.4-8.0) in never smokers to 9-fold (HR 9.3, 95% CI 7.8-11.0) among smokers, when compared to non-smoking cancer-free subjects. Active cancer and smoking demonstrated a synergistic effect on VTE-risk
(S=1.5, 95% CI 1.1-1.9).

Conclusions: Our findings showed that smoking in combination with cancer yielded a modest synergistic effect on the risk of VTE.

PB 635 | The Impact of Traditional Triggers on the Risk of Venous Thromboembolism in Cancer - Results from a Population-based Case-crossover Study

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Background: Cancer is a well known risk factor for venous thromboembolism (VTE). Although several patient-, cancer- and treatment-related risk factors for VTE have been identified, the relative magnitude of these triggers of VTE risk in cancer is not well defined.

Aims: To investigate the impact of various VTE-triggers in subjects with cancer.

Methods: From a case-crossover study of VTE patients (n=707) recruited from the general population (The Tromsø study), VTE-patients with a history of cancer longer than six months prior to VTE were selected (n=96). All hospitalizations and triggers were registered within each subject during the last 90-day period before the VTE diagnosis and in up to four preceding 90-day comparison periods (n=332). A 90-day washout period between the comparison- and VTE-periods was used to avoid potential carry-over effects. Conditional logistic regression was used to obtain odds ratios (ORs) for each trigger. Each patient served as their own control allowing for within-subject adjustment for fixed covariates.

Results: When comparing the VTE-period to the control periods, immobilization (OR 22.1, 95% CI 10.0-48.8), infection (OR 10.9, 95% CI 5.9-20.1), central venous catheters (OR 11.0, 95% CI 4.0-30.1), chemotherapy (OR 4.4, 95% CI 2.2-8.8) and radiotherapy (OR 4.3, 95% CI 2.0-9.4) had the greatest impact on VTE in cancer, while surgery (OR 0.8, 95% CI 0.4-1.5) had no significant effect. The impact of radiotherapy came predominately from palliative (OR 10.4, 95% CI 3.4-31.7), rather than therapeutic radiotherapy (OR 0.9, 95% CI 0.2-4.5).

Conclusions: Our results imply that VTE-triggers related to cancer and its complications (i.e. immobilization, infection) generally have a greater impact on VTE than factors related to cancer treatment (i.e. surgery, radiotherapy, chemotherapy).

PB 636 | Impact of a Novel Version 2.0 of an Electronic Alert System for Venous Thromboembolism (VTE) Prevention in Hospitalised Cancer Patients

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Background: Hospitalised cancer patients have high VTE risk. Prophylaxis with Low Molecular Weight Heparin (LMWH) is widely recommended, although underuse is a problem worldwide. Electronic alerts (e-alerts) are effective tools to increase its prescription.

Aims: To evaluate the impact of a new version (v2.0) of our e-alert system for VTE prevention compared with the initial software.

Methods: Prospective study including consecutive adult cancer patients admitted at our centre. During the first period (April 2014-June 2015) the initial e-alert system remained operative. During the second phase (July 2015-December 2016) the v2.0 was active. The v2.0 software interrogated physicians about reasons for not using prophylaxis. Patients were followed for 30 days after discharge. Approval by institutional ethics committee was obtained.

Results: 1128 patients were included, 767 patients in the first period and 461 in the second (Table 1). E-alerts v2.0 associated with an increase of the use of LMWH prophylaxis during hospitalisation (65.1% vs 72.2%; p=0.007). However, this improvement did not result in a reduction of VTE during admission or follow-up (2.5% vs 2.6%). 80% of VTE events occurred despite LMWH use. Major bleeding was similar (3.2% vs 3.4%), while mortality was higher in Group 2 (10.1% vs 15.6%; p=0.016), probably due to a higher proportion of patients with advanced disease. The main reason for not prescribing LMWH prophylaxis was bleeding risk, but in 17% of cases physicians did not consider the patient to have high VTE risk.

Conclusions: The new e-alert system further increases the use of VTE prophylaxis in hospitalised cancer patients, although this was not associated with a reduction of the incidence of VTE. A relevant number of VTE events occurs despite prophylaxis with standard LMWH. Identification of risk factors for thromboprophylaxis failure is needed.

PB 637 | Platelet-related Thrombin Generation in Adult Survivors of Childhood Cancer

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Background: Adult survivors of childhood or adolescent cancer have significant long term morbidity related to their cancer therapy. Vascular conditions including premature atherosclerosis, hypertension, arterial and venous thrombosis are commonly described in these subjects.

Aims: This study aims to characterize thrombin generation (TG) in presence of platelets, as an assay of global hyper- or hypo-coagulability, in individuals from the Cardiac and Vascular late sequelae in long-term survivors of childhood cancer (CVSS) study.

Methods: TG, investigated in platelet rich plasma (PRP) at 1pM TF, was available in 200 individuals from the CVSS and in 407 individuals from the population-based Gutenberg Health study (GHS), as a control group. Lag time, endogenous thrombin potential (ETP) and TG peak were the analysed parameters of a TG curve.

Results: TG lag time was shorter (p=0.00056) and TG peak was higher (p=0.0027) in cancer survivors compared to GHS individuals. Cancer survivors with any cardiovascular risk factor (CVRF) presented with shorter lag time and higher TG peak compared to GHS individuals with any CVRF. The multivariable linear regression analysis adjusted for age, sex and CVRFs showed that female sex (beta estimate, ß: 193.3

[95%CI: 113.9; 272.7], p< 0.001), obesity (ß: 162.1 [64.7; 259.5], p=0.0013) diabetes (ß: 470.3 [98.5; 842.1], p=0.014) and particularly hypertension (ß: 174.6 [80.6; 268.5], p< 0.001) were associated with higher ETP in cancer survivors.

Conclusions: Cancer survivors presented with higher TG potential in comparison to population sample. Survivors with CVRFs had a worse TG profile compared to the control group with CVRFs. Hypertension in survivors was strongly associated with higher TG potential, independent of age, sex and traditional CVRFs. These results substantiate an interaction between cancer (treatment), CVRF like hypertension, and hypercoagulability that may be pathophysiologically relevant.

PB 639 | Clinical History of Thrombosis before Diagnosis of Overt Myeloproliferative Neoplasms in Triple Negative Patients

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Background: Thromboses are the most important preventable risk factors for morbidity and mortality in chronic myeloproliferative neoplasms (MPN).

Aims: To analyze the prevalence of thrombotic events in the past clinical history of patients with a diagnosis of MPN,even in absence of blood cells count abnormalities leading to hematology consult at the time of thrombosis diagnosis.

Methods: We here performed a retrospective cross sectional study of patients with a diagnosis of Philadelphia negative MPN and a prior history of thrombosis, analyzed from electronic charts. Available thrombophilia screening included the following assays: PT, aPTT, antithrombin, protein C and protein S, coagulation Factor VIII, anticardiolipin antibodies, Lupus Anticoagulant. Coagulation Factor VIII levels > 150IU/dL were considered elevated. These tests are part of a routine work-up in our institution. The local institutional review board approved the protocol and written informed consent was obtained from all patients.

Results: Among a cohort of 260 patients with MPNs (78PV, 102ET, 80 MF), forty four were found triple negative for JAK-2, calreticulin and MPL gene mutations. Sixty-nine (26.54%) patients (29F, 40M) had a personal past clinical history of arterial or venous thrombosis. Among patients with thrombosis, 13(18.8%) cases (11 ET, 2MF) were triple negative (median age:60 years). Most events, in particular in triple negative patients, occurred within 18 months before overt .MPN. At the time of thrombosis diagnosis, only a minority of patients showed blood tests abnormalities suggestive of MPN or indicating hematological consultation.History of thrombosis had a strong positive correlation with higher risk stages of MPN.

Conclusions: We here report the occurrence of ischemic and thromboembolic events in 20% cases of triple negative MPNs. Further evidences are needed to support the development of risk stratification scores or the detection of early molecular markers in diagnosis of MPNs.

PB 640 | Importance of the Association Cancer Cells and Microparticles on the Hypercoagulable Power of Cancer

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Background: The hypercoagulable state of malignancy occurs due to the ability of tumor cells to activate the coagulation system. The prominent role is attributed to exposure of tissue factor (TF) and procoagulant phospholipids (PPL) by the tumor cell. We recently showed that contact of human plasma with pancreas adenocarcinoma cells (BXPC3) or breast cancer cells (MCF7) enhances thrombin generation (TG). We demonstrated that the procoagulant activity of the cancer cells alone was not sufficient to induce hypercoagulability (TG higher than the upper normal limit).

Aims: We analyzed the specific procoagulant role of microparticles (MVs) originate from the cancer cells and we estimated their association with cancer cells for cancer induced hypercoagulability.

Methods: BXCP3 and MCF7 cells were cultured in 96-well plates. Primary human umbilical vein cells (HUVEC) were used as normal control experiment. The CAT® assay (Stago France) was used to study TG of normal platelet poor plasma added in wells carrying

(a) cancer cells,

(b) cancer cells in presence of their respectively isolated MVs, or

(c) MVs alone. TF activity (TFa) of cells and MVs was assessed with a specific clotting assay.

Results: The TFa were found in abundant amounts BXPC3 cells, and BXPC3 MVs compared to MCF7 cells and MCF7 MVs. The HUVEC

TABLE 1 Thrombin generation on cells in presence and absence of MVs in Normal Pool Plasma

	Pool	HUVEC	BXPC3	MCF7	HUVEC +MVs	BXPC3 +MVs	MCF7 +MVs	Pool +MVs(BXPC3)	Pool +MVs(MCF7)
Lag-time (min)	8.9±1.6	7.7±0.9	4.8±0.6	7.5±0.7	7.9±0.9	1.2±0.5	5.1±0.7	2.2±0.6	6.1±0.4
tt-Peak (min)	14.6±1.3	12.1±0.9	5.7±0.8	9.3±1.0	11.7±0.9	2.6±0.6	6.9±1.1	3.3±0.4	8.2±0.8
Peak(nM)	136±11	150±11	220±12	179±11	166±10	410±12	220±10	380±14	210±11
MRI (nM/min)	24±9	35±10	244±13	99±12	45±10	294±11	120±12	247±14	100±13

cells and HUVEC MVs showed TF activity comparable to a normal pool. Analytical data of TG are depicted in Table 1.

Conclusions: This experiment showed that hypercoagulability induced by cancer cells is the resultant of the combination of the procoagulant properties of cancer cells with procoagulant elements of the plasma microenvironment. To the best of our knowledge, the present study showed for the first time that the inherent procoagulant properties of cancer cells are not sufficient to induce hypercoagulability and documents that procoagulant elements of the microenvironment, namely MVs are necessary elements for cancer induced hypercoagulability.

PB 641 | Variability of Khorana Score and its Validity after First Chemotherapy in Patients with Gastric Cancer

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Background: Venous thromboembolism (VTE) is a leading cause of morbidity and mortality in patients with gastric cancer (GC). Risk stratification with the Khorana score (KRS) coupled with targeted prevention is one of the most validated approaches. However, the variability and validity of the KRS after chemotherapy (CT) is unknown.

Aims: To determine Variability of the KRS and its Validity after first CT in GC.

Methods: Single institution cohort of GC (2010-15). VTE events were objectively confirmed. GC was ascertained if biopsy proven and metastatic, or on active CT. We defined stage as limited (stage I to II) and advanced (stage III to IV). Along with cancer specific data, we abstracted the KRS before and after first CT. The KRS was dichotomized into intermediate and high based on the original description. The primary outcome was VTE prediction. Continuous and categorical variables are expressed by the median (interquartile range) and by percentages. We used SPSS version 23 specifically McNemar test, paired sample t-test, Wilcoxon and cox regression with forward modeling to analyze main objectives.

Results: We included 112 pts in the analysis who were men (66%), 58 (51-64) yo, with adenocarcinoma (84%) and advanced disease (59%). The median follow-up was 21.3 months (9.5-42.6). VTE occurred in 12% of patients. The median time from diagnosis to VTE occurrence was 59 days (3-258). Pre-CT KRS classified 53% of patients as high risk, but ~30% of them were reclassified as intermediate risk after CT. We found that only leucocytes counts changed after CT (P< 0.01). Performance Status (PS) and post-CT KRS were independent predictors of VTE after adjusting for stage in multivariate analysis. The strongest predictor was PS (HR: 7.6; 95%CI: 2.27-25.33; p< 0.01) followed by post-CT KRS (HR: 3.69; 95%CI: 1.17-11.65; p=0.03).

Conclusions: After further validation, post-CT KRS appears a valid tool identify patients with GC at high risk of VTE after first CT.

PB 642 | Venous Thromboembolism in Patients Undergoing Allogeneic Stem Cell Transplantation with Umbilical Cord Blood

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Background: Venous thromboembolism (VTE) after hematopoietic stem cell transplantation (HSCT) has been shown to occur in approximately 4.6% of patients and is associated with graft versus host disease (GVHD) (Gerber D, et al. Blood 2008). We hypothesized that HSCT with use of umbilical cord blood, which is associated with less GVHD, would be associated with fewer post-transplant VTE events when compared to HSCT with matched related or unrelated donor stem cells.

Aims: Our primary aim was to compare the incidence of VTE following allogeneic HSCT between those who received umbilical cord blood stem cells and those who received matched related or unrelated donor stem cells.

Methods: A retrospective chart review was performed on all patients who underwent allogeneic HSCT for a hematologic malignancy between 1/2010 and 3/2016 at the University of Colorado Hospital. Data regarding baseline characteristics, VTE characteristics and diagnostic interventions were collected. A two-tailed Fisher's exact test was used to compare GVHD incidence and VTE characteristics between groups.

Results: Of 253 charts reviewed, 115 patients received cord blood and 138 patients received non-cord blood. Among those who received cord blood, fewer patients developed either acute or chronic GVHD compared to the non-cord cohort (37% vs 63%; p = 0.0001); however, VTE events and the number of patients with GVHD at time of VTE were similar between groups (Table 1). The number of positive and negative evaluations for VTE are described in Figure 1.

TABLE 1 VTE Characteristics

	Cord	Matched Related or Unrelated	P-Value
	115	138	
Total patients with VTE	37 (32%)	40 (29%)	0.5866
VTE with pulmonary embolism	7 (19%)	7 (17.5%)	1.0000
VTE with catheter-associated DVT	21 (57%)	22 (55%)	1.0000
Platelet count <50,000 at VTE diagnosis	15 (40.5%)	11 (27.5%)	0.2405
Total VTE recurrence or progression	10 (27%)	14 (35%)	0.4725
VTE recurrence with pulmonary embolism	4 (11%)	8 (20%)	0.3517
Bleeding event requiring anticoagulant discontinuation	4 (11%)	6 (15%)	0.7385
Active GVHD at time of VTE	6 (16%)	10 (15%)	0.4072

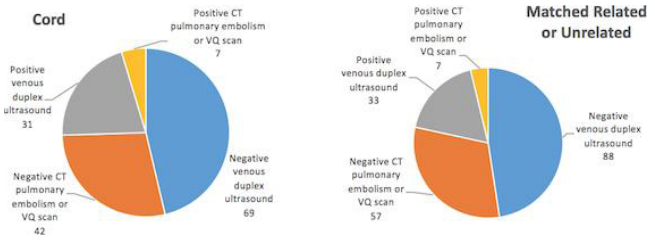

FIGURE 1 Fraction of positive and negative VTE investigations

Conclusions: Our analysis demonstrates that despite a lower overall rate of GVHD in those receiving cord blood, there was no difference in the incidence of VTE between groups, possibly due to a greater influence of GVHD on the development of VTE in those who received cord blood. Further investigation into this possibility and other contributing variables is required. A longer duration of follow-up and greater number of VTE investigations may account for the increased VTE incidence seen at our institution.

PB 643 | Joint Effects of FGG rs2066865 and Pre-cancer Fibrinogen Level on Cancer-related Venous Thromboembolism

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Background: Studies on the association between plasma fibrinogen and risk of venous thromboembolism (VTE) are conflicting. Fibrinogen gamma (FGG) rs2066865 is a missense mutation that alters fibrinogen conformation and function, and is known to be associated with VTE. It is not known, however, to what extent the FGG rs2066865 affects the risk of VTE in cancer.

Aims: To study the joint effect of FGG rs2066865 and cancer on VTE risk in subjects with high and low pre-cancer fibrinogen levels.

Methods: Cases with a first VTE (n=648) and an age-weighted subcohort (n=1893) were recruited from 3 surveys of the Tromsø study (1994-95, 2001-02 and 2007-08) and followed until 31.12.2012. VTE-events were considered cancer-related if occuring from 6 months before until 2 years following a cancer diagnosis. Pre-cancer fibrinogen levels were dichotomized using the median value in the study population (3.5g/L). Age- and sex-adjusted Cox regression models were used to obtain hazard ratios (HRs) for VTE by cancer status, FGG and fibrinogen levels.

Results: There were 167 cancer-related VTE-events and 359 subcohort members diagnosed with cancer during the study period. In cancer patients, the risk of VTE increased per additional risk allele at FGG from 12-fold (HR 12.2, 95% CI 9.1-15.9) higher for 1 risk allele to 20-fold (HR 20.0, 95% CI 11.4-35.1) higher for 2 risk alleles, when compared to cancer-free subjects without risk alleles. However, in subjects with fibrinogen>3.5g/L, a joint effect of cancer and FGG was seen. The HRs were 13.2 (95% CI 7.9-22.2) and 32.3 (95% CI 11.5-90.8) for 1 and 2 risk alleles, respectively, when compared to cancer-free subjects with no risk alleles.

Conclusions: We showed an allele-dependent increased risk of VTE in cancer patients with FGG rs2066865 and a joint effect of FGG and cancer on the risk of VTE in subjects with high pre-cancer fibrinogen levels. Our findings imply an important role of fibrinogen in the pathogenesis of VTE in cancer.

PB 644 | Performance of Current Risk Assessment Models for Prediction of Venous Thromboembolism in Patients with Gastric Cancer

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Background: Cancer-associated thrombosis is a leading cause of morbidity and mortality. Gastric Cancer (GC) is among the most prothrombotic solid tumors. There is a paucity of data on the predictive value of current risk assessment tools (RATs) in GC.

Aims: To determine the predictive performance of current RATs in patients with GC.

Methods: Single institution cohort of GC treated patients (2010-15). VTE events were objectively confirmed. Active GC was defined as biopsy proven metastatic disease or on active chemotherapy. We defined stage as limited (stage I to II) and advanced (stage III to IV). Along with GC specific data, we abstracted the Khorana Score (KRS), platelet lymphocyte ratio (PLR) and neutrophil lymphocyte ratio (NLR). We divided the KRS into intermediate and high based on the original description; and used proposed values for NLR (>3) and PLR (>260) for dichotomization. The primary outcome was VTE prediction. Continuous and categorical data are expressed by the median (interquartile range) and percentages. We used SPSS version 23 to estimate prediction rates of VTE for each RAT in independent models using Cox proportional regression and receiver operating characteristic curves (ROC).

Results: We included 112 pts in the analysis who were predominantly men (66%), 58 (51-64) yo, with adenocarcinoma (84%) and advanced disease (59%). The median follow-up was 21.3 mo (9.5-42.6). VTE occurrence was 12%. The median time from diagnosis to VTE occurrence was 59 days (3-258). High KRS (53%) had a higher risk of developing VTE in univariate analysis but did not reach statistical difference (HR: 2.28; 95% CI: 0.7-7.4, ; p< 0.01). After adjusting for performance status (PS) and stage, only PS remained predictive of VTE (HR:6.2; 95%

CI: 1.9 - 20.2; p< 0.01). We did not find any VTE predictive value for NLR (AUC=0.466, p=0.07) and PLR (AUC=0.41, p=0.33) in our cohort.

Conclusions: Although potentially predictive in other cancer types, KRS NLR and PLR did not predict the occurrence of VTE in our GC database.

PB 645 | Clinical Course of Venous Thromboembolism in Patients with Pancreatic Cancer: Insights from the RIETE Registry

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Background: Epidemiological studies have consistently highlighted an association between pancreatic cancer (PC), especially locally advanced or metastatic disease, and the highest risk of venous thromboembolism (VTE), including symptomatic deep-vein thrombosis and/or pulmonary embolism. However, few data are available regarding it's clinical course once the initial VTE event has occurred.

Aims: We aim to analyze the clinical VTE-related outcomes incidence and characteristics in PC patients throughout the whole duration of anticoagulation, as compared to other cancer patients.

Methods: We analyzed clinical data for cancer patients with VTE obtained from the RIETE registry.

Results: As of September 2016, 10951 cancer patients were recruited, of whom 497 had PC (103 non metastatic disease and 394 metastatic disease). Due to lower survival, the duration of anticoagulation was significantly shorter in metastatic as compared to non metastatic PC patients (median duration, 49 vs. 103 days; p < 0.001). In metastatic as well as in non metastatic PC patients, the rate of VTE recurrences nearly doubled the rate of major bleedings (31.65 [95%CI: 21.03-45.74] VTE recurrences per 100 patient-years vs. 15.82 [95%CI: 8.65-26.55] major bleedings per 100 patient-years, and 15.51 [95%CI: 6.22-31.97] vs. 8.87 [95%CI: 2.39-22.70] events per 100 patient-years, respectively). In PC patients, both VTE recurrences and major bleedings tend to be more frequent than in other cancer patients (14.70 [95%CI: 13.09-16.45] and 12.86 [95%CI: 11.36-14.50] events per 100 patient-years respectively in metastatic disease vs. 9.58 [95%CI: 8.42-10.85] and 6.83 [95%CI: 5.86-7.91] events per 100 patient-years respectively in non metastatic disease).

Conclusions: Our results highlight significant differences in the clinical profile of VTE-related outcomes in PC as compared to other cancers and suggest the need of developing specific anticoagulant strategies in PC patients.

PB 646 | Mean Platelet Volume (MPV) and MPV/Platelet (plt) Identify Essential Thrombocythemia (ET) Patients at High Thrombotic Risk

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Background: ET, a BCR/ABL-negative myeloproliferative neoplasm, is hampered by a high risk of thrombotic complications. The persistent enhanced platelet activation contributes to the acquired thrombophilic condition typical of ET. Different studies have investigated MPV and MPV/plt ratio as emerging platelet activation biomarkers in other thrombophilic diseases.

Aims: To investigate whether in ET patients, MPV and MPV/plt correlate with other known thrombotic risk factors (i.e. glycoproteinV (GPV), mutational status, history of thrombosis) or with treatments.

Methods: Eighty-nine consecutive ET patients (56F/33M; median age 60 yrs) were enrolled and clinical data recorded. Fourty-six patients were JAK2V617F+, 23 CALR+, 3 MPL+, and 17 triple negative. Patients treatments included: hydroxyurea (HU) (n=6), aspirin (ASA) (n=25), HU+ASA (n=38), and none of them (n=20). MPV was measured by an automated hematology analyzer and plasma GPV antigen levels by commercial ELISA.

Results: MPV values were inversely related to platelet count (p< 0.05). Patients JAK2V617F+ showed higher MPV values than JAK2 wild type (p< 0.05) or other mutation carriers (p=ns). Higher MPV values and MPV/plt were also observed in patients with a history of thrombosis (n=15) *vs* those without
(p< 0.05). No differences in MPV values and MPV/plt were found in relation to therapies. GPV levels normalized for platelet count were greater in JAK2V617F+ patients, suggesting an increased shedding of this membrane receptor from platelets.

Conclusions: Data show that JAK2V617F mutation or a positive history of thrombosis are significantly associated with MPV values in ET patients: this may contribute to the prothrombotic status. In view of the increasing importance of MPV and MPV/plt as markers of platelet reactivity, prospective studies are warranted to evaluate the role of these hematological biomarkers in risk assessment models.

Project funded by „AIRC-IG2013“ grant Nr. 14505 from the “Italian Association for Cancer Research” (A.I.R.C.).

PB 647 | Interleukin Profile in Polycythemia Vera and its Correlation with the Risk of Thrombosis

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Background: Polycythemia Vera (PV) is a myeloproliferative neoplasm characterized by dysregulated hematopoiesis and a hypercoagulable state. PV patients are at risk of premature and accelerated thromboembolic events (arterial & venous), that can lead to significant morbidity & sudden deaths.

Aims: To evaluate the inflammatory cytokines in PV patients and correlate the cytokine profile with the clinical risk of thrombosis.

Methods: This was a prospective study carried out at a tertiary care hospital of North India from June 2015 to October 2016. PV patients were diagnosed by the WHO 2008 diagnostic criteria. A detailed history of PV patients was taken for past or current episodes of thrombosis. The thrombotic episodes were further divided into arterial, venous or both. Age and sex matched healthy controls were selected from the normal population. These patients underwent various cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-11, IL-12/23p40, TNF-α and IFN-γ) evaluation in blood by flow cytometry (Fig.1a) bead based assays (CBA kits, BD Biosciences™). Informed consents were obtained & this study was approved by the institute ethics committee. Statistical analysis were done using SPSS Statistics 22.0.

Results: A total of 52 PV patients and 20 healthy controls were included in the study. The median age of patients was 52 years with male:female ratio of 2:1. JAK2 mutations were positive in 49 patients. Thrombotic events were seen in 19(36.5%) patients(12 arterial & 7 venous). Levels of IL-6 (p< 0.001), 8(p=0.001), 12/23p40 (p=0.009) were higher in PV patients than controls (Fig.1b). IL-6 & IL-8 levels were significantly associated with the risk of thrombosis in PV (Table1). There was no association of thrombosis with leucocytosis or thrombocytosis.

Conclusions: An abnormal inflammatory cytokine milieu was seen in PV patients suggesting a possible role of IL-6, IL-8 & IL-12/23p40 in the pathogenesis of PV patients. In addition, IL-6 and IL-8 were significantly higher in PV patients with thrombosis.

Fig.1(a). flow cytometer images obtained after sample processing;
Fig.1(b). showing levels of IL-6, IL-8 & IL-12/23p40 in PV patients & controls

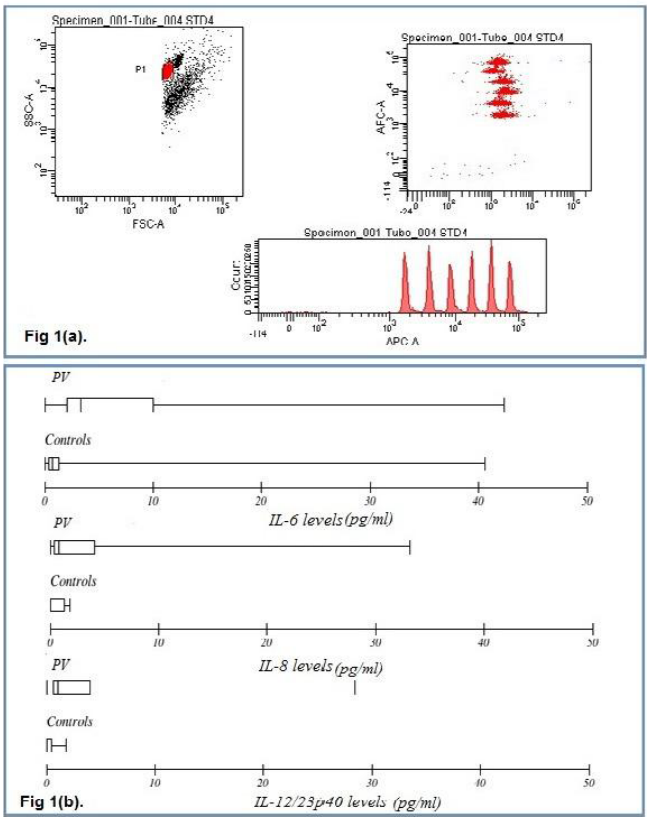

FIGURE 1 (a). flow cytometer images obtained after sample processing; Fig.1(b). showing levels of IL-6, IL-8 & IL-12/23p40 in PV patients & controls

TABLE 1 Association of IL-6 & 8 with thrombotic events (Mann U Whitney test)

Cytokines (mean±S.D)	Thrombotic events present(n=19)	Thrombotic events absent(n=33)	´p´ values
IL-6 (pg/ml)	13.88±12.60	2.55±2.13	<0.001
IL-8 (pg/ml)	3.66±7.17	1.06±1.33	0.021

PB 648 | Joint Modeling of Longitudinal Haemostatic and Inflammatory Biomarker Trajectories and Mortality in Patients with Cancer

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Background: Elevated biomarkers of hypercoagulability, such as D-Dimer, and inflammation, such as C-reactive protein (CRP), are associated with an increased risk of death in patients (pts) with cancer. However, the relationship between longitudinal changes of these biomarkers over time and mortality remains incompletely understood.

Aims: To explore the role of longitudinal changes in haemostatic and inflammatory biomarkers for dynamic overall mortality prediction in pts with cancer.

Methods: 105 pts with active malignancy were included in this prospective cohort study (**Table 1**). D-Dimer, Prothrombin fragment 1.2 (F1.2), CRP, and others (to be presented at the meeting) were measured at study inclusion, as well as during 6 follow-up visits, each 1 month apart. The prognostic impact of longitudinal biomarker trajectories on mortality was quantified with a so-called joint model.

Results: During the observation period of 250 days, we observed 17 deaths, for a 250-day mortality of 16.6% (95%CI: 10.6-25.3). Graphical inspection of biomarker trajectories suggested that levels of D-Dimer (**Figure 1A**), F1.2, and CRP (**Figure 1B**) increased before death. In joint modeling, a doubling of the D-Dimer trajectory was associated with a 2.3-fold increase in the risk of death (Hazard ratio (HR)=2.28, 95%CI: 1.54-3.37, p< 0.0001). A corresponding increase in F1.2 and CRP was associated with a 4.2-fold increase (HR=4.23, 1.43-12.53, p=0.009) and a 3.2-fold increase (HR=3.18, 1.75-5.81, p< 0.0001) in the risk of death, respectively. These associations

prevailed after adjusting for age and metastatic disease at baseline (Adjusted HR for D-Dimer=2.33, 1.54-3.53, p< 0.0001, others not shown).

Conclusions: Longitudinal trajectories of haemostatic and inflammatory biomarkers predict the occurrence of death in pts with cancer. The evolving biomarker profile of cancer pts over time is consistent with the co-occurrence of increased thrombin generation and systemic inflammation at the end of life.

TABLE 1 Characteristics of the study population (n=105)

Variable	Median [25th-75th percentile], or Absolute count (percent)
Age at entry (years)	62.7 [53.1-68.6]
Metastatic disease at entry	45 (43%)
D-Dimer at entry (µg/mL)	1.0 [0.5-2.1]
Prothrombin Fragment 1.2 at entry (pmol/L)	220 [153-277]
C-reactive protein at entry (mg/dL)	0.6 [0.1-1.8]
Number of follow-up visits per patient	6 [3-6]
Remission during antineoplastic treatment	36 (34%)

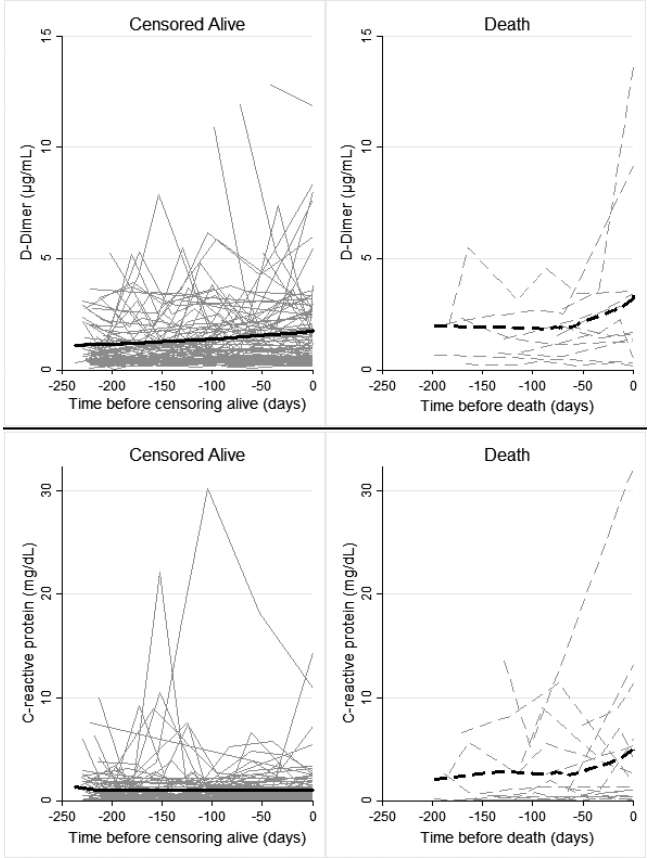

FIGURE 1 Trajectories of D-Dimer and CRP in patients with cancer who were censored alive or died during the study period

PB 649 | LMWH First Dose Program to treat Cancer-associated Thrombosis in a Pharmacy Setting

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Background: Currently, clinically stable patients diagnosed with a pulmonary embolism or deep vein thrombosis are referred to the emergency department (ED) for management. This practice strains an already overburdened ED and is associated with long wait times and poor injection education. Despite their elevated risk of venous thromboembolism (VTE), patients with cancer commonly receive little to no information to help them recognize the symptoms of thrombosis.

Aims: This pilot study sought to determine if clinically stable patients with a newly diagnosed blood clot could be effectively managed by a community pharmacist following a guidelines-based algorithm to prescribe and initiate low molecular weight heparin (LWMH) therapy.

Methods: Clinically stable cancer patients, with newly diagnosed VTE, were enrolled into this pilot study by their oncologist. A pharmacist trained in the algorithm prescribed a 14-day supply of LMWH, as well as provided injection teaching, VTE education and follow-up.

Results: Patients (N=48) were prescribed a LMWH (pre-filled dose-specific syringe) for a minimum of 10 days. Self-injection training was well received, < 5% of patients were unable to self-inject following training, with most confident in their ability to self-inject. No known occurrences of bleeding or other side-effects were observed in the patients enrolled in the study. Physician and patient satisfaction was high with the program.

Conclusions: This pilot study demonstrates that cancer patients with newly diagnosed VTE can be safely and effectively managed by pharmacists in the outpatient setting. This study also shows that pharmacists are capable of doing more than their traditional role of dispensing medications and are capable of delivering complex care services, particularly in the management of VTE. Stable patients with CAT can be managed in a local pharmacy with no need to visit the ED, overall affording the patient better care with a lower cost to the healthcare system.

PB 650 | Biomarkers for Venous Thromboembolism (VTE) in Newly Diagnosed Multiple Myeloma (NDMM): Early Results from the Thromboprophylaxis in Multiple Myeloma (TiMM) Trial at King's College Hospital

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Background: There is a well-established link between myeloma and VTE risk, however there is limited information regarding the use of

TABLE 1 Mean Biomarker Results at each Visit

	Baseline	Visit 1 (Week 1)	Visit 2 (Week 3)	Visit 4 (Week 9)	Visit 7 (Week 18)
Mean (SD) F8	188 (87)	229 (39)	245 (58)	267 (98)	157 (57)
Mean (SD) VWF:Ag	188 (55)	224 (38)	244 (45)	260 (92)	168 (35)
Mean (SD) VWF:Act	128 (66)	172 (71)	193 (67)	250 (111)	124 (25)

biomarkers to identify alterations in risk. Currently myeloma patients receiving chemotherapy are the only group of ambulatory cancer patients who routinely receive thromboprophylaxis which takes the form of either aspirin or low molecular weight heparin, depending on risk stratification.

Aims: To describe Factor 8 (F8), von Willebrand Antigen (VWF:Ag) and Activity (VWF:Act) in NDMM patients as they progress through treatment as part of the TiMM trial.

Methods: The TiMM trial randomises patients to either standard thromboprophylaxis or apixaban 2.5mg BD. F8, VWF:Ag and VWF:Act were measured before starting treatment (baseline) and at weeks 1, 3, 9 and 18 into their myeloma treatment.

Results: To date 10 patients have been recruited to the TiMM trial. Two have been classified as high risk and randomised to apixaban as thromboprophylaxis. Eight patients have been classified as low risk, 4 randomised to receive aspirin and 4 apixaban. F8, VWF:Ag and VWF:Act concentrations increase during the first 2 months of treatment and normalise by week 18.

Conclusions: Our early findings indicate that the risk of VTE, as assessed by biomarkers, is elevated, and increases during the first 9 weeks of chemotherapy. This risk appears to reduce by week 18. This could be clinically useful to establish the period of highest risk and the optimal duration of thromboprophylaxis and also which patients may be at highest risk of VTE. Further results are required to assess the significance of this, along with additional biomarkers that are currently being assessed as part of the TiMM trial.

PB 651 | The CATs out of the Bag: Alternatives to Low Molecular Weight Heparin (LMWH) in Patients with Cancer Associated Thrombosis (CAT)

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Background: CAT occurs in up to 19% of patients with malignancy and is a leading cause of death(1-4). The clot study (5) established the superiority of LMWH over coumarins for the treatment of CAT. Direct oral anticoagulants (DOAC) have become first line in the treatment of venous thromboembolism (VTE) but their role in patients with cancer associated thrombosis is unclear.

TABLES

Table 1 – Timing of DOAC

Timing of DOAC therapy	N
DOAC within the first 6 months of treatment	10
Rivaroxaban	9
Apixaban	1
DOAC after 6 months	24
Rivaroxaban	17
Apixaban	7

Table 2 – Complications of anticoagulation

	Treatment Phase	Secondary prophylaxis phase
Bleeding	2 - Haematuria on LMWH – 1 required red cell transfusion 1 - Rectal bleeding on LMWH (minor) 1 – Muscle bleed (post surgery) on LMWH	1 - Rivaroxaban associated menorrhagia 1 - Rivaroxaban associated upper GI bleed in the setting of peptic ulcers
Thrombosis	2 – New DVT after dose reduction in month 2 3 – Extension of DVT on LMWH	1 – New PE on prophylactic LMWH 1 – MI on prophylactic LMWH

Aims: To review anticoagulant choice and complications in patients with CAT.

Methods: A review of patient records of patients with a CAT at a tertiary hospital between July 2015 and June 2016 was undertaken.

Results: 145 patients (76M /69F) were identified for analysis. The median age was 66 (Range 22-90). 46% were PE and 32% DVT. A wide variety of cancer types were seen. 127 patients completed 6 months of anticoagulation, 16 died within 6 months of CAT, 1 patient was on anti-platelet therapy (MI) and 1 stopped anticoagulation against medical advice. 3 patients started a DOAC as part of a clinical trial and 2 were commenced on warfarin prior to the diagnosis of malignancy. 140 started LMWH and 115 patients completed 6 months of LMWH. Of these, 43 stopped anticoagulation after 6 months, 31 continued on prophylactic LMWH, 15 continued on therapeutic LMWH because of progressive thrombosis, 24 changed to a DOAC and 9 changed to warfarin. 34 patients (23%) received DOACs at some stage (Table 1). 24 were switched to a DOAC after completing 6 months of LMWH to continue secondary prevention. 13 patients reported complications while on anticoagulation (Table 2). Only one patient required a red cell transfusion.

Conclusions: LWMH remains the agent of choice in the treatment of CAT. In this cohort, DOACs appear to be as effective in secondary prevention as LWMH with no excess adverse events.

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PB 652 | Additional Testing Following Screening Strategies for Occult Malignancy Diagnosis in Patients with Unprovoked Venous Thromboembolism

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Background: ^{18}F-Fluorodesoxyglucose Positron-Emission-Tomography combined with Computed-Tomography (FDG PET/CT) might be an attractive tool for cancer screening in patients with venous thromboembolism (VTE), allowing non-invasive whole-body imaging. One of the frequent criticisms to the use of FDG PET/CT for screening is the potential for false positive results leading to unnecessary/invasive investigations.

Aims: To compare the frequency and invasiveness of additional testing following extensive and limited screening strategies for occult malignancy in patients with unprovoked VTE.

Methods: We analysed patients included in the MVTEP study, a randomized trial that compared a screening strategy based on FDG-PET/CT with a limited screening strategy for occult malignancy diagnosis in patients with unprovoked VTE. All additional diagnostic procedures following screening were recorded and classified as invasive or non invasive.

Results: A total of 394 patients were analysed. The mean number of additional tests per patient was 0.30 in the FDG PET/CT arm, vs. 0.27 in the control arm (p=0.65). Overall, 45 (22.8%) patients in the FDG PET/CT group underwent additional diagnostic tests, versus 32 (16.2%) in the limited screening group (absolute risk difference +6.6%, 95% CI -1.3 to +14.4%, p=0.13). Sixteen (8.1%) patients in the FDG PET/CT group underwent invasive procedures, versus 6 (3%) in the limited screening group (absolute risk difference +5.1%, 95% CI +0.5 to +10.0%, p=0.03).

Conclusions: We found no statistical difference in the number of additional procedures following each screening strategy. However, a higher number of invasive tests were performed in the FDG PET/CT group, but mostly leading to an underlying disease.

PB 653 | Assessing the Effects of Pre-analytical Variables on Thrombosis Biomarkers in Cancer Patients: Study Planning and Launch

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Background: Thrombosis is a major cause of morbidity in cancer patients with an incidence that varies widely, impacted by factors related to the cancer, treatment, and patient characteristics. To accurately assess patient risk and build upon existing risk assessment tools, further biomarker development and standardization are needed.

Aims: To advance standardization and provide guidance for measurement of thrombosis biomarkers in cancer patients, a pilot study was designed to identify steps during biospecimen procurement, handling, and processing which are critical for optimal specimen preservation and accurate marker detection.

Methods: The study was designed with input from an NCI/NHLBI working group; a request for information to the research community; guidance from a Scientific Advisory Committee comprised of experts in clinical pathology, pre-analytical variables, biospecimen science, and thrombosis in cancer patients; and with an experimental design developed by investigators from Boston Medical Center.

Results: Blood will be collected from cancer patients and healthy donors using standard operating procedures. The impact of pre-analytical variables experienced in the clinical setting, including delay to blood processing, delay to assay, and freeze-thaw cycles, will be measured on markers of coagulation (factor VIII activity, prothrombin fragment 1+2), fibrinolysis (D-dimer, plasminogen activator inhibitor-1, plasmin-antiplasmin complex), cell injury (plasma DNA, nucleosomes), and inflammation (soluble P-selectin, myeloperoxidase). Participants will be followed for six months for cancer and thrombotic outcomes.

Conclusions: The study will provide best practices for biomarker assessment, a potential biospecimen source to enhance future NIH funding opportunities, and a foundation for future biomarker studies to assess thrombotic risk and predict efficacy of anti-thrombotics. The data generated will be widely shared with the research community through publications and deposition in a public data repository.

PB 654 | Predictors of Prolonged Hospitalization Deep Vein Thrombosis Patients

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Background: Deep Vein thrombosis (DVT) patient usually need hospitalization after diagnosed approximately 5-7 days to get proper dose of anticoagulant and bridging into targeted dose vitamin K antagonist (VKA) at home. Several risk factors of prolonged hospitalization are well established, but prediction model of prolonged hospitalization DVT patients is still limited.

Aims: To Identify predictors of prolonged hospitalization DVT patients.

Methods: This was a cohort study conducted in our second referral general hospital, Karawaci, Tangerang, Banten, Indonesia from 2014-2015. We followed DVT patients after they were diagnosed using compression Doppler ultrasound. Prolonged hospitalization is defined as hospitalization more than 7 days after DVT was diagnosed. Eighteen potential predictors were evaluated using Binary Logistic statistical analysis.

Results: During 59 hospitalization DVT patients, 22 (37.3%) patients went home more than 7 days. Thirty-three (55.9%) patients were female with mean of age 47.1± 14.7 years old. Mostly the location of DVT was on popliteal vein 61.4%, followed by femoral (29.8%) vein and the rest was iliac vein. Most patients (81%) got weight based doses unfractionated heparin (UFH) followed by VKA. The rest were using Low Molecular Weight Heparin (LMWH) followed by VKA. Eight factors were included to be evaluated by binary logistic model. After adjusted for comorbidity conditions, cancer (OR 17.24; 95% CI: 2.11-142.28), hemoglobin level (OR 1.962; 95% CI: 1.242-3.100), high D-dimer (OR: 1.422; 95% CI: 0.94- 2.156), and high creatinine level (OR: 9.70; 95% CI: 1.03-90.90) were an independent predictors of prolonged hospitalization more than 7 days. The model was statistically significant p< 0.000. Those four variables could predict hospitalization for more than 7 days around 59,5%.

Conclusions: Predictors of prolonged hospitalization DVT patients were cancer, hemoglobin level, high D-dimer and creatinine level.

PB 655 | Incidence of Central Venous Catheter-related Venous Thrombosis in Japanese Inpatients with Hematological Malignancies: Single Center Experience

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Background: Symptomatic catheter-related thrombosis (CRT) occurs in 3-5% of cancer patients with central venous catheter (CVC) and the incidence of CRT could reach 30% when including asymptomatic cases. However, little is known about the incidence of CRT in patients with hematological malignancies. Some of them might need CRT prophylaxis, but on the other hand have more bleeding risk. It is important to identify the risk factors for thrombosis in such patients with central venous catheters.

Aims: To determine the incidence of CRT, and review the characteristics of CRT patients and current practice of CRT management.

Methods: We conducted a retrospective chart review of adult patients with hematological malignancies at Kitasato University Hospital, Japan from December 2013 to November 2016.

Results: A total of 142 patients who received central venous catheter (CVC) insertion. Twenty-eight patients (19.7%) were diagnosed with deep vein thrombosis, of whom 8 (5.6%)were associated with CVC insertion, including 7 cases of non-Hodgkin lymphoma and one of acute myeloid leukemia. The platelet counts at CRT diagnosis were more than 100 x 10^9/L except one case of leukemia, whose platelet count was 14 x10^9/L. The ECOG performance status of 6 patients (75%) was 3-4. The median time from CVC insertion to CRT diagnosis was 22days (range 10-27days). The insertion site of CVCs was femoral vein in 6 and jugular vein in 2 patients. Three patients have local inflammation at the insertion site due to catheter infection. Seven patients (87.5%) were treated with therapeutic anticoagulation using unfractionated heparin, and one using edoxaban. A single bleeding event of melena during anticoagulation occurred in each treatment.

Conclusions: In this study, the incidence of CRT among hematological malignancies was relatively lower, compared with the previous investigations. Further large scale prospective studies will elucidate some risk factors for CRT and proper anticoagulation among patients with hematological malignancies.

PB 656 | Inaugural Thrombotic Events in Myeloproliferative Neoplasms

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Background: BCR ABL negatives myeloproliferative neoplasms (MPN) including polycythemia vera (PV), essentiel thrombocythemia (ET) and primitive myelofibrosis (PMF) are clonal disorders characterized by hematopoietic progenitor cell proliferation. Thrombosis is a well known complication. It occurs particularly at diagnosis and involve both arterial and thrombotic vessels.

Aims: This study aimed to assess patients profile with inaugural thrombosis.

Methods: In a retrospective study, 67 patients with BCR ABL negatives MPN (WHO criteria 2016) were enrolled from January 2010 to December 2015. Clinical and biological data of patients with thrombosis were recorded.

Results: Among 67 patients (19 PV, 42 ET and 6 PMF), thrombotic events were reported in 20 (30%): 8 with PV, 9 with ET, and 3 with PMF. Mean age of patients with thrombosis was 59 years old [31-79], the sex ratio was 0.8. Cardiovascular risk factors were identified in

seven (35%) patients. Thrombotic localizations were venous thrombosis (n=7) and arterial thrombosis (n=13). Portal vein and peripheral arteries were the most frequent sites. The JAK2 mutation was found in 17 patients (85%). Two patients had recurrent thrombotic events during the follow up despite the cytoreductive therapy associated to antiplatelet drugs.

Conclusions: According to these results, in MPN, thrombotic events occur at diagnosis. Peripheral arteries and Intraabdominal vein are the most frequent localizations and usually reveal the diagnosis.

PB 657 | Biological Pro-coagulant and Pro-inflammatory Effect of Interferon Alpha in Myeloproliferative Neoplasms

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Background: Myeloproliferative neoplasms (MPN) are associated with an increased risk of arterial and venous thrombosis. Pegylated-interferon alpha (IFN) has obviously a great therapeutic efficacy on hematopoietic cell proliferation but its effects on hemostasis and inflammation have not been studied.

Aims: To determine whether IFN impacts the biological profile of MPN patients comparing endothelial, platelet and coagulation parameters in IFN or hydroxyurea (HU) treated and non treated (NT) patients.

Methods: 88 patients were included: 28 treated by IFN : 20 polycythemia vera (PV) and 8 essential thrombocythemia (ET), 38 treated by HU (27 PV and 11 ET) and 22 NT (6 PV and 16 ET).

Results: We observed significant effects of HU on von Willebrand factor (vWF) levels and a more pronounced effect of IFN:vWF antigen increased from 111.7±9.5 % in NT to 162.9±10.3% in HU and 227±17.1% in IFN-patients (p< 0.01). VWF activity increased to a similar extend. We observed only in IFN patients significant decreased protein S activity compared to NT patients (62.2±2.5% vs 88.7±3.9%, p< 0.01) and also significant increased levels of factor VIII:C and fibrinogen.

We had the opportunity to test again 10 patients at least 6 months after IFN discontinuation. IFN was stopped for side effects in 6 patients, for complete molecular response in 2 and for normalization of hematological parameters in 2 others. VWF activity and antigen, protein S activity, FVIIIC and fibrinogen returned to levels similar to those of NT patients and were significantly different from levels observed in IFN-treated patients.

Conclusions: We observed an increase of procoagulant and inflammatory markers in IFN-treated MPN patients that could be considered as a part of IFN biological effects. Though minimal, these alterations could require more intensive preventive anticoagulation than aspirin in patients with associated minor thrombotic risk factors such as FV Leiden or FII G20210A.

PB 659 | Audit: Use of Khorana Score in Oncology Patients in Belfast Trust

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Background: Venous Thromboembolism (VTE) is a well-documented cause of morbidity and mortality in cancer patients. The Khorana score has been validated as a means of risk assessing patients who would benefit from primary prophylaxis; advising low molecular weight heparin if the Khorana score is ≥3.

Aims: To audit if cancer patients in the Belfast trust were prescribed primary prophylaxis if they had a Khorana score of ≥3.

Methods: This audit retrospectively looked at all positive CT pulmonary angiography (CTPA) cases between January 2014 and December 2017. Cancer patients were identified and their Khorana score and evidence of VTE prophylaxis was gathered from their notes.

Results: 5321 CTPAs were performed in this period. 613 were identified with pulmonary embolisms (PE) of which 124 had a diagnosis of cancer and 54 of whom the Khorana score could apply. The mean age was 63, 56% were female, 44% were male. Of these 13% were local cancers, 24% with nodal spread, 44% were metastatic and extent of the disease was not clearly documented in 19%. 67% resulted in admission, with a mean hospital stay of 11 days. 15% had previously documented VTE and family history was poorly recorded in all cases.

7 patients were identified with a khorana score of 3 or more. Only 1 was on primary prophylaxis, 1 already on an anticoagulant.

Conclusions: This audit looked only at PE´s found by CTPA, it did not encompass incidental findings on staging CTs. The Khorana score was only applicable to small numbers of patients, however in these the standard of primary prophylaxis in high risk groups was not met. The Khorona score was not predictive in this group and of interest 2 patients who were prescribed primary prophylaxis still had a PE.

PB 660 | Treatment Tactics of Ulcerous Hemorrhage in Patients with Coronary Disease

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Background: Frequency of ulcerous gastroduodenal hemorrhage (UGDH) combining with coronary disease has no tendency for decreasing in spite of the successful drug treatment of ulcerous disease (CD).At occurrence of such combination there is a severe influence of one pathology to another one and this fact stipulates a high mortality from 37 to 55% at conservative and operative treatments of such patients.

Aims: Aim of this research is the analysis of treatment results ulcerous hemorrhage in patients with CD.

Methods: The treatment results of 72 patients with UGDH combining with CD for the period from 2010 till 2016 has been analyzed.

There were 51 men and 21 women. Average age was 53,2±4,2 years. Ulcerous hemorrhage activity has been determined by J. Forrest-Rosch (1974) classification and hemorrhagic shock level - by Algover index. In 34 patients the reason of hemorrhage was duodenum chronic ulcer (DCU) and in 38 patients - acute gastric and duodenum ulcers. As concomitant CD in 58 patients there was stable angina, in 14 cases - acute coronary syndrome (ACS) with rising (5) and without ST rising (9). In 2 patients ulcerous hemorrhage has been combined with acute cardiac infarction.

Results: As endoscopic ways of a hemostasis at 52 patients have been performed electric coagulation as endoscopic ways of hemostasis in 20 patients - introduction of 70° solution of ethanol around the ulcer. Reliable endoscopic hemostasis has been pointed in 61 patients. In 5,6% of patients it was impossible to get a primary hemostasis and in 9,6% of cases there was a recurrent hemorrhage. All those patients have been operated. Performed operations in 8 patients were palliative and in 3 of them - radical (resection of 2/3 of stomach by Bilrot 2). Mortality was 2,78 %.

Conclusions: The main treatment methods of such patients are conservative therapy and endoscopic ways of hemostasis. Surgical interventions should be performed at impossibility of achieving a primary hemostasis or at recurrent hemorrhage.

PB 661 | Venous Thrombosis and Thromboembolic Risk Factors in Outpatients with Lymphoma on Chemotherapy

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Background: Prevalence of venous thrombotic events (VTE) in patients with lymphomas is approximately 4%. There are a number of thrombotic risk factors (TRF) identified in this group of patients in general, but not exactly in those with ambulatory chemotherapy.

Aims: To evaluate the prevalence, TRF and characteristics of VTE in patients with lymphomas under outhospital chemotherapy.

Methods: Retrospective unicenter study, single physician for the treatment and follow-up of patients. Data from all patients with lymphoma treated at our center between January 2014 and December 2015 were collected. We analized age at diagnosis, previous antithrombotic prophylaxis, histology, stage, chemotherapy scheme, use of epo, Hb< 10g/dl, platelets (Plat)>350x10^9/L, leucocite (LEU)>11x10^9/L, immobilization, Charlson, IMC >35, use of dexamethasone, radiotherapy or catheters, VTE.

Results: We analyzed 94 subjects, median age 52.5 years (IQR 36.7 to 72 years). The most prevalent histologies were diffuse large B-cell lymphoma (LDCGB) (34%) and Hodgkin (EH) (23.4%). Three patients were under secondary antiplatelet prophylaxis for prior arterial ischemia. 34% of patients had one or more TRF. 3 (3,2%) patients presented VTE:

· Female 19 years old, EH 2B, plat>350x10^9/L, Hb <10gr/L, leu 11x10^9/L, doxorubicin. Upper limb thrombosis 22 months after diagnosis. Treatment LMWH 3 months.

· Patient 2: Female 74 years old, LDCGB 2A, plat>350x10^9/L, Hb <10gr/L, charlson 4. Lower limb thrombosis 8 months arter diagnosis. Treatment antivitamin-K 6 months.

· Patient 3: Male 40 years old, LDCGB 1A, plat>350x10^9/L, doxorubicin. Upper limb thrombosis 8 months after lymphoma. Treatment LMWH 3 months. Digestive hemorrhage 20 days after iniciate anticoagulation.

Conclusions: Prevalence of VTE in patients with outpatient with lymphoma looks similar to the global lymphoma. Three VTE affected patients presented TRF. Upper limb thormbosis was the most frequent one, probably related with the local administration of chemotherapy.

PB 662 | Investigation of Plasma Microparticles in Patients with Type 1 Diabetes May Be Interfered by Elevated Endogenous Lactadherin Levels in this Patient Group

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Background: Type 1 diabetes is a prothrombotic state with elevated levels of procoagulant microparticles (MPs) in plasma. MPs may express phosphatidylserine (PS), a negatively charged phospholipid which can bind to coagulation factors and facilitate thrombin generation. Lactadherin is a membrane glycoprotein that binds to PS with high affinity and is used to detect PS expression on MPs.

Aims: To investigate levels of PS+ MPs in patients with type 1 diabetes and healthy controls and how this relates to endogenous lactadherin levels.

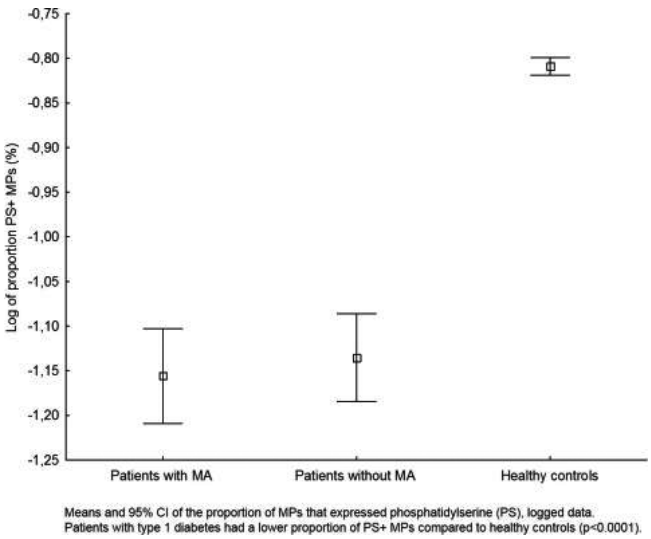

FIGURE 1 Proportion of total microparticles (MPs) expressing phosphatidylserine (PS+) among patients with type 1 diabetes and healthy controls.

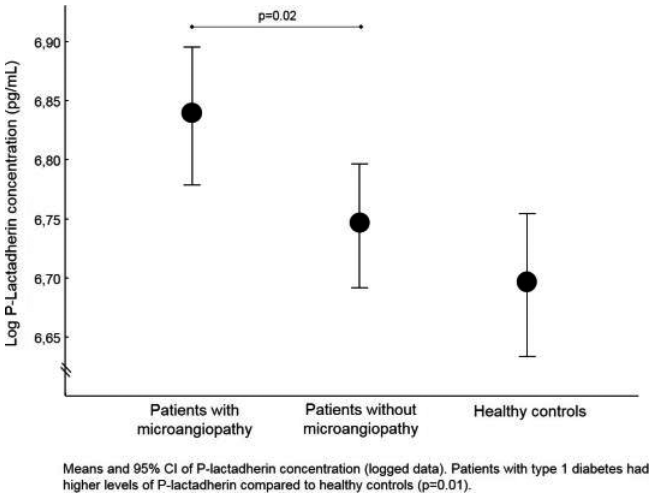

FIGURE 2 Endogenous plasma lactadherin levels in patients with type 1 diabetes and healthy controls.

Methods: We recruited 236 patients with type 1 diabetes aged 20 to 70 years with no history of cardiovascular disease, and 100 healthy individuals matched for age, BMI and sex. Plasma MP levels were assessed by flow cytometry, with lactadherin used as a marker of PS expression. Endogenous P-lactadherin levels were measured using ELISA (R&D systems, UK).

Results: Patients had higher levels of total circulating MPs compared to controls (41.5 (IQR 24.6 - 68.5) x 10^9/L vs. 23.2 (IQR 15.3 - 31.8) x 10^9/L, p< 0.0001), while the proportion of PS+ MPs was lower among patients compared to controls (p< 0.0001), see Figure 1.

Patients with type 1 diabetes had higher P-lactadherin levels compared to controls (median 874 pg/mL (IQR 747-1011) vs. 828 pg/mL (IQR 683-959), p=0.01). In addition, patients with microangiopathy (n=106) had higher P-lactadherin than patients without microangiopathy (p=0.02), see Figure 2. There was no significant correlation between P-lactadherin and number of PS+ MPs.

Conclusions: In our study, patients with type 1 diabetes had higher levels of circulating MPs compared to healthy controls, while a smaller proportion of MPs expressed procoagulant PS. However, the patients also had higher endogenous P-lactadherin levels, which could potentially bind to PS on MPs and interfere with analysis, giving false low PS positive MP levels. Future studies should take endogenous levels of P-lactadherin into account when analyzing PS expression on MPs using lactadherin as a marker.

PB 663 | Circulating Platelet Microparticles with Regards to Microvascular Complications and Sex Differences in Patients with Type 1 Diabetes

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Background: Patients with type 1 diabetes have high rates of vascular complications and women with diabetes lose their natural protection against cardiovascular disease. Platelet microparticles (PMPs) play an important role in inflammation and thrombosis, and high levels of circulating PMPs have been noted in patients with type 1 diabetes.

Aims: The aim of this study was to investigate the role of PMPs in the pathogenesis of diabetic microangiopathy.

Methods: We investigated PMP levels in plasma samples of 236 patients (107 women) with type 1 diabetes using flow cytometry and monoclonal antibodies against Glycoprotein IX (CD42a). The patients were aged between 20 to 70 years and had no history of cardiovascular disease. 106 patients had microvascular complications and 130 patients had no evidence of microvascular complications except for simplex retinopathy. PMP levels of 100 healthy individuals matched for sex, age and BMI were also analyzed. Data were log transformed and analyzed by independent t-test or ANOVA and contrasts.

Results: Patients with type 1 diabetes had higher levels of circulating PMPs compared to healthy controls 11.35 (IQR 7.29 - 19.49) x 109/L vs. 9.23 (IQR 5.68 - 12.10) x 109/L), p< 0.0001). PMP levels did not differ between patients with and without microvascular complications. However, healthy women had significantly lower PMP levels compared to healthy men (p=0.03), whereas PMP levels between men and women with type 1 diabetes were similar, see figure.

Conclusions: Our study confirms previous findings that patients with type 1 diabetes have higher PMP levels compared to healthy controls, but shows no clear relation between PMPs and microvascular complications. Interestingly, the favorable lower levels of PMPs among women compared to men among healthy controls are absent in women with type 1 diabetes. If this sex difference is of importance for the higher risk of cardiovascular disease in women with type 1 diabetes has to be investigated further.

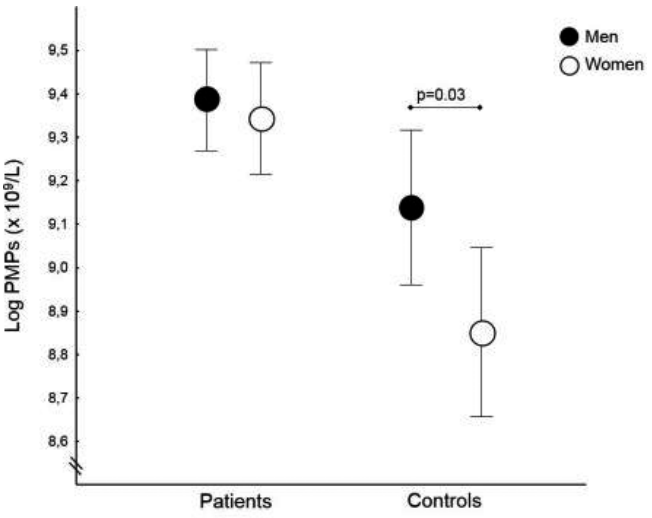

FIGURE 1 Platelet microparticles (PMPs) in men and women with type 1 diabetes and healthy controls

PB 664 | Effects of Low- and High-dose Doxorubicin and Paclitaxel on Tumorigenic, Thrombogenic and Angiogenic Properties of Extracellular Vesicles Derived from Breast Cancer Cell Lines

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Background: Extracellular vesicles (EVs) are shed from membranes of both normal and malignant cells. Various types of cell stimulation, including exposure to drugs, can affect the EV profile.

Aims: Eexplore the properties of EVs derived from breast cancer (BC) cells following exposure to high or low doses of chemotherapeutic drugs used for BC treatment and to evaluate ,thrombogenic and angiogenic effects of these EVs on endothelial cells (EC).

Methods: EVs were isolated from BC cell lines (MCF7 and MDA231), pre-exposed to increasing doses of doxorubicin or paclitaxel**.** Structure and size of cells and EVs were studied using electron microscopy and nano-tracking analysis. Antigen levels were measured by FACS. EV effects on EC thrombogenicity, proliferation, migration and tube formation were assessed by FACS, FXa chromogenic assay, XTT method and time-lapse microscopy.

Results: BC cell stimulation caused massive EV shedding. EV populations expressed reduced levels of the tumorigenic marker EpCAM compared to their parental cells. MDA231-EVs obtained after high-dose stimulation with both drugs demonstrated a 6-fold increase in procoagulant activity. High-dose doxorubicin induced a 2-fold increase in the expression of negatively charged phospholipids and eliminated TFPI from MDA231 EVs (P< 0.001). BC-EVs used nanotubes as a bridge for intra-cellular communication, and penetrated into ECs. EVs obtained from both BC cells increased EC thrombogenicity. EVs derived from doxorubicin-stimulated MDA231 cells induced EC proliferation and migration. EVs originating from both cell lines exposed to high-dose paclitaxel restricted EC proliferation and migration. EVs derived from high dose of paclitaxel-stimulated MDA231 cells induced massive tube formation similar to stimulation with VEGF.

Conclusions: These in vitro findings suggest that the tumorigenic, thrombogenic and angiogenic properties of cancer cell EVs are dependent on the type and dose of the applied agent, which could have relevance to tailoring chemotherapy

PB 665 | Functional Tissue Factor Activity on Endothelial Cells Conveyed by Bloodcell Microparticles

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Background: Microparticles (MP) can transfer their surface markers to other cells. Tissue factor (TF) can be detected on the surface of cells without mRNA upregulation, suggesting that TF is conveyed by MP. Systemic inflammation triggers higher levels of TF, resulting in a pro-coagulant state *in vivo*, contributing to dysregulated haemostasis in patients with inflammatory syndromes.

Aims: The aim of this project is to test MP derived from human primary blood cells and cell lines concerning their ability to induce functionally active TF on endothelial cells (EC) and identify the cellular source of the most potent MP.

Methods: MP were isolated from red blood cells, platelets, peripheral mononuclear cells and granulocytes of healthy donors. Endothelial MP were obtained from human umbilical vein EC (HUVEC). MP from untreated cells versus TNFalpha stimulated cells were compared to model inflammation. FIIa generation of the isolated MP was tested against a phospholipid standard for quantification. To test FX activation, HUVEC were cocultured with cellspecific MP for six. Total TF levels of MP, supernatants and cell lysates were tested.

Results: MP isolated from TNFalpha treated cells had higher procoagulant activity. Functional TF was detected both on MP and treated EC. FX activation on EC was elevated when incubated with MP from inflamed cells compared to MP from untreated cells. TF-specific FX activation was inhibited by antiCD142. Total TFlevels were higher in isolated MP derived from TNFalpha treated cells. Correspondingly total TF concentration was elevated in EC lysates and supernatants after stimulation with MP of stimulated cells. Leucocyte MP showed the highest procoagulant potential.

Conclusions: HUVEC exposed to MP of TNFalpha treated EC and leucocytes show the highest TF levels. The data suggest that TF is not only transferred from MP but might also stimulate expression of TF. This test system allows an *in vitro* evaluation of the interaction between MP and the endothelium of procoagulant mechanisms.

PB 666 | Impact of All Trans Retinoic Acid on the Plasmin Generation of Leukemic Cells and Microparticles in Acute Promyelocytic Leukemia

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Background: Acute promyelocytic leukemia (APL) is associated with a high rate of coagulopathy leading to hemorrhagic events. We previously demonstrated that microparticle-derived from APL blast cells (APL-MPs) have the capacity to generate plasmin in an

urokinase-dependent manner. All Trans Retinoic Acid (ATRA) is the main treatment associated with chemotherapy in this disease.

Aims: The aim of this study was to investigate the influence of ATRA on the plasmin generation capacity of leukemic cells and microparticles in acute promyelocytic leukemia

Methods: To that aim, NB4 cells (APL cell line) were cultured with or without ATRA for 5 days. Plasmin generation capacity (PGC) from both cells and MPs were measured using a functional chromogenic assay. MP-PGC from 20 APL patients were compared before and after ATRA treatment.

Results: A 5 day-ATRA treatment significantly decreased the NB4 cell proliferation compared to control without ATRA ($3.2x10^5$*vs* $1.2x10^5$ cells, p=0.01) Interestingly, NB4 cells generated also significantly less plasmin in presence of ATRA compared to cells cultured without ATRA (20 *vs* 745 mOD/min, p=0.03) whereas no qualitative difference was observed on NB4-MPs. However, a significant increase of MP counts was observed in the supernatant of treated cells ($3.5x10^6$*vs* $0.9x10^6$ MPs/ml, p=0.04) resulting in a global increase in the NB4-MP PGC after release of the cellular plasmin activator on MPs. When, the MP-PGC of 20 APL patients was compared before (J0) and after ATRA treatment (J7-J14), the plasmin generation was significantly decreased (6.9 [4.8-9.0] mOD/min *vs* 5.1 [3.5-6.2] mOD/min, p=0.009) which may be explained both by the impact of ATRA on APL cells and the short half time of APL-MPs in the circulation.

Conclusions: Altogether these data show for the first time an impact of APL treatment by ATRA on microvesiculation which could contributes to reduce the risk of coagulopathy.

PB 667 | Circulating Microparticles Trend in Patients with HCV-related Liver Cirrhosis who Underwent Direct-acting Antiviral Therapy

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Background: Plasma microparticles (MP) have been reported in cirrhosis. Since they own the ability of induce the activation of coagulation cascade, high MPs levels have been associated with a higher risk of portal vein thrombosis (PVT).

Aims: To investigate the presence of MP in patients with HCV related-cirrhosis treated with direct-acting antiviral (DAA) therapy as well as to correlate the MP profile changes with the risk of PVT development.

Methods: Patients with HCV related-cirrhosis treated with DAA were prospectively enrolled. Plasma levels of Annexin V-MP, endothelial (E)-MP, platelet (P)-MP and tissue factor (TF) bearing MP were measured by cytofluorimetry at baseline, at the end of therapy and 12 weeks (12W) after the end of therapy. During follow-up, PVT onset was recorded. Fifty healthy subjects were enrolled as controls.

Results: Sixty patients were enrolled (Child A/B 50/10). All of them reached 12W after the end of therapy follow-up. Baseline median levels of Annexin V-MP (6640 MP/μL) and TF-bearing MP (32 MP/μL) were significantly higher than end of therapy levels (4280 MP/μL, p< 0.05; 24 MP/μL, p< 0.01, respectively) and 12W after end of therapy-levels (2747 MP/μL, p< 0.01; 21 MP/μL, p< 0.01, respectively). A statically significant increase in E-MP levels was observed after antiviral therapy (12W after end of therapy: 1269 MP/μL in comparison to baseline levels 395 MP/μL; p< 0.05). Baseline median levels of P-MP (164 MP/μL) were higher than 12W time point-levels (125 MP/μL), however the difference was not statistically significant (p=0.15). Median follow up was 9 months (4-14). One PVT episode was recorded (incidence 2%).

Conclusions: Eradication of HCV is associated with a significant change in Annexin V and endothelial MP profile, possibly related to both reduction of inflammatory systemic condition and to improvement of liver function. This amelioration may partially correct the disequilibrium of the hemostatic imbalance of liver cirrhosis, leading to a reduction in the risk of PVT development.

PB 668 | P2Y1 and P2Y12 Antagonists Mediate the Release and Composition of Platelet Extracellular Vesicles

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Background: Activated platelets contribute to inflammation and thrombosis by release of platelet extracellular vesicles (PEV). Because the P2Y1 and P2Y12 receptors for adenosine diphosphate (ADP) regulate platelet activation, we hypothesize that inhibition of these receptors affects the concentration and composition of PEV.

Aims: To investigate the role of platelet ADP receptors in the release of PEV.

Methods: Platelet-rich plasma from 6 healthy volunteers was incubated with saline, P2Y1 antagonist MRS2179 (100 μM), and/or P2Y12 antagonist ticagrelor (1 μM). Platelets were activated by ADP (10 μM) under stirring conditions at 37 °C. The reactivity was assessed by impedance aggregometry. Concentrations of PEV exposing glycoprotein IIIa (CD61), P-selectin (CD62p) and phosphatidylserine (PS) were determined by a state-of-the-art flow cytometer (A60-Micro, Apogee). Size distributions of PEV were obtained by calibration with nanoparticles of known optical properties and light scattering theory. Data were analysed using Student´s t-test.

Results: ADP-induced aggregation was decreased 73% by P2Y1 antagonist, 86% by P2Y12 antagonist, and 95% by both antagonists (p<

0.001 for all).The release of PEV participating in haemostasis ($CD61^+$/$CD62p^-$/PS^-; 3.1±0.8x10^8 events/ml) was inhibited by 50% in presence of both antagonists (p=0.003). The release of proinflammatory and prothrombotic PEV ($CD61^+$/$CD62p^+$/PS^+; 2.4±1.6x10^7 events/ml) was unaffected by P2Y1 antagonist (p=0.59), but decreased 62% by P2Y12 antagonist (p=0.04) and 72% by both antagonists (p=0.03). The size distributions confirmed that flow cytometry measured PEV ranging from 150 nm to 1000 nm in diameter.

Conclusions: Inhibition of P2Y1 and P2Y12 receptors affects the release of distinct subpopulations of PEV upon activation by ADP. Our findings suggest that patients treated with P2Y12 antagonists have decreased concentrations of proinflammatory and prothrombotic PEV, and thereby attenuated inflammation and thrombosis.

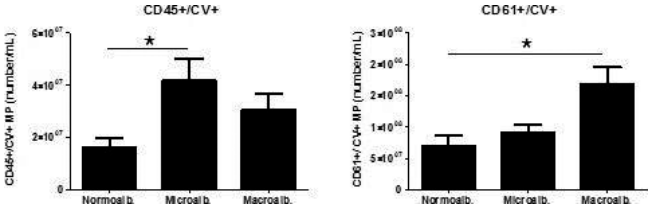

FIGURE 1 CD45+ and CD61+ mirovesicles in plasma. *p<0.05 with ANOVA

possibly reflects the pro-coagulant and pro-inflammatory state which accompanies DN. More research will be needed to further investigate the role of these MV in the progression of DN.

PB 669 | Characterization of Plasma Microvesicles in Patients with Diabetic Nephropathy

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Background: Diabetic nephropathy (DN) is a major complication of diabetes and is characterized by a hyper-coagulative and -inflammatory state. Microvesicles (MV) are small cell-derived vesicles that are secreted under physiological conditions, including inflammation and coagulation. MV numbers, size, cellular origin, composition and function can alter during disease. Evidence shows that MV can actively regulate cellular processes influencing disease progression.

Aims: To characterize plasma MV in type 2 diabetes mellitus (T2DM) patients with albuminuria (alb.).

Methods: Thirty T2DM patients were divided in three groups based on 24h urinary albumin levels; normoalb. (< 30mg/day), microalb. (30-300mg/day), and macroalb. (>300mg/day). MV from citrated plasma were stained with the live-cell labeling dye Calcein violet AM (CV), and simultaneously labelled for cell surface molecules CD14, CD235a, CD34, CD3, CD45, CD61, CD62p, CD62e, CD66b or IgG1 control antibody. The number of double positive MV were determined using flow cytometry (Apogee). This study was approved by a medical ethics committee and informed consent was obtained from all patients.

Results: The number of $CD61^+$ (platelet) MV were increased in patients with macroalb. compared with normoalb. patients (figure). Also the number of $CD45^+$ (leukocyte) MV were increased in patients with microalb. (figure), compared with normoalb. patients. In addition, both micro- and macroalb. patients showed mildly increased MV that are $CD14^+$ (monocyte) and $CD235a^+$ (erythrocyte), however, increased $CD62e^+$ (endothelium) MV were observed in microalb. patients only.

Conclusions: Our data show that MV from T2DM patients with micro- and macroalb. display different profiles of cellular origin. DN patients have increased numbers of leukocyte- and platelet derived MV, which

PB 670 | Coagulo-fibrinolytic Properties of Circulating Microparticles, and Subsequent Intravascular Coagulation, in Women with Ovarian Hyper Stimulation for *In vitro* Fertilization

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Background: Ovarian hyper stimulation for *in vitro* fertilization (IVF) is a risk factor for intravascular thrombosis during the first trimester of pregnancy. Several modifications of coagulation factors have been invoked to explain the procoagulant state following IVF; however, the involved mechanisms remain poorly understood. Recently, microparticles (MPs) have emerged as new cell-derived effectors with a key role in regulating haemostasis.

Aims: We aimed to determine if MP circulating subsets and activities are associated with the procoagulant state observed after ovarian hyper stimulation for IVF

Methods: Fifty one women were included in a prospective cohort study. Blood sampling was scheduled at 6 different times of the IVF cycle, including day 3 basal hormonal assessments, first day of FSH stimulation, ovarian triggering day, day 2 embryo transfer, mid-luteal phase, and pregnancy test. For each sample, MP circulating subsets, MP functional properties (Tissue Factor-Dependent Procoagulant Activity (MP-TF), Plasmin Generation Capacity (MP-PGC)), Fibrin monomer and D-dimer were measured.

Results: MP subsets showed no quantitative variation throughout IVF cycle. In contrast, MP-TF showed an early burst in activity, from 13.2 [5.9-20.3] fM to 32.5 [17.3-56.7] fM, limited to the time of embryo

transfer (p=0.01). A wave of MP-PGC subsequently occurred during the luteal phase, with maximum activity of 2.8 [1.6-4.6] mDO/min, compared to basal activity of 1.7 [1.2-2.7] mDO/min (p=0.028). This thrombolytic reaction was significantly associated with the peak of TF-MPs activity (r^2=0.63, p=0.001). Finally, a subclinical intravascular clot formation and degradation followed as showed by a significant increase in fibrin monomer (p=0.001) and D-dimer (p=0.001), at mid to late-luteal phase.

Conclusions: We highlighted for the first time a procoagulant process, occurring throughout IVF cycle, for which microparticles are mediators or early markers.

PB 671 | Immune Response Favors the Release of Leukocyte-derived Microparticles Mediating Endothelial Senescence, and Pro-coagulant and Pro-inflammatory Phenotype

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Background: Microparticles (MP) are surrogate markers of vascular endothelial cell injury and possible pro-inflammatory and pro-coagulant mediators in degenerative diseases with thrombotic disorders. MP expose phosphatidylserine that enhances the activity of tissue factor (TF) expressed by inflamed endothelium and activated leukocytes. Leukocyte-derived MP (LMP) are key in the coupling between thrombosis and inflammation.

Aims: To define the LMP-driven signalling pathways in endothelial cells (ECs) and to identify the LMP subsets involved in the crosstalk.

Methods: MP (1-30 nM) generated from isolated spleen rat leukocytes (LMP) by 24 h incubation with 5 mg/ml LPS or 25 ng/ml PMA + 1 μM A23187 ionophore, were applied to porcine coronary ECs. MP integration (PKH26), target cell apoptosis (a-5/IP), senescence (C12FDG SA-β-galactosidase probe), ROS formation (dihydroethidium probe) were assessed by flow cytometry, the expression of proteins by western blot.

Results: After 24h, a maximum of 82% ECs integrated $PKH26^+$-LMP. After 48 h, LMP induced a significant raise in SA-β-galactosidase activity in young ECs (18±5 *vs* 58±6 MFI) with p53, p21, p16 up-regulation, the two latter showing significant differences between LPS and PMA + A23187 stimuli. The 2-fold raise in NADPH oxidase expression and 3-fold down-expression of eNOS indicated MP-mediated oxidative stress. LMP prompted VCAM1, ICAM1, COX-2, TF, AT1, ACE up-regulation, indicating a pro-inflammatory and pro-coagulant phenotype, and a secondary generation of procoagulant ECs-derived MP. LMP induced MAPKs phosphorylation. The absent ECs apoptosis indicated a specific pro-senescent action.

Conclusions: LMP induce oxidative stress, premature senescence, pro-coagulant and pro-inflammatory responses in ECs. Because alteration of ECs function prematurely or with age could impair graft success, our data bring new information on circulating leukocyte MP as possible contributors to endothelial dysfunction in response to innate or adaptive immune system.

PB 672 | Increase in Microparticles TF/TFPI Procoagulant Ratio in Carriers of Inherited Bleeding Disorders

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Background: Although the presence of Microparticles (MPs) has been widely described in hypercoagulable states, their role in haemorrhagic disorders has been poorly evaluated.

Aims: To evaluate the presence and impact of prothrombotic MPs in congenital bleeding disorders.

Methods: Seventy-one consecutive carriers of inherited bleeding disorders [18 with haemophilia A, 3 with haemophilia B, 19 with factor (F)VII deficiency, 10 with FXII deficiency, 8 with FV deficiency, 8 with hypo/dysfibrinogenemia, and 5 with FXIII deficiency] were enrolled. Samples were performed prior to any administration of factor replacement therapy or plasma. Seventy healthy volunteers, age and gender-matched, unrelated to the cases acted as controls. MPs expressing phosphatidylserine (PS), tissue factor-bearing MPs (TF+MPs) and TF pathway inhibitor-bearing MPs (TFPI+MPs) were measured by flow-cytometry. The ratio of the TF/TFPI expression levels was calculated as the MPs procoagulant potential.

Results: Cases showed significantly higher median levels of PS-MPs (3608 [1666-5750] MPs/uL) than controls (2042 [985-3853] MPs/uL, p=0.04) The TF/TFPI MPs ratio was higher in cases (1.19 [0.9-1.4]) than in controls (0.94 [0.85-1.13], p=0.02). Compared to controls: FVII deficiency and hypo/dysfibrinogenemia showed reduced levels of TFPI+MPs (p=0.002) and an increased ratio (p=0.019); FXII deficiency showed higher levels of PS-MPs (p=0.035) and an increased ratio (p=0.015); FV deficiency (two homozygous) showed reduced MPs levels and ratio (p=0.04); FXIII deficiency carriers (one homozygous) presented higher PS-MPs (p=0.0139) levels and a reduced ratio (p=0.02); Haemophilia A and B showed significantly higher levels of PS-MPs (p=0.002) and no difference in other MPs.

Conclusions: Patients with congenital bleeding disorders had an increased TF/TFPI MPs ratio. MPs may constitute a procoagulant "rescue" mechanism that contribute to inhibit the TFPI pathway and protect carriers from bleeding diathesis.

PB 673 | Factor Xa Inhibitors Apixaban and Rivaroxaban Suppress the Release of TF-bearing Microvesicles from Cancer Cell Lines

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Background: The release of procoagulant tumour-derived microvesicles into the bloodstream is associated with increased risk of thrombosis in cancer patients. One factor capable of promoting tissue factor (TF)-bearing microvesicle release is the activation of protease activated receptor 2 (PAR2) on the surface of cells. PAR2 is activated by factor Xa (fXa) and TF-factor VIIa (fVIIa) complex. Therefore the use of fXa inhibitors including Apixaban and Rivaroxaban may interfere with PAR2 activation and suppress the release of microvesicles.
Aims: To investigate the ability of Apixaban and Rivaroxaban to suppress the release of TF-bearing microvesicles from cancer cell lines.
Methods: MDA-MB-231 and AsPC-1 cell lines were activated with fXa (10 nM), PAR2-activating peptide (20 μM) or used without activation, in the presence and absence of therapeutic and sub-therapeutic concentrations of Apixaban (0-100 ng/ml) or Rivaroxaban (0-600 ng/ml). Microvesicles were collected from the conditioned media by ultracentrifugation and quantified. In addition, the released TF antigen, was measured by TF ELISA and microvesicle-associated TF activity was measured using a chromogenic thrombin generating assay, and by Calibrated Automated Thrombogram.
Results: Both test cells lines responded to activation by factor Xa or PAR2-AP releasing increased amounts of TF-bearing microvesicles. Pre-incubation of cells with therapeutic concentrations of either Apixaban or Rivaroxaban reduced the fXa-induced microvesicle release, and the associated TF antigen and activity, to levels comparable to those observed from non-activated cells. In contrast, PAR2-AP activation remained unaffected by DOACs. Basal release of TF-bearing microvesicles was attenuated to a lower extent.
Conclusions: Both Apixaban and Rivaroxaban can suppress the release of microvesicles from cancer cell lines following fXa activation.

PB 674 | Endothelial Nitric Oxide Synthase Gene Polymorphisms Influence on Endothelial Microparticles Levels in Renal Transplantation

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Background: Endothelial Microparticles (EMPs) are noninvasive cellular markers of endothelial function. Origin and size of MPs may predict the intensity of inflammation. Nitric oxide is an important vasodilator produced by endothelial cells. Decrease of NO levels may induce the occurrence of allograft injury in renal transplanted patients. eNOS gene polymorphisms can influence NO production and release
Aims: We investigated the relationship between eNOS gene polymorphisms and EMPs(number and size) in Renal Transplanted recipients (RTx).
Methods: Ninety-two renal transplant recipients were included. The polymorphisms in eNOS gene were determined by simple polymerase chain reaction (VNTR b/a) and polymerase chain reaction-restriction fragment-length polymorphism (G894T and T786C) analysis. EMPs were tested by flow cytometry (annexinV$^+$/CD51/61$^+$). Calibration beads were used to distribute the EMPs according to their size (≤or>0.7μm). Groups were distributed according to genotype and model allelic carriers model. Results are presented as median and interquartile range (Kruskall-Wallis followed by Dunn`s and Mann-Whitney U-test). p< 0.05 significative.

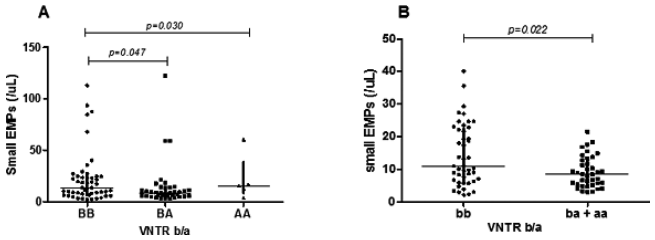

FIGURE 1 Levels of small EMPs according to polymorphisms of eNOS gene in Kidney Transplanted Recipients. (A: genotype. B: Allelic carrier model)

Results: The distribution of genotypes for the 3 polymorphisms studied showed no deviation from Hardy-Weinberg equilibrium (all P>0.05). EMPs numbers were not different according to G894T, VNTR b\a and T786C polymorphisms (p=0.749, 0.908 and 0.306). For EMPs size, we found more small EMPs (≤0.7μm) in bb (13.30;IQR=17,70) genotype (VNTR b\a) compared to ba (8.47; IQR=8.03)(Fig.1A). According to allelic carrier, presence of b (bb,13.30;IQR=17.76) showed higher number of small EMPs relation to allelic a (aa + ba, 8.96;IQR=15.6) (Fig 1B)
Conclusions: Since the genetic inheritance pattern of the eNOS polymorphisms is not known. Our data showed an association of VNTR b/a with small EMPs levels. The presence of allele b was related to higher levels of small EMPs and it reflects a pro inflammatory profile, characteristic of renal transplant patients. **Support**: Capes, CNPq, FAPEMIG

PB 675 | Effects of Chemotherapy on Extracellular Vesicles and Coagulation Activation in Colorectal Cancer Patients

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Background: Cancer patients are at risk of thrombosis and exhibit a hypercoagulable state. Extracellular vesicles (EV) which affect coagulation activation by exposing tissue factor (TF) and by providing a phospholipid rich surface are elevated in patients with colorectal cancer (CRC) (Hron, Thromb Haemost 2007).
Aims: We aimed to evaluate the long-term effect of chemotherapy (ChTx) on number and source of EV and D-Dimer as a parameter of coagulation activation.

TABLE 1 Number of extracellular vesicles (EV), levels of D-dimer and CEA in venous blood of colorectal cancer patients before and during chemotherapy

	Before 1st ChTx (baseline)	Before 2nd ChTx	p-value 1st vs 2nd	Before 3rd ChTx	p-value 1st vs 3rd
n	41	40		35	
EV (x 10^3 mL^{-1})	429 (288; 664)	376 (225; 584)	0.13	255 (181; 474)	0.007
PLT+EV (x 10^3 mL^{-1})	216 (129; 287)	171 (104;248)	0.056	119 (74; 210)	0.001
% of EV	50	45		47	
TF+EV (x 10^3 mL^{-1})	19 (10; 38)	20 (9; 32)	0.58	15 (9; 29)	0.101
% of EV	6	7		6	
D-Dimer (µg mL^{-1})	0.99 (0.48; 2.52)	0.69 (0.50; 3.06)	0.95	0.99 (0.52; 2.17)	0.95
CEA (µg L^{-1})	9.4 (1.9; 42.8)	9.7 (2.6; 70.6)	0.086	9.4 (3.0; 54.9)	0.39

Methods: Advanced CRC patients receiving 5-fluorouracil based ChTx were eligible. The number of EV was assessed by flow cytometry in fresh platelet poor plasma obtained from venous blood collected immediately before ChTx. EV were defined by size (forward scatter, < 1 µm) and annexin V binding and labeled using antibodies (anti-CD41a: platelet positive EV [PLT+EV]; anti-CD142: TF positive EV [TF+EV]). D-Dimer was assessed by ELISA. The paired *t*-test was used to compare baseline (BL) levels with levels obtained before ChTx. Data are given in absolute numbers (median [quartiles]) if not otherwise stated.
Results: 41 patients (mean age 64 years, 68% men) were included and 35 patients completed 3 cycles of ChTx. Table 1 shows the number of EV, the levels of D-dimer and CEA at BL and before 2nd and 3rd ChTx, respectively. EV significantly decreased from 429 (288; 664)x10^3 mL^{-1} at BL to 255 (181; 474)x10^3 mL^{-1} before the 3rd cycle. PLT+EV significantly decreased from 216 (129; 287)x10^3 mL^{-1} at BL to 119 (74; 210) x10^3 mL^{-1} before the 3rd cycle. The proportion of PLT+EV ranged from 45%-50% throughout ChTx. Number and proportion of TF+EV were small at all time points. D-dimer levels were 0.99 (0.48; 2.52) µg mL^{-1} at BL and did not decrease over the course of ChTx. D-dimer levels did not correlate with the number of EV.
Conclusions: In patients with advanced CRC, ChTx attenuates coagulation activation as indicated by a decline of the number of EV.

PB 676 | The Impact of the Eculizumab on the Thrombogenicity Induced by Extracellular Vesicles in Paroxysmal Nocturnal Hemoglobinuria Patients

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by a complement-mediated hemolysis. Complement attack induces extracellular vesicles (EV) production. These EV can be implied in thrombus formation, the leading cause of death in PNH patients. Eculizumab, an anti-C5 monoclonal antibody, decreases the thrombosis frequency in PNH.
Aims: We wanted to assess the impact of eculizumab on the EV quantification and on their procoagulant activity. The purpose is to check, if the antithrombotic activity of the eculizumab could be, in part, explained by its interaction with the EV.
Methods: We recruited 6 PNH patients. Informed consents were obtained for each patient. The study was led according to the declaration of Helsinki and approved by the local Ethic Committee. We collected platelet free plasma (PFP) for each patient before the start of eculizumab, after 4 weeks and after 11 weeks of treatment. We assessed the amount of platelets EV (flow cytometry), the procoagulant activity on PFP (Thrombin generation-TGA), provided by phospholipids (STA®-Procoag-PPL) and by isolated EV (TGA). We used mixed-effects linear regression (R 3.1.2 with nlme package) with logarithmic transformation for flow cytometry results.
Results: We compared the results obtained after 4 weeks or after 11 weeks of treatment compared to the value before the start of the treatment. The results on the patient's PFP show a decrease in the amount of platelet EV with the treatment. About the procoagulant activity of the EV, we observed a decrease in the procoagulant profile (PL and EV-induced) with the eculizumab.
Conclusions: Eculizumab can play its anti-thrombotic role by an action on the EV. With a bigger cohort, we could estimate a threshold value of the procoagulant activity induced by the EV. The purpose will be to measure the EV activity by STA®-Procoag PPL or TGA on isolated EV in order to assess the thrombotic risk of PNH patients treated with eculizumab.

PB 677 | Magnetic Capture of Extracellular Vesicles: A Quantitative Flow Cytometry Study

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Background: Cells release small membrane vesicles, called microparticles, exosomes or extracellular vesicles (EV), which attract interest for their diverse properties and potential applications. However, EV found in body fluids are heterogeneous, and there is yet no reliable method allowing the isolation of selected EV populations.

Aims: We aim at developing a simple method of EV isolation, based on their specific capture with magnetic particles (MagP) conjugated with EV ligands. We focused first on EV exposing phosphatidylserine (PS), a major EV population in plasma[1] with procoagulant properties. EV derived from lyzed red blood cells (L-RBC) were used as a model of PS+ EV. The efficiency of EV isolation was evaluated qualitatively by electron microscopy (EM) and quantitatively by flow cytometry (FC).

Methods: Small (50 nm) MagP conjugated to annexin-5 (Anx5) were synthesized. FC and EM were performed on a Gallios (Beckman Coulter) and a CM120 (FEI), respectively.

Results: The specific binding of Anx5-MagP to L-RBC was demonstrated by EM. To quantify EV capture, we used two complementary approaches of FC, namely light-scatter triggering to detect large (~µm) EV and fluorescence triggering to detect small (~100 nm-1 µm) EV[2]. The influence of the following parameters on the efficiency of EV capture was investigated:

1) concentration of Anx5-MagP (from 10^{11} to 10^{15} MagP/L),

2) ratio between RBC and Anx5-MagP concentrations,

3) conditions of extraction.

The relationship between the amount of captured PS+ EV and the total concentration of Anx5-MagP was determined. At maximal Anx5-MagP concentration (10^{15}/L), nearly 100% PS+ EV -both large and small- were captured up to 10^5 L-RBC/µL.

The protocol of extraction optimized with L-RBC is currently applied to PS+ EV from plasma.

Conclusions: This method of isolation is simple, quantitative and of general application, opening the way toward EV omic analysis and biomarker discovery.

1 Arraud et al., *J. Thromb. Haemost*. 2014;12:614-27

2 Arraud et al., *Cytom. A* 2016;89:184-95

PB 678 | Procoagulant Extracellular Vesicles in Amniotic Fluid

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Background: Embolization of amniotic fluid (AF) into the blood circulation leads to disseminated intravascular coagulation (DIC). Procoagulant phosphatidylserine (PS)- and tissue factor (TF) exposing extracellular vesicles (EVs) might play an important role in AF embolism induced DIC.

Aims: It was the aim of the present study to perform analyses of the procoagulant properties of AF with a panel of functional coagulation assays and flow cytometry.

Methods: We applied a prothrombinase assay (that quantifies PS exposure on EVs), an EV-associated TF activity assay, a fibrin generation assay, a thrombin generation assay, a whole blood clotting model and flow cytometry in AF and control plasma.

Results: We found that PS exposure on EVs was 21-fold increased in AF compared to plasma. Also EV-associated TF activity was highly increased in AF compared to plasma. AF derived EVs activated the blood coagulation cascade via PS and TF in the fibrin- and thrombin generation assay. In a whole blood clotting model AF derived EVs significantly shortened the clotting time from 734 ± 139 seconds in the presence- to 232 ± 139 seconds in the absence of an anti-TF antibody. The contact activation pathway via factor FXII was not affected. Applying flow cytometry, a sub-population of PS+ and TF+ EVs was identified in AF but not in control plasma.

Conclusions: We investigated the effect of AF on blood coagulation and found that PS+ and TF+ EVs determine its procoagulant potential. Taken together our data further delineate the pathomechanisms underlying AF induced coagulopathy.

PB 679 | Thrombin Generation Associated with Extracellular Vesicles is Increased Two Weeks Post-partum

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Background: In pregnancy, the incidence of venous thromboembolism (VTE) is highest 2-3 weeks post-partum. Contradictory to this, most of the procoagulant markers in plasma increase with time of pregnancy, peak right after delivery and then decrease. Extracellular vesicles (EVs) are suggested to be important contributors to thrombosis due to the presence of surface-bound tissue factor (TF) and phosphatidylserine (PS). The role of EVs in healthy pregnancy is unclear.

Aims: To measure thrombin generation (TG) associated with EVs, and in plasma, in 20 healthy pregnant women and in healthy controls.

Methods: Citrated whole blood was drawn from healthy pregnant women at 6 time-points (3 before delivery and 3 after delivery). Citrated plasma was prepared by centrifugation at 2500 g, 15 min and then frozen. TG was measured directly in thawed plasma (with PPP-reagent). Furthermore, EVs were isolated from citrated plasma by sequential centrifugation (17000 g, 30 min), added to pooled normal plasma (PNP), and EV-associated TG was measured. EV-bound PS activity was measured with the Zymuphen PS activity kit.

Results: In plasma, the TG parameters peak and ETP increased with time of pregnancy, peaked right after delivery and then decreased. On the other hand, EV-associated thrombin generation and EV-bound PS activity peaked 2 weeks after delivery.

Conclusions: In healthy pregnancy and puerperium, EV-associated TG, but not plasma-based TG, is increased two weeks post-partum, where also the incidence of VTE is the highest. Future studies will show if measurements of EV-associated thrombin generation may be useful to assess the thrombotic risk in healthy- and "at-risk" pregnancies.

PB 680 | Enumeration of Circulating Microparticles in Healthy Medical Workers Occupationally Exposed to Low Doses of Ionizing Radiation

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Background: Strong evidence suggests microparticles (MP) as a biomarker of vascular injury. It may also contribute to the initiation and the development of atherosclerosis. In fact, several biological mechanisms might be responsible of atherosclerosis caused by radiation exposure even at a low dose. Therefore, the measurement of MP might be of a great potential for providing new insight into low-dose radiation cardiovascular risk.

Aims: We investigated the effect of long-term medical occupational exposure to low doses of ionizing radiation (LDIR) on MP populations levels.

Methods: The current study was conducted in accordance with the Helsinki Declaration on 38 healthy medical workers occupationally exposed to LDIR and 29 matched controls by gender, age, and smoking habits. The measured MP by flow cytometry were classified as positive or negative phosphatidylserine (PS^+ or PS^-), and phenotyped according to their cellular origin.

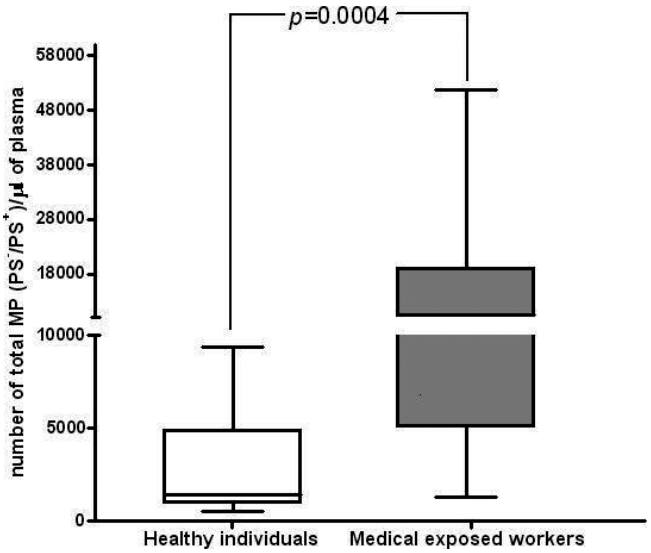

FIGURE 1 Levels of circulating total microparticles (PS−/PS+) in medical workers exposed to low doses of ionizing radiation and in healthy individuals

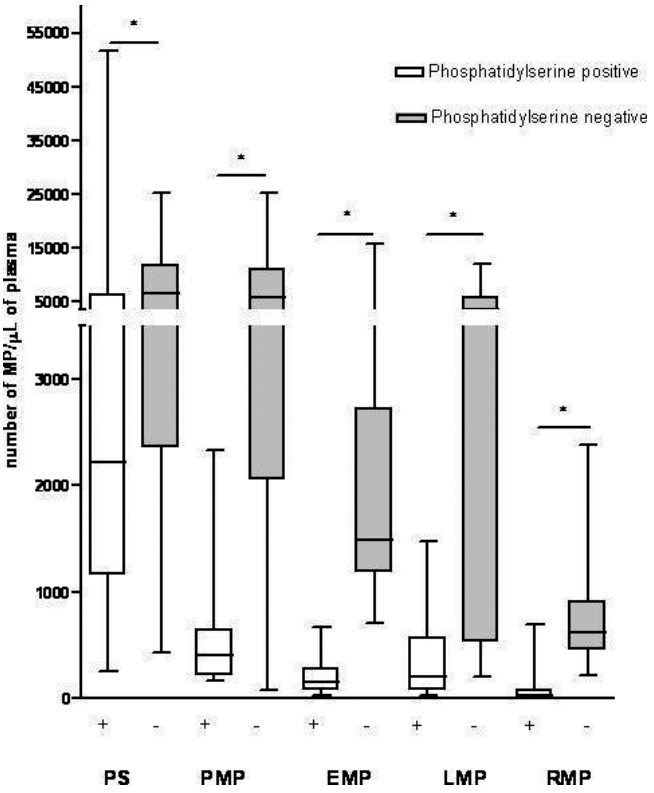

FIGURE 2 Distribution of phosphatidylserine negative and phosphatidylserine positive MP in medical workers exposed to LDIR

Results: Total MP (PS^-/PS^+), regardless of phenotype, were significantly higher in workers occupationally exposed to LDIR than controls (p=0.0004).

In medical exposed workers, PS^- MP predominated (68%) and were approximately three times more common than PS^+ MP.

Concerning the measurement of MP according to their cellular origin, we found that MP derived from platelets ($CD41a^+$ PMP), endothelial ($CD146^+$ EMP), leucocytes ($CD45^+$ LMP) and erythrocytes ($CD235a^+$ RMP) are more abundant in exposed workers than those in controls (p< 0.0001). However, no significant difference was found in the proportion of the other blood elements in the peripheral circulation between the two groups. In addition, no association was observed between MP levels and the studied confounding factors.

Conclusions: Our results suggest that elevated circulating MP populations levels represent an indicator of cellular damage caused by medical exposure to LDIR. By consequence, the quantification of MP seems to a useful biomarker for assessing the negative effects of occupational exposure to ionizing radiation.

PB 681 | A High Throughput Flow Cytometry Assay for the Detection of Platelet Microparticles

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Background: Platelet-derived microparticles (PMP) are crucial in disease states such as cancer, signalling through surface markers and promoting coagulation. There is a need for detection methods for PMP which are sensitive, specific and attainable by clinical and research laboratories.

Aims: To develop a high throughput and simple detection method for PMP.

Methods: A novel flow cytometry assay was adapted from previous methods (Nielsen et al., *J Extracell Vesicles*, 2014; 3, Pasalic et al., *Nanomedicine*, 2016; 12:977-86) on the BD LSRFortessa X-20 equipped with a volumetric 96-well plate sampler and 200mW 488nm laser. Platelet-free plasma (PFP, citrated blood, 2x15 min 2500g, stored -80°C) was stained with lactadherin (L)-FITC + antibodies for 20 min, diluted 1/10 in DPBS, and 15 µL sampled (0.5 µL/s). All buffers were 0.1 µm filtered. MP event collection was triggered on FITC fluorescence threshold to reduce noise. Negative control: ultracentrifuged plasma supernatant (22 min 19,610g then supernatant 47 min 158,397g). Positive control: platelet-rich plasma (PRP, citrated blood, 10 min 200g) stimulated with 20 µM ADP or 10 µM epinephrine for 2.5h at 37°C (n=4), then PFP prepared as above. Results are mean±SD, significance by paired t-test.

Results: Volumetric sampling accuracy was confirmed both by sampling a consistent volume of varying bead concentration and by sampling varying volumes of known bead counts (linear regression r^2>0.99 for each); run-to-run count beads were used per plate. Plasma PMP events were predominantly removed by prior ultracentrifugation (e.g. plasma L+/CD41+ count 1102 ± 270 before vs 104.3 ± 6 after spinning, p=0.02, n=3), indicating specific binding of fluorescent labels (Fig 1A-B). ADP or epinephrine with PRP (Fig 1C-D) increased CD41a+, CD61+, CD42b+, CD31+ and CD62P+ PMP plasma counts (e.g. ADP increased L+/CD41+ MP from 370±90 to 1012±211, p < 0.01).

Conclusions: This simple flow cytometry assay produces absolute counts of PMP in plasma, and shows sensitivity to changes in MP levels.

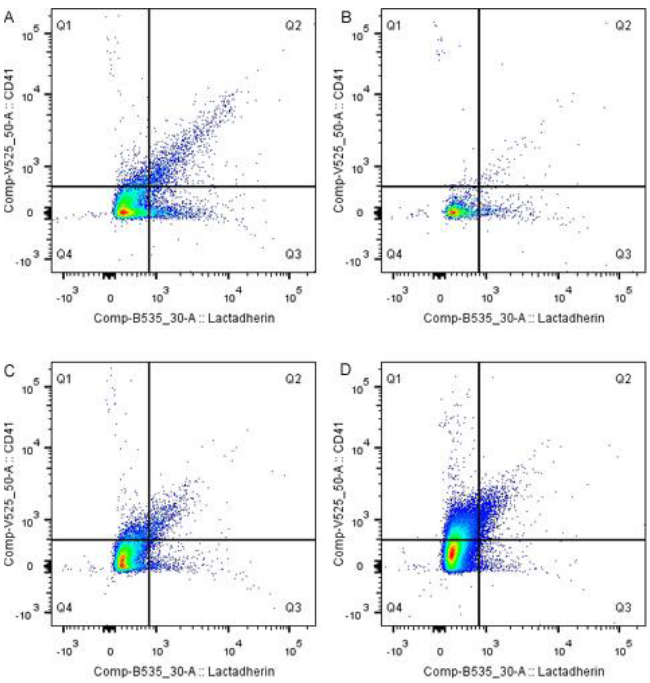

FIGURE 1 Plasma PMP events (A), showing 90% removed by prior ultracentrifugation (B). Plasma PMP from PRP (C) increased 270% by ADP stimulation (D).

PB 682 | Visualization of Tissue Factor-positive Microvesicles from Patients with Pancreatic Cancer Using Laser Scanning Confocal Microscopy

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Background: Elevated levels of microvesicle-tissue factor (MV-TF) activity is associated with venous thrombosis in patients with pancreatic cancer (PC) patients. There were not any methods to detect antigens on MVs. In a recent study, tissue type plasminogen-positive MVs were visualized using laser scanning confocal microscopy (LSCM).

Aims: To visualize and quantitate TF-positive MVs (TF+MVs) from the culture supernatant of PC cell lines and plasma of PC patients using LSCM.

Methods: MVs were collected from the supernatant of the high and low TF-expressing human PC cell lines (BxPc-3 and Panc-1, respectively), from untreated or lipopolysaccharide (LPS) treated whole blood, and from plasma of PC patients. MV-TF activity was measured using an in-house assay. MVs were labeled with 5(6)-carboxyfluorescein diacetate N-succinimidyl ester, which is changed to the green fluorescent molecule carboxyfluorescein inside the MVs. MVs were either captured using annexin V and detected using a fluorescent-labeled anti-TF antibody, or captured using an anti-TF antibody and detected using fluorescent-labeled annexin V. Tyramide signal amplification (TSA) was used for some studies.

Results: TF+MVs were detected from BxPc-3 cells using annexin V capture, whereas TSA was required to detect TF+MVs from Panc-1 cells. Visualization of TF+MVs in plasma from LPS treated whole blood and in plasma from PC patients required either capture with annexin V and detection with a fluorescent-labeled anti-TF antibody with TSA, or capture with an anti-TF antibody and detection with a fluorescent-labeled annexin V. The coefficient of determination of fluorescent signal and concentration of EVs or fluorescent signal of TF were R^2 = 0.642 and R^2 = 0.679, respectively.

Conclusions: We successfully visualized TF+MVs from the supernatant of PC cell lines and plasma of PC patients. LSCM may be a useful tool to characterize various antigens expressing on MVs from cell lines and clinical samples.

PB 683 | New Specific and Highly Sensitive Procoagulant Test to Measure Tissue Factor Activity on Microparticles

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Background: Tissue factor-dependent procoagulant activity of microparticles (MP-TF) is associated with an increase risk of thrombosis in different clinical settings. However, MP-TF remains difficult to measure due to a lack sensitivity of the current functional assays.

Aims: Therefore, the aim of this work was to develop a new test to measure MP-TF dependent procoagulant activity, optimizing sensitivity while maintening a good specificity.

Methods: The assay works on total MPs washed from platelet-free plasma (PFP) samples by high-speed centrifugation. FXa is generated by incubating MPs with FVII, FX and $Ca^{2}+$. FXa is measured by monitoring the degradation of a fluorogenic substrate. TF specificity is ensured by running the same MPs pre-incubated with control IgG or a high affinity blocking anti-TF antibody (SBTF1, BioCytex).

Results: A high sensitivity (< 3 fM) was obtained by using i) FVII instead of FVIIa (+47±3% at 10nM), ii) a supra-physiological dose (760nM) of factor X, iii) high initial volume of PFP: 500 µL, iv) low plasma viscosity before centrifugation by an optimal 1:2 dilution and v) a centrifugation of 24000g, 1h. A good specificity was maintained using SBTF1 which shows a more potent inhibition than HTF1 (94±1,7% versus 70±6,2% at 2,5µg/mL). Specificity of this antibody was confirmed by the absence of activity on MPs derived from knock out-TF cell line. Taken together, these optimizations allow us to detect a measurable level of MP-TF activity in normal PFP samples (~10 fM) and a significant increase of MP-TF activity after LPS activation of whole blood samples (1.2 to 58 fold, n=27, p=0,0077). The assay shows a good linearity (r^2 = 0,99) and a repeatability at 17±11%.

Conclusions: This optimized test specifically measures MP-TF due to specific inhibition by SBTF1. The high sensitivity given by optimal conditions allow to measure TF activity in normal samples and to demonstrate increased MP-TF activity after LPS activation of whole blood samples.

PB 684 | Circulating Microparticles in Women with Polycystic Ovary Syndrome: Preliminary Results

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Background: The impact of hormonal imbalance in the production of circulating Microparticles (MPs) is still a matter of debate.

Aims: The aim of our study was to evaluate the presence and cellular origin of circulating MPs in a consecutive cohort of women with and without polycystic ovary syndrome (PCOS).

Methods: Fifteen consecutive hyperandrogenic women and 23 healthy women age and BMI-matched were enrolled. Blood samples were collected during the luteal phase both in cases and in controls. Moreover, in cases we also collected sample in the follicular phase before (T0) and 24 h after (T24) stimulation test with GnRH analogue (Triptarelin 0.1 mg s.c.). MPs expressing phosphatidylserine (AnnexinV-MP), MPs derived from endothelial cells (CD62E+), platelet (CD61+), and tissue factor-bearing MPs (TF+) were measured by flow-cytometry.

Results: Hyperandrogenic women showed significantly higher median levels of Annexin V (p 0.001), endothelial-(p< 0.001), platelet-(p< 0.001) and TF+MPs (p 0.003) than healthy controls during the luteal phase. When we considered women with hyperandrogenism, we found that median levels of Annexin V MPs and platelet-MPs significantly increased at T0 compared to T24 (p=0.014 and 0.03, respectively). Moreover, levels of Annexin V and platelet-MPs were significantly higher at T24 versus basal levels (p=0.02 for both comparisons). Finally, we did not detect any difference in women who had confirmed PCOS after stimulation test (n.8) compared to women without PCOS diagnosis (n.7). In PCOS levels of MPs did not correlate with insulin levels, but Annexin V MPs positively correlated with androgen hormone levels (testosterone and dehydroepiandrosterone).

Conclusions: Our preliminary results suggest that hormonal disorders impair MPs levels in women. Moreover, hormonal stimulation seemed to affect mainly Annexin V and platelet-MPs.

PB 685 | Plasma Platelets Microparticles Evaluation in Thrombotic Thrombocytopenic Purpura

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Background: ´Platelet microparticles (PMPs)′ have a procoagulant activity about 100-50 times more than active platelets because of high expression of negative charge phospholipids on their surfaces. PMPs assay in TTP patients could be apply as a diagnostic biomarker of patients clinical status.

Aims: This study investigate the role of microparticles in thrombotic thrombocytopenic purpura by immunophenotyping and plasma PMPs count in Iranian patients.

Methods: We had 2 study groups, 15 TTP patients and 15 healthy control group, Microparticles prepared in two step by low (2000g) and high (18000g) centrifugation and then size confirmation, was done by ´Dynamic Light Scattering (DLS)´ Zetasizer, PMPs immunophenotyping was done by FITC Anti-Human CD41a, FITC Anti-Human CD42b, FITC Anti-Human CD61, FITC Mouse Anti-Human CD31

and PE Anti-Human CD62P, with FACS Calibur flow cytometer (BD, USA) and also PMPs count was determined by PE Anti-Human CD61, and Polysciences Microbeads (1 micron in diameter) with Partec-cyflow and results analyzed by FlowJo 7.6 (Treestar, USA) and Partec FlowMax software.

Results: Our results showed that the majority of microparticles in TTP patients and normal individuals were platelet-derived microparticles (PMPs) but indicated that PMPs in TTP patients originated from activated platelets because a high percentage of them were positive for CD62p (p-selectin). While in normal healthy controls PMPs had low expression of this activity marker. PMPs plasma level results also demonstrated that plasma PMPs level in TTP patients was higher than normal control group
(P-value< 0.001).

Conclusions: Our results from the count of microparticles showed that PMP's plasma level in TTP patients was significantly higher than normal healthy individuals, also we suggest that elevated PMPs level in plasma with considering the patients history, may be related to thrombotic events in TTP patients, but for confirmation of this hypothesis requires more evaluations and investigations with the larger size of study groups.

PB 686 | Endothelial and Platelet Microparticles in Kidney Transplanted Recipients: Influence of Time Post Transplantation and Donor Type

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Background: Microparticles (MPs) are membrane fragments shed from damaged or activated cells. Origin and size of MPs may predict the intensity of inflammation and the cell type involved in several conditions. Vascular endothelial cells of renal allograft are mainly recognized by receptor's immune system after transplantation and allograft rejection is the main cause of post-transplantation complication.

Aims: To investigate whether time post transplantation and donor type may influence on number and size of Endothelial (EMPs) and Platelet (PMPs) microparticles in kidney transplanted patients (KTx).

Methods: The study was conducted in accordance with the declaration of Helsinki and local committee of ethics. EMPs and PMPs were isolated from whole blood samples of 97 KTx and analyzed by flow cytometry. Patients were distributed in groups according to time post transplant (≤ or > 60 months) and donor type (Deceased or Living). All data are presented as median and interquartile range (Mann-Whitney U-test). $p < 0.05$ was considered significant.

Results: The number of EMPs was higher in recipients with ≤ 60 months postgraft (p=0.049) and no difference was found for PMPs (p=0.109). Concerning to the type of allograft donor, patients that received kidney from deceased donor had higher number of EMPs comparing to living donor (p=0.050) and no difference was found for PMPs (p=0.170). No difference was found between groups for size of MPs. Table 1.

Conclusions: It has been admitted that the endothelial injury provided by the surgical act can contribute to allograft rejection risk. Our data corroborate with this hypothesis, since patients before 60 months post transplant showed higher number of EMPs. Our data also support that allograft derived from living donor is associated with better vascular conditions, as showed by lower number of EMPs. **Funding agency:** CAPES; CNPq; FAPEMIG.

PB 687 | The Relation between Circulating Endothelial Microparticles and Asymetric Dimethylarginine in Vitamin B12 Deficient

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Background: Hyperhomocysteinemia, increased RDW, MCV and the proliferation of smooth muscle cells induced by hypomethylation are cardiovascular disease risk factors in vitamin B12 deficienct adolescents.

Aims: The aim of our study is to investigate the levels of cIMT (carotid intima media thickness), EMP (endothelial microparticle) and ADMA (asymmetric dimethylarginine) which are indicative of susceptibility to atherosclerosis and endothelial dysfunction in adolescents with deficiency of vitamin B12.

Methods: Adolescent with age ranging between 11-17 years age eighty-eight patients detected; 50 patients of vitamine B12 deficiency (B12< 130 pg/ml) and 38 healthy controls (B12>200 pg/ml) were included in the study. In all cases number of EMPs (CD144+EMP,

TABLE 1 Time post transplantation and donor type in studied patients.

Parameters	Groups	EMPs (/μL)	P	PMPs (/μL)	P	MPs (/μL)	P
Time post-transplant (months)	T1≤60(N=41) T2>60(N=54)	36.5 (19.5) 30.4 (19.3)	0.049	158.8(124.8) 191.8(175.8)	0.109	232.6(149.8) 275.0(274.4)	0.691
Donor	Deceased(N=52) Living(N=45)	33.6 (19.7) 29.1 (12.5)	0.050	188.3(308.22) 155.8 (119.6)	0.170	270.5(302.6) 238.5(229.1)	0.638

CD146+EMP, CD105+EMP) were measured in the flow cytometry and ADMA levels were measured with High Performance Liquid Chromatography method. The cIMT and LVMİ were assessed using high-resolution echocardiography.

Results: The levels of ADMA, cIMT, CD144+EMP and CD146+EMP in vitamine B12 deficiency group were significantly higher than control group ($p < 0.05$). In patients, the vitamine B12 has negatively relationship with homocysteine, ADMA, CD144+EMP, CD146+EMP and cIMT ($p < 0.05$). Homocysteine and ADMA were identified as the parameters affecting vitamin B12 deficiency ($p < 0.001$). Also cIMT, triglycerides, HDL and CD144+/CD146+EMP are other risk factors for vitamine B12 deficiency.

Conclusions: The demonstration of increased EMP and ADMA in adolescents with vitamin B12 deficiency indicates that these markers may be used as markers for predicting atherosclerosis and endothelial dysfunction in adolescents with vitamin B12 deficiency. One of the important variable affecting atherosclerosis and endothelial dysfunction is vitamin B12, which is important to emphasize the need for close vitamin B12 control in the prevention of cardiovascular disease-related problems in adolescents with vitamin B12 deficiency.

PB 688 | Circulating Microparticles as Predictive Biomarkers for Tumor Progression in Advanced Non-small Cell Lung Cancer

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Background: Microparticles (MPs) are proposed as useful biosensors for membrane damage in cancer patients.

Aims: We investigated the clinical significance circulating MPs for the prediction of progression disease (PD) and prognostic outcome in patients with advanced non-small cell lung cancer (NSCLC) treated with cytotoxic chemotherapy.

Methods: This study was performed according to the guidelines of Helsinki Declaration. All parents signed a written informed consent. Peripheral blood samples were obtained from 60 patients with advanced NSCLC before and after first-line platinum-based chemotherapy. Flow cytometry measurement (FCM) was used to quantify circulating MPs. Receiver operating characteristic (ROC) analysis was performed to determine the optimal cutoff values for plasma levels of MPs to discriminate between patients with clinical benefit and non-responders after treatment. The relationship between baseline MPs levels and tumor response was investigated in advanced NSCLC patients.

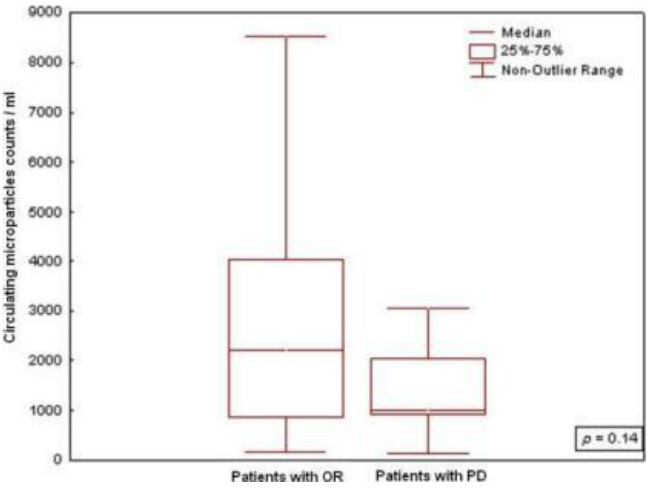

FIGURE 1 Baseline circulating MPs values in advanced NSCLC patients with OR and those with PD after chemotherapy

Results: The median pretreatment MPs number was notably higher but without significant difference in patients with objective response (OR) than that in patients with PD after chemotherapy (2983 ± 384 vs. 1839 ± 478, $p = 0.14$).

Using multivariate analysis, it was found that treatment response was independent of clinical features (age, gender, smoking status, histological sub-types, clinical stage) ($P > 0.05$)

TABLE 1 Correlation of treatment response with clinical features in advanced NSCLC patients. SCC: sqamous cell carcinoma, ADK: adenocarcinoma

Variables	OR	PD	X^2	P value
Gender (Female/Male)	40/7	10/3	0.491	0.48
Age (<60 /≥60) years	26/21	8/5	0.16	0.68
Smoking (yes/no)	43/4	10/3	2.88	0.11
Histopathology (SCC/ADK)	25/22	4/9	2.15	0.34
Staging (III/IV)	19/28	3/10	1.46	0.48

By contrast, increased levels of MPs in patients with clinical benefit during or after chemotherapy correctly predicted PD radiologically confirmed in 61% of evaluable cases. However, there was no significant difference in the median duration of time to progression (TTP) between patients with high percentage change and those with low percentage change in MPs counts after chemotherapy ($p = 0.39$).

Conclusions: The increase in MPs numbers during or after chemotherapy might be a predictive factor of PD in advanced NSCLC patients. Furthermore, baseline MPs levels could be considered for tumor response in advanced NSCLC patients.

PB 689 | Microparticles with Platelet Pro-aggregation Potential Are Present in Patients with Acute Coronary Syndrome and No Perfusion (TIMI-0)

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Background: Hyperactivity of platelets play an important role in acute coronary syndromes and therapy is oriented to decrease it. The presence of microparticles (MPs) signs a pro-coagulant prone condition.
Aims: To study MPs with platelet pro-aggregation potential in patients with acute coronary syndrome and flow grade TIMI-0 or TIMI-3.
Methods: Fifty six male patients were recluted at the Hemodynamic Department of Instituto Nacional de Cardiologia; only was considered patients who had a percutaneous transluminal coronary angioplasty (PTCA) at the anterior descending coronary artery. Patients were under antiplatelet (clopidogrel, aspirin) medication. Blood was obtained in sodium citrate as anticoagulant and MPs were separated by high speed centrifugation of platelet-free plasma. The amount of MPs was expressed as mg/mL of protein.
A volume of 40 uL of platelet rich plasma (PRP) obtained from blood bank healthy donors was supplemented with 30 ug/mL of MPs from each patient and incubated during 5 min at 37°C. We used a 96-wells plate, placed on a EON™ Microplate Spectrophotometer (BioTek) and absorbance at 595 nm was recorded. Platelet aggregation was induced with 10 uM ADP. Results were calculated considering the absorbance obtained with platelet poor plasma as 100% of aggregation.
Results: Table 1 shows the median value of the platelet aggregation test in TIMI 0 and TIMI 3 groups.

TABLE 1 Shows the median value of the platelet aggregation percentage in each group.

TIMI	Median	Range	p*
0	61.2	41-73	0.03
3	53.3	40-77	

Conclusions: This approach allow us to observe that TIMI 0 patients have more platelet pro-aggregation MPs as compared to patients in the TIMI 3 group.
Project supported by DGAPA UNAM IN219117

PB 1442 | Anti-β2-Glycoprotein I Antibodies Cause Lupus Anticoagulant and Activated Protein C Resistance via Coagulation Factor V

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Background: The presence of lupus anticoagulant (LA), measured as a prolonged clotting time, correlates best with thrombotic events in the antiphospholipid syndrome. The mechanism behind the paradoxical association between thrombosis and LA is not understood. It is assumed that antibodies against β2-glycoprotein I (β2GPI) prolong clotting time by competing with coagulation factors for anionic phospholipids, but this does not explain the prothrombotic phenotype in patients with LA.
Aims: To understand the mechanism behind the thrombotic tendency associated with lupus anticoagulant.
Methods: We assessed the effects of monoclonal anti-β2GPI IgG on coagulation in plasma and with purified coagulation factors.
Results: Addition of anti-β2GPI IgG to plasma caused a dose-dependent prolongation of clotting time, which was corrected in the presence of excess phospholipids, confirming LA activity of the antibodies. Experiments with factor (F)V-depleted plasma indicated that the LA effect depends on the presence of FV. Binding studies showed that β2GPI binds to both FV and FVa. Further exploration of the effects of anti-β2GPI IgG on the prothrombinase complex showed that IgG-β2GPI complexes inhibit the activation of FV by FXa in a phospholipid-dependent manner, thereby interfering with the initiation of coagulation. Thrombin generation experiments showed that anti-β2GPI IgG only prolongs the lag time, confirming these observations. In addition, the interaction between β2GPI and FV caused activated protein C (APC) resistance. Although the antibodies did not impair degradation of FVa by APC, they interfered with the cofactor role of FV in the APC-mediated degradation of FVIIIa.
Conclusions: Here, we provide an explanation for the LA paradox. The interaction between antibody-β2GPI complexes and FV causes a prolonged clotting time in sensitive assays in vitro. This interaction also results in APC resistance, a well-known risk factor for thrombosis.

PB 1443 | Plasma Clot Properties, Disease Phenotype, and Thrombotic Risk in Patients with a Lupus Anticoagulant: Results from the Vienna Lupus Anticoagulant and Thrombosis Study (LATS)

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Background: Patients (pts) with a lupus anticoagulant (LA) have a high risk of thrombosis. Altered properties of *ex vivo* plasma clot formation and lysis have been associated with thrombotic risk in several human studies.

Aims: To explore the relationship between plasma clot properties, LA phenotype and thrombosis in pts with a persistent LA.

Methods: In our prospective LA and thrombosis study we measured plasma clot properties using a tissue-factor-based turbidimetric assay with concurrent addition of rt-PA in 97 healthy controls and 171 pts with the LA.

Results: As compared to healthy controls, pts with the LA had significantly longer lagphases, lower clot formation rates (CFR), and longer clot lysis times (CLT, all p< 0.0001, **Table 1**). This finding was even more pronounced in LA pts with a prior history of thrombosis, and prevailed after adjusting for vitamin K antagonist (VKA) exposure. In LA pts, a prolonged lupus-sensitive aPTT (aPTT-LA) was strongly correlated with a longer lagphase (rho=0.54, p< 0.0001) and a lower CFR (rho=-0.46, p< 0.0001). 13 pts were started on VKA during follow-up, and this exposure significantly increased lagphase (mean: 20.5 vs. 14.2 minutes, p=0.02) while decreasing CFR (mean: 0.09 vs. 0.14 OD/min, p=0.01). 148 LA pts were followed-up for a median of 9.0 years, during which we observed 34 thrombotic events. In univariable time-to-thrombosis regression, a higher CFR was associated with a lower risk of thrombosis (Hazard ratio per 0.1 OD/min increase=0.55, 95%CI: 0.30-0.98, p=0.04). This association did not prevail after adjusting for the aPTT-LA and VKA.

Conclusions: The plasma clot property-assay is highly lupus-sensitive. Interestingly, *ex vivo* analysis of plasma clot formation could distinguish between healthy controls and LA pts with and without prior thrombosis and indicated an increased risk for future thrombosis. Obviously, the observed associations with higher thrombosis risk are not causal, but seem to reflect the confounding influence of a more aggressive LA phenotype.

PB 1444 | Biophysical Characterization of Clot Retraction in Platelet Rich Plasma of Patients with Primary Anti-phospholipid Syndrome

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Background: Anti-phospholipid syndrome (APS) is an autoimmune process, leads to thrombotic disorders. The most significant autoantibody is the one against beta-2-glycoprotein I (β2GPI). The complex of the β2GPI and its antibody is targeted at the platelet thrombus.

Aims: Our aim is to use a nano-thrombelastographic (nTEG) method based on atomic force microscopy that gives information about the viscoelasticity and contractility of platelet rich fibrin network during clot formation and degradation in platelet rich plasma (PRP) of primary APS patients.

Methods: Patients with primary APS or VTE as controls were selected in the study. Both groups (age and therapy matched; n=60) were under vitamin K antagonists (VKA) or direct oral anticoagulants (DOAC; II- or Xa-Inhibitors) therapy. None of them had inter-current disease in the previous one year. Citrated blood was centrifuged for PRP at room temperature, 150g, 10 minutes.

An AFM cantilever was submerged in a 300µL sample and cyclically moved up and down with 1 µm amplitude and 1 µm/s speed. The sample contained PRP and Ca^{2+} 10 mM, clotting was initiated with thrombin, 1 IU/ml final activity. As the sample clotted the cantilever increasingly deflected during its vertical travel.

Results: Preliminary results of APS vs. controls are given in median. The force difference of each cycle -calculated from the maximum

TABLE 1 Selected plasma clot properties of the study population (n=268). Data represent medians [25th-75th percentile].

Variable	Healthy controls (n=97)	LA patients without prior history of thrombosis (n=57)	LA patients with prior history of thrombosis (n=114)
Baseline absorbance (OD)	0.21 [0.19-0.26]	0.22 [0.18-0.28]	0.23 [0.19-0.30]
Lagphase (minutes)	3.6 [3.0-4.1]	9.3 [6.8-15.4]	12.6 [9.3-18.6]
Maximum clot formation rate (OD/minute)	0.20 [0.18-0.25]	0.14 [0.08-0.20]	0.11 [0.06-0.16]
Peak Optical Density (OD)	0.80 [0.70-0.90]	0.74 [0.61-0.87]	0.78 [0.55-0.89]
Time to Peak Optical Density (minutes)	8.4 [7.7-9.0]	15.6 [12.1-23.7]	21.6 [15.4-29.6]
Clot lysis time (minutes)	11.1 [10.0-12.3]	12.4 [10.7-15.2]	14.9 [12.5-18.5]
Maximum clot lysis rate (OD/min)	0.05 [0.04-0.05]	0.04 [0.03-0.05]	0.04 [0.03-0.05]

deflection signal- for the APS group (n=9) was 56 vs. 221nN of the controls (n=8); delay until the first force signals was 248 vs. 140sec; slope of the force generation was 0.15 vs. 0.41nN/sec. Platelet count and fibrinogen content was equal.

Conclusions: APS clots were much later formed and less retracted than VTE clots. These data are the first to evidence the nanoscale changes in the viscoelastic properties of platelet rich clot affected by β2GPI and its antibody complex.

PB 1445 | Patient-derived Anti-β2GP1 Antibodies Recognize a Peptide Motif Pattern and Not a Specific Sequence of Residues

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Background: Antiphospholipid antibody syndrome (APS) is an autoimmune disease characterized by the presence of so-called antiphospholipid antibodies (aPLA) and clinical manifestations such as recurrent thromboembolic or pregnancy complications. Although the main antigenic determinant for aPLA has been identified as the beta-2-glycoprotein 1 (β2GP1), the precise epitope recognized by aPLA still remains largely unknown.

Aims: Characterization of the epitope of antiphospholipid antibodies

Methods: Proliferation assay was performed on APS patient-derived $CD4^+$ T cell in presence of different domains and peptides. We generated an alanine-scanning library to perform and ELISA epitope-mapping assay to identify the aPLA epitope.

Results: In the present study we identify a sequence of β2GP1 able to induce the proliferation of $CD4^+$ T cells isolated from APS patients but not from healthy donors. A comparison of this sequence with previously reported epitopes recognized by aPLA antibodies reveals the presence of a motif in β2GP1 in which the polarity but not the sequence of amino acids determines the characteristics and specificity of aPLA-interacting motifs. We further determined the sequence of polar and nonpolar residues in the main aPLA-interacting motif using point mutations. We have further demonstrated in vitro that peptides and domains of β2GP1 containing these motifs were able to interact with aPLA and to inhibit their monocyte activating activity.

Conclusions: We have characterized the main aPLA-interacting motif, which is present, at least once, in all aPLA-related receptors described so far. These data could also contribute to the future development of more sensitive and specific diagnostic tools for APS determination and potential peptide- or β2GP1 domain-based clinical therapies.

PB 1446 | Longitudinal Anti-phospholipid-Antibody Trajectories for Dynamic Thrombotic Risk Prediction in Patients with the Lupus Anticoagulant: A Joint Model

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Background: Thrombosis is a frequent complication in patients (pts) with the antiphospholipid syndrome (APS). Longitudinal trajectories of antiphospholipid-antibodies (aPLAs) may harbor important prognostic information on this risk over time.

Aims: To define the role of repeated aPLAs measurement for dynamic thrombosis risk prediction in pts with a lupus anticoagulant (LA).

Methods: In this prospective cohort study, we measured 5 aPLAs (Lupus-sensitive aPTT (APTT-LA, Diagnostica Stago), IgM & IgG anti-Cardiolipin (aCL) and anti-β2-glycoprotein-I (aβ2-GPI)) in 165 pts with persistently positive LA at study inclusion (**Table 1**), and during 1,047 follow-up visits. The prognostic impact of the longitudinal aPLAs trajectory on thrombosis was analyzed with a so-called joint model.

Results: During a median follow-up of 9.2 years, we observed 41 thrombotic events (arterial n=20, venous n=21). The aPTT-LA (mean±SD at baseline: 95 seconds (sec) ± 34) declined by 1.1 sec/year in pts who did not develop thrombosis, and increased by 1.6 sec/year in pts who developed thrombosis (difference/year=2.6 sec, 95%CI:1.6-5.8, p=0.001, **Figure 1A**). In joint modeling, a 5 sec prolongation of the aPTT-LA over time was associated with a 9% increase in the risk of thrombosis (Hazard ratio (HR)=1.09, 95%CI:1.04-1.15, p=0.001). This association prevailed after adjusting for the time-dependent exposure to vitamin-K-antagonists (Adjusted HR=1.14, 1.03-1.14, p=0.001),

TABLE 1 Baseline characteristics of the study population (n=165)

Variable	Median [25th-75th percentile], or Absolute count (percent)
Age at entry (years)	41 [30-60]
Female Gender	136 (82%)
Prior history of thrombosis	105 (64%)
Prior history of pregnancy complications	54 (33%)
aPTT-LA (seconds)	87 [70-117]
β2GPI IgM (MPL)	5.6 [2.5-15.2]
β2GPI IgG (GPL)	9.4 [2.1-48.6]
aCL IgM (MPL)	9.1 [3.5-23.0]
aCL IgG (GPL)	18.6 [6.3-71.7]

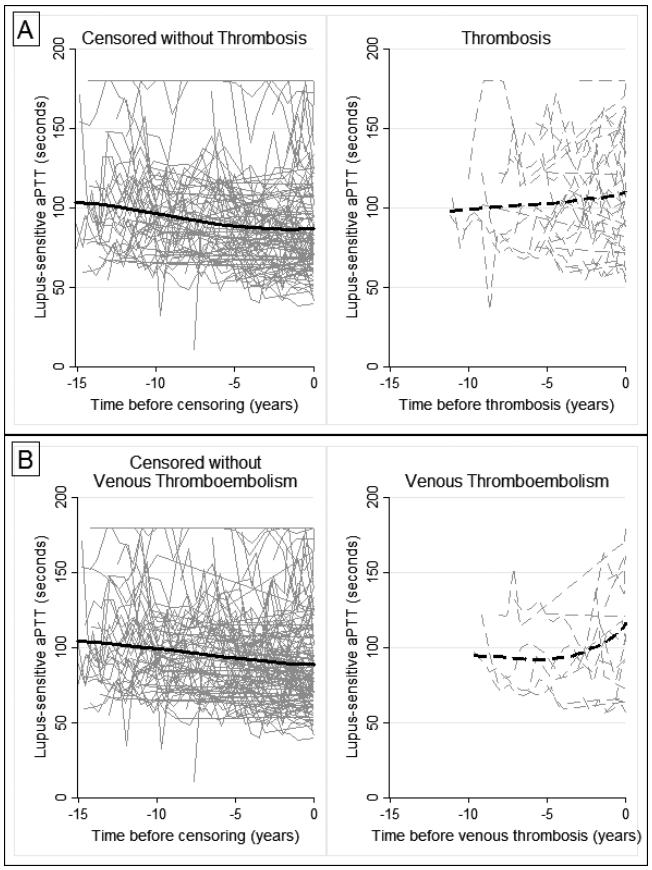

FIGURE 1 Trajectories of the aPTT-LA in LA-positive patients without and with thrombosis (Fig 1A - All events. Fig 1B - Venous Thromboembolism only)

and was even more pronounced for venous events (**Figure 1B**). No prognostic information on thrombotic risk could be extracted from the APLAs trajectory of IgM/IgG aCL or aβ2-GPI.

Conclusions: An increasing aPTT-LA over time predicts the occurrence of thrombosis in pts with the LA. This defines a critical link between a more aggressive APS phenotype *in vivo* and the most important disease-defining laboratory test, the LA. Clinically, routine monitoring of the aPTT-LA may help in dynamically stratifiying LA pts according to their thrombotic risk.

PB 1447 | Impact of Adding Antiphosphatidylserine/Prothrombin Antibodies in the Thrombotic Risk of Patients with APS Laboratory Criteria: A Prospective Study

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Background: APS is an autoimmune disorder characterized by thrombosis/pregnancy morbidity associated with lupus anticoagulant (LA) and/or anticardiolipin (aCL) and/or anti-b2-glycoprotein I (ab2GPI) antibodies. At least two other novel antibodies seem to be important markers of the thrombotic risk in patients with aPL: antibodies against phosphatidylserine/prothrombin complex (aPS/PT) and ab2GPI directed to domain I.

Aims: There is evidence that patients having triple positivity of conventional aPL have a greater risk of thrombosis than those having two or one positive aPL tests. We assessed the contribution of aPS/PT to the thrombotic risk in a prospective study.

Methods: This cohort enrolled 180 consecutive patients (59 men, 121 women; 40 with SLE; median age 40 years) with persistent aPL. Median follow-up was 31 months (range 3-60). The APS group includes 99 patients while there were 81 aPL subjects belonging to the non-APS group. LA, IgG and IgM aCL and ab2GPI were measured following ISTH guidelines. IgG and IgM aPS/PT were evaluated using the ELISA QUANTA Lite from INOVA Diagnostics, USA (positive results >30 Units for both isotypes).

Results: 29 patients (16.1%) had at least one thrombotic episode during follow-up, 10 without previous thrombosis and 19 had recurrences of thrombosis (16 venous and 13 arterial) and the overall incidence of thrombosis was 4.7% per patient-year. First event was arterial in 5 and venous in 5 non-APS subjects. Positive aPS/PT was found in 48% of APS patients and in 12% of non-APS patients. High levels of IgG aPS/PT were predictive of thrombotic events (HR 2.6, CI95% 1.2-3.9). The highest incidence of thrombosis was found in patients with triple positivity (LA/aCL/ab2GPI) also having positive results in the aPS/PT assay (HR 2.9, CI95% 1.5-4.9) as compared with aPL patients with triple or double positivity.

Conclusions: Adding the aPS/PT assay to the panel of conventional aPL is an important tool to achieve a better stratification of patients with high risk prediction of thrombosis.

PB 1448 | Triple Positive Antiphospholipid Antibodies: Away with Titres and Ratios?

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Background: Triple antiphospholipid antibody (aPL) positivity (TP) is associated with worse clinical outcomes than non TP subjects.

Aims: TP aPL positivity (TP) [lupus anticoagulant (LA) plus anticardiolipin (aCL) plus anti-beta-2-glycoprotein antibodies ($a\beta_2$GPI) of either isotype] was evaluated in persistent aPL carriers who never suffered thrombosis (NTHR), in thrombotic (THR) primary antiphospholipid syndrome (PAPS) and deceased (D) PAPS patients.

Methods: Activated partial thromboplastin time (aPTT), dilute Russell's viper venom time (DRVVT) (coagulation assays) IgM/IgG aCL and $a\beta_2$GPI (immune assays) were measured in 33 NTHR (M/F 5/26, age 48.5±15), in 64 THR APS (M/F 22/42, age 46.2±13) and 11 D PAPS (M/F 5/6, age 55.5±8) before initiating of warfarin anticoagulation.

TABLE 1 triple and non triple positive aPL in non thrombotic, thrombotic and deceased PAPS

	NTHR			THR			D		
	TP	NTP	p	TP	NTP	p	TP	NTP	p
DRVVTr $\bar{x}$±SD)	1.63±0.3	1.22±0.2	0.001	1.84±0.4	1.49±0.3	0.0006	2.47±0.8	2.40±0.6	0.8
IgGaCL($\bar{x}$±SD)	165±77	20±13	<0.0001	153±125	36±51	<0.0001	445±440	113±139	0.07
IgGaβ2GPI $\bar{x}$SD)	70±27	4±3	<0.0001	90±76	16±30	<0.0001	153±103	10±8.500000	0.006
aPTTr ($\bar{x}$±SD)	2.59±1.0	1.57±0.7	0.02	2.31±0.71	1.7±0.53	0.0005	2.31±0.49	1.93±0.32	0.2
IgGaCL ($\bar{x}$±SD)	169±83	24±27	<0.0001	162±131	45±56	<0.0001	445±440	113±139	0.07
IgGaβ2GPI ($\bar{x}$±SD)	63±23	6±9	<0.0001	86±67	22±33	<0.0001	153±103	10±8.5	0.006

Results:
The frequency of TP expressed as any LA and/or aPL increased was 25% in NTHR, 55% in THR and 100% in D PAPS (p< 0.0001); that of aPTTr with any IgG aPL was 15.6%, 45.3% and 63.5% in the same groups (p=0.003); that of DRVVTr with any IgG aPL was 22%, 53% and 63.6% in the same groups (p=0.006). The combination of either DRVVTr or aPTTr with IgM aPL was not significant.Within each group (NTHR, THR and D PAPS) mean DRVVTr, aPTTr, IgG aCL and IgG aβ_2GPI titres were always greater in TP patients than in NTP patients (Table 1). The TP tests were split according to DRVVTr and aPTTr and according to their triple positive partners, IgG aCL and IgG β_2GPI; the DRVVTr was progressively higher across the three groups (p< 0.01) compared to the aPTTr; the average IgG aβ_2GPI progressively increased across the three groups (p=0.006) regardless of its partnership with DRVVTr or aPTTr; the IgG aCL was significantly higher in the D than in other groups.
Conclusions: Although TP frequency was progressively higher across NTHR, THR and D groups, the intensity of the TP tests was much greater in the D than in other groups: the concept of TP must not part with that of intensity, as they may both retain prognostic relevance.

PB 1449 | oThe Effect of Treatment with Hydroxychloroquine on Soluble Tissue Factor Levels in Patients with Antiphospholipid Antibodies and Antiphospholipid Syndrome

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Background: Antiphospholipid syndrome (APS) is characterised by venous, microvascular and/or arterial and/or obstetric morbidity in patients who are persistently positive for antiphospholipid antibodies (aPL) (Miyakis 2006) . The mainstay of treatment is based on anticoagulation therapy; however, increasing interest is currently received by the antimalarial hydroxychloroquine (HCQ). The use of HCQ has been associated with a reduced risk of thrombosis but HCQ's antithrombotic mechanism of action is unclear particularly in patients with aPL and APS.

Aims: The aim of our study was to assess soluble tissue factor (TF) levels in HCQ naïve-patients with persistent aPL or APS at baseline and 12 weeks after commencing HCQ. We hypothesise that HCQ lowers levels of soluble TF.
Methods: Twenty- two individuals with APS with or without other associated autoimmune disease had blood samples taken before and 12 weeks after starting HCQ 200mg (see table 1). Plasma was stored at -80°C and thawed to measure TF using Imubind TF kit (Invitech Ltd, Cambridgeshire, UK) according to the manufacturer's instructions. A two-tailed student's paired t-test was performed and p=0.05 was

TABLE 1 Patient Characteristics

Table 1: Demographic data	
Variables	**APS (N=22)**
Age: median (range)	47 (18-69)
Sex (female:male)	20:02
Ethnicity (white:asian:black)	(20:1:1)
aPL subtype	
LA* positive	19
IgG or IgM aCL positive (>99th centile)	5
IgG or IgM anti-Beta2GPI antibody positive (>99th centile)	0
aCL and anti-Beta2GPI antibody positive (>99th centile)	0
aCL antibody and LA* positive (>99th centile)	3
Anti-Beta2GPI antibody and LA* positive (>99th centile)	0
aPL complication	
Thrombotic APS	
Previous arterial thrombosis	10
Previous venous thrombosis	7
Previous arterial and venous thromboses	4
Obstetric APS	
Previous recurrent 1st trimester pregnancy loss	2
Previous pre-eclampsia	1
Previous intrauterine growth restriction	1
Previous stillbirth	3
Treatment	
Warfarin	14
Heparin	0
Aspirin	3
Autoimmune profile	
Antinuclear antibodies (ANA)	10
Extractable nuclear antibodies (ENA)	2
Double stranded DNA antibodies (dsDNA)	1
Key: aCL - anticardiolipin; anti-Beta2GPI - anti-Beta2glycoprotein1; LA - lupus anticoagulant. *LA detected by either DRVVT, dilute aPTT or TSVT	

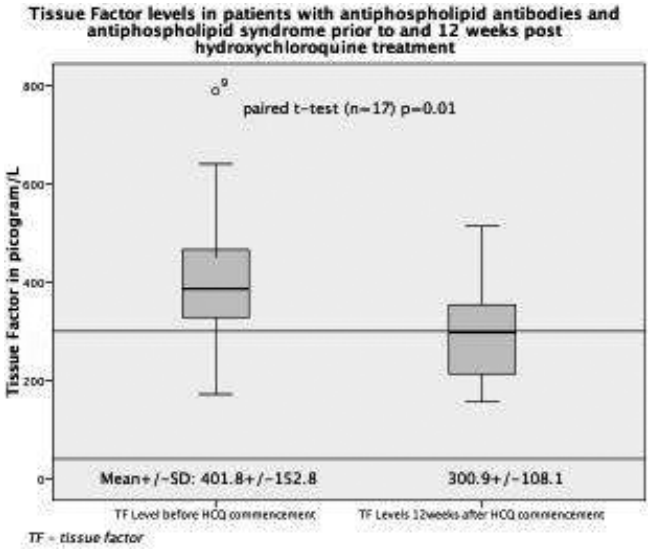

FIGURE 1 Soluble tissue factor prior to and post hydroxychloroquine commencement

considered as significant. There are no previous data in this area, and our study is therefore a pilot study.

Results: Soluble TF levels were above our normal range (40-300 pg/ml) prior to the commencement of HCQ and were significantly reduced (pre level mean (SD) 401.8 (152.8) pg/ml versus post 300.9 (108) pg/ml (p = 0.010) (see graph).

Conclusions: There was a significant reduction in soluble TF levels in this patient cohort of patients with apL and APS after commencing HCQ. Our previous work has shown that HCQ has not affected complement turnover, VEGF levels, thromboelastometry findings or CRP levels. Our findings of a reduction of soluble TF levels in aPL positive patients after the commencement of HCQ maybe a key mechanisim by which HCQ reduces thrombotic risk. Further studies of a larger patient cohort are required to confirm our observation.

PB 1450 | Increased Plasma Cell-free DNA (cfDNA) and Myeloperoxidase (MPO) and Impaired Fibrinolysis in High-risk Patients with Antiphospholipid Antibodies (aPL)

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Background: NETosis and impaired fibrinolysis have been implicated in the pathogenesis of thrombosis associated with aPL.

Aims: To evaluate plasma cfDNA and MPO, and their possible influence on fibrinolytic resistance in individuals positive for aPL at different risk of thromboembolic events.

Methods: Forty-nine patients with different aPL profiles (11 single, 13 double, 25 triple positive) and 20 controls were studied. Plasma cfDNA and MPO were evaluated by fluorometric and chromogenic assays, respectively. Clot formation and clot lysis were evaluated by turbidimetric assay in tissue factor (5 pM)-activated plasma challenged with t-PA (30 ng/ml).

Results: Positive aPL individuals had higher levels of cfDNA and MPO than controls (Table 1). Clot time was longer in patients (many were on warfarin) whereas clot lysis time did not differ from controls. Upon addition of activated protein C (APC, 0.3 µg/ml), clot time prolongation (APC clot ratio) was similar in patients and controls whereas lysis time shortening (APC lysis ratio) was much weaker in aPL, indicating a resistance to the profibrinolytic activity of APC. Levels of cfDNA and MPO, and resistance to APC profibrinolytic activity were markedly higher in triple positive than single or double positive aPL (table 1). There was an inverse correlation between APC lysis ratio and either cfDNA or MPO (rho -0.38 and -0.41, respectively, p< 0.002). Moreover, ROC curve analysis (aPL only) revealed a good accuracy of MPO and resistance to APC profibrinolytic activity to identify patients with triple positivity (AUC >0.7, P≤ 0.01).

TABLE 1 Markers of NETosis and responses to APC in patients with different aPL profiles

Assay	Controls (n=20)	aPL profiles			P (aPL vs Controls)	P (Single+double vs triple)
		Single positivity (n=11)	Double positivity (n=13)	Triple positivity (n=25)		
cfDNA, ng/ml	210 (181-234)	243 (189-303)	242 (208-401)	284 (236-363)	0.002	ns
MPO, mOD	68 (38-81)	73 (46-136)	96 (55-122)	161 (74-459)	0.005	0.01
Clot time, min	3.5 (3.0-3.8)	3.5 (2.6-4.3)	3.5 (3.0-4.8)	11 (9.3-13)	0.0004	< 0.0001
APC clot ratio	1.39 (1.33-1.46)	1.38 (1.27-1.50)	1.26 (1.16-1.55)	1.33 (1.12-1.49)	ns	ns
Lysis time, min	76 (69-83)	76 (63-81)	71 (63-74)	71 (64-78)	ns	ns
APC lysis ratio	1.57 (1.47-1.88)	1.32 (1.12-1.46)	1.22 (1.09-1.32)	1.08 (1.06-1.16)	< 0.0001	0.015
Warfarin treatment, n (%)	__	0 (0)	3 (23.1)	21 (84)		< 0.0001

Conclusions: aPL positive individuals at the highest risk of thrombosis (triple positive) display markedly increased cell activation/damage and NETosis and enhanced resistance to the profibrinolytic activity of APC, despite warfarin treatment (which enhances the response to APC). These phenomena may represent new pathogenetic mechanisms leading to increased thrombotic risk in aPL positive individuals.

PB 1451 | Alteration of Beta 2 Glycoprotein I Conformation upon Domain V Disulfide Bond Reduction

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of anti-beta 2 glycoprotein I (anti-beta2GPI) antibodies in serum. It was previously found, that APS patients have a higher proportion of beta2GPI in oxidized state than healthy people. Beta2GPI exists in two main structural conformations: the closed (circular) form where the first domain (DI) interacts with domain V (DV), and the open (linear) form which exposes a cryptic epitope within DI. The open and potentially antigenic conformation may lead to formation of antibody-protein complexes, resulting in disease progression.

Aims: It is not known whether reduction/oxidation of beta2GPI is related to changes in its structure. In this study we investigate the influence of redox changes at a disulfide bond in DV (which is considered to be located at the DI/DV interaction surface) on the conformation of beta2GPI.

Methods: We prepared enzymatically reduced and labelled beta2GPI and compared its structure and properties to those of beta2GPI treated by pH shift to induce an open or closed conformation. Comparisons were made using tryptophan fluorescence quenching experiments, western blot and atomic force microscopy (AFM). ELISA was used to test the binding of anti-DI antibodies from APS patients to each of the beta2GPI species.

Results: For the first time, AFM confirmed the conformation of beta2GPI in open or closed state. These results were further correlated to fluorescence quenching experiments and ELISA. After reduction of the DV disulfide bond, a mixture of beta2GPI conformational populations was found. This finding is supported by western blot analyses showing multiple bands.

Conclusions: Beta2GPI conformation may be altered by reduction of the disulfide bond in DV. Therefore, oxidation of the free thiol groups in this position could trigger APS antibody binding by causing beta2GPI to adopt an open conformation. This paradigm can be further translated to proteins involved in other autoimmune diseases.

PB 1452 | High Prevalence of Activated Protein C Resistance Associated with High Avidity Anti-protein C Antibodies in Various Antiphospholipid Syndrome Clinical Phenotypes

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Background: Acquired activated protein C resistance (APCr) in association with anti-protein C antibodies (anti-PC) may provide a marker for a more severe thrombotic phenotype in antiphospholipid syndrome (APS) patients with venous thromboembolism (VTE). The role of APCr and anti-PC in other APS subgroups is unknown.

Aims: To determine the prevalence of anti-PC and association with APCr in APS clinical phenotypes: VTE or arterial thrombosis (AT) ± pregnancy morbidity (PM), PM only; and asymptomatic antiphospholipid antibodies (aPL).

Methods: Patient samples were collected locally and from the international APS ACTION cohort. Anti-PC and avidity were determined by in-house ELISA. APCr was measured as % inhibition of ETP by thrombin generation (5 pM TF and 4 μM phospholipids +/- exogenous APC (rh-APC; NR 56-132%) or endogenous PC (using Protac; NR 63-141%).

Results: In this ongoing study, 380 patients have been tested (325 for anti-PC; 289 for APCr): 158 VTE, 116 AT (27 AT + VTE), 36 PM, 70 aPL. 39% (126/325) had anti-PC, with a higher prevalence in PM (53%) compared to the other groups (34-38%) (p=0.003, one way ANOVA). High avidity anti-PC were detected in 19% of 325 patients (63/126 [50%] of patients with anti-PC); these high avidity antibodies were observed in 20%, 19%, 33% and 11%, of VTE, AT, PM and aPL patients, respectively. In APCr tests, a higher proportion of patients showed resistance to activation of endogenous PC (62%) vs exogenous APC (39%; p< 0.0001). Overall, 39% of 289 patients were resistant to both, with APCr associated with high avidity anti-PC in 70% VTE, 78% AT, 92% PM and 44% aPL patients (Fisher´s exact test p < 0.0001).

Conclusions: APS (VTE, AT and PM) and aPL patients showed a high prevalence of APCr (using activation of endogenous PC or exogenous APC), and this in association with high avidity anti-PC may provide a pathogenic marker. Associations with severity and course of disease in various APS clinical phenotypes remain to be established.

PB 1453 | Clinical Impact of Triple Positivity of Antiphospholipid Antibodies in Primary Thrombotic Antiphospholipid Syndrome

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Background: Lupus anticoagulant (LAC), anticardiolipin (aCL) or anti-b2-glycoprotein I (aB2GP1) are the antiphospholipid antibodies (aPL) required for the diagnosis of antiphospholipid syndrome (APS). Triple positivity (TP) for aPL is a suggested marker of disease severity. However, the clinical impact of TP in primary thrombotic APS (PAPS) is controversial and further clinical evidences are needed.

Aims: The aim of this study was to evaluate the impact of TP on the clinical presentation of PAPS, comparing TP with other aPL profiles.

Methods: Our cohort included 96 PAPS patients followed in the Hematology Center at Unicamp, Brazil.

Results: Twenty patients (21%) presented TP, 10.4% had double positivity, 49% were only LAC positive and 19.6% were aB2GP1 or aCL. Patients with double and single positivity were grouped as non-TP. The distribution of gender, age, site of the first thrombosis (arterial x venous) and incidence of thrombosis recurrence were not different between TP and non-TP groups. Clinically, the TP group presented a higher incidence of pregnancy morbidity (total=85% vs 50%, P=0.04). Compared to non-TP patients, the aCL-IgG title was higher in TP patients (50 vs. 35U, P< 0.0001), as well as the DRVVT-R for those with positive LAC (2.44 vs. 1.57, P< 0.0001). Patients with TP had higher frequency of positive ANA test (50% vs 18%, P=0.007), and lower complement C3 levels (median=1.04 vs. 1.29, P=0.001) when compared with non-TP. The levels of C4 were similar between the groups (median= 0.23 vs. 0.24, P=0.21). In multivariate analysis, positive ANA and C3 levels were independently associated with TP, odds ratio of 4.7 (95%CI=1.2-18.9) and 0.15 (95%CI= 0.0-0.63, P=0.02).

Conclusions: In conclusion, in thrombotic PAPS the clinical presentation in TP was similar to non-TP. However, we observed that the presence of TP in patients with PAPS was associated with a more complex immune profile. That may suggest the need for specialized follow-up, attending to the development of systemic autoimmune manifestations.

TABLE 1 Demographic and clinical features of thrombotic PAPS according to the antibody profile

	Triple positive n= 20	Non-triple positive n= 76	P
GENDER F/M	15/ 5	49/27	0.43
AGE AT DIAGNOSIS, years	28.9 (24-33.2)	32.7 (22.2-47.5)	0.29
PERIOD OF TIME SINCE DIAGNOSIS in years	8.0 (3.5-13.0)	5.2 (2.2-9.4)	0.29
VENOUS/ARTERIAL THROMBOSIS	15/5	50/26	0.59
THROMBOSIS RECURRENCE	7 (35%)	27 (35.5%)	1
OBSTETRIC MORBIDITY*	11 (84.6%)	15 (50%)	0.04

Continuous data are expressed as median and interquartile; categorical data as number and percentage *43 patients got pregnant (13 TP e 30 non TP)

TABLE 2 Laboratory results from PAPS patients according to their aPL profile

	TRIPLE POSITIVE n= 20	NON-TRIPLE POSITIVE n= 76	P
DRVVT R	2.44 (2.09-2.80)	1.57 (1.46-2.0)	>0.0001
aCL IgG title	50 (42.5-60)	35 (22.5-40)	>0.0001
Positive ANA test	10 (50%)	14 (18.4%)	0.007
C3	1.04 (0.97-1.20)	1.29 (1.15-1.44)	0.001
C4	0.23 (0.13-0.27)	0.24 (0.20-0.31)	0.09

Continuous data are expressed as median and interquartile; categorical data as number and percentage

PB 1454 | β2GpI Binds to Fibrinogen and Alters Fibrin Generation and Degradation

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Background: β-2Glycoprotein I (β2GpI) is an abundant plasma protein identified as the major autoantigen in the antiphospholipid syndrome (APS), an autoimmune disease characterized by venous and arterial thrombosis. Nonetheless, the physiological functions of this protein are still unclear. We have recently discovered that β2GpI binds to thrombin (αT) with high affinity (K_d = 60 nM), while altering fibrin generation and inhibiting platelet aggregation [Pozzi et al., 2013; Acquasaliente et al., 2016]. Although it seems clear that β2GpI alters the αT-fibrinogen axis by interacting with the protease, no information is available for the possible binding of β2GpI to fibrinogen.

Aims: Probe the interaction of β2GpI to fibrinogen and investigate the effect of β2GpI binding on fibrin generation by αT and its degradation by the fibrinolytic system.

Methods: Binding measurement were performed by fluorescence, Surface Plasmon Resonance (SPR) and Dynamic Light Scattering (DLS). Fibrin generation and degradation was monitored by turbidimetry in the presence of αT alone or with tissue-plasminogen activator (TPA) and plasminogen. Fibrin structure was studied by DLS and scanning electron microscopy (SEM).

Results: Fluorescence and SPR data concurrently indicate that β2GpI binds to fibrinogen with high affinity, with K_d values of 48±4 nM or 179±20 nM. Turbidimetric, DLS and SEM analyses indicate that β2GpI induces the formation of thinner and shorter fibrin fibers from fibrinogen and αT. Intriguingly, when fibrin clot formation was carried out also in the presence of TPA and plasminogen, β2GpI strongly accelerated fibrinolysis.

Conclusions: β2GpI has strong affinity for fibrinogen and it is likely that at physiological concentrations of the two proteins (4 and 7 μM) it circulates in the bloodstream bound to fibrinogen, altering the structure of the ensuing fibrin clot and its degradation by the fibrinolytic system. These results are consistent with the prothrombotic effect of anti-β2GpI autoantibodies found in APS patients.

PB 1455 | Circulating Microparticles in Pregnant Patients with Primary Antiphospholipid Antibody Syndrome: An Explorative Study

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Background: Identifying biomarkers associated with a high risk of maternal/fetal complications in patients with antiphospholipid antibody (aPL) syndrome (APS) can help to better define clinical management.

Aims: Our aim was to evaluate the presence, cellular origin and trend during pregnancy of circulating microparticles (MPs) in a consecutive cohort of APS women.

Methods: Eleven pregnant women affected by primary APS and 15 healthy women age and BMI-matched were enrolled and blood samples were longitudinally collected at three time points (I, II and III trimester). MPs expressing phosphatidylserine (AnnexinV-MP), MPs derived from endothelial (CD62E+), platelet (CD61+), trophoblast (CD105+) and tissue factor-bearing MPs (TF+) were measured by flow-cytometry.

Results: APS pregnant patients showed increased levels of endothelium, platelet, TF+ and trophoblast-MPs during pregnancy compared to healthy pregnant controls

. High risk APS patients (triple aPL positivity and a history of vascular thrombosis and/or severe pregnancy complications) showed significantly higher levels of Annexin V-MPs ($p < 0.001$), platelet-derived ($p < 0.001$) and trophoblast-derived MPs ($p < 0.0001$) compared to healthy controls in all trimesters probably due to a global activation of coagulation (platelets and TF-driven) as well as a damage to the trophoblast. Low risk APS patients (only history of pregnancy morbidity or vascular thrombosis) showed significantly higher trophoblast-derived MP levels in II ($p=0.018$) and III trimesters ($p < 0.01$) confirming the pathological effect of aPL on the trophoblast. The ratios of trophoblast and platelet-derived MPs were significantly higher in high versus low risk APS patients since the I trimester ($p=0.02$).

Fig. 1 Circulating MPs levels in the study population. Data are expressed as median levels of MPs. p are calculated between APS and healthy pregnant women * <0.05; ** < 0.01; *** < 0.001. Trim: trimester; APS: antiphospholipid antibody syndrome

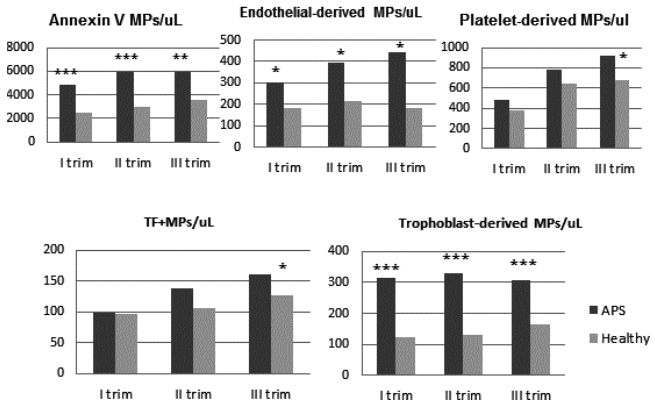

FIGURE 1 Circulating MPs levels in the study population.

Conclusions: This explorative study showed high levels of MPs in pregnant women with primary APS. Platelet and trophoblast-derived MPs detected in the I trimester might constitute a novel laboratory marker to assess the risk for pregnancy complications.

PB 1456 | Outcomes of Anticoagulant Therapy in Patients with Antiphospholipid Syndrome Managed in an Anticoagulation Clinic

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Background: Prolonged oral anticoagulation with vitamin K antagonists (VKA) is the treatment of choice for secondary prevention of thrombosis in patients with antiphospholipid syndrome (APS). However, data on long-term safety and efficacy of anticoagulant therapy in APS patients is scarce.

Aims: To analyse the risks and benefits of extended oral anticoagulation in patients with thrombotic APS.

Methods: Anticoagulant treatment was assessed in 237 consecutive patients with definite APS (revised Sapporo criteria) and previous thrombosis treated in our anticoagulation clinic from 2000 to 2017. Bleeding events (major and minor) and recurrent thrombotic events were identified by reviewing the data from the anticoagulant therapy management computer program Trombo.

Results: The clinical profile of our cohort is summarized in table 1. 230 (97%) of patients were treated with VKA (77% with a target international normalized ratio (INR) of 2.0-3.0 and 23% with a target INR of 2.5-3.5). The time in therapeutic range was 60 ± 18%. Only four patients were treated with low molecular weight heparin and three with rivaroxaban. During a total follow-up of 1564 patient-years the rate of minor bleeding was 19.6 cases per 100 patient-years and the rate of major bleeding was 1.2 cases per 100 patient-years. None of the bleeding episodes were fatal. The rate of thrombotic recurrence was 1.8 cases per 100 patient-years. Two patients died of thrombosis. Details on major bleeding and thrombotic complications are given in table 2.

TABLE 1 Clinical features of patients (VT - vein thrombosis, PE - pulmonary embolism, TIA - transient ischaemic attack, TE - thromboembolism).

Age (mean ± S.D.) (years)	**47 ± 16**
Sex (female) N (%)	128 (54)
Type of APS (primary/secondary) N (%)	212/25 (78/22)
Types of thrombotic events N Arterial system (Stroke/ TIA/Peripheral TE) Venous system (VT/PE/both) Arterial and venous thrombosis Microthrombosis	 62 (47/4/11) 148 (77/28/43) 2 25
Additional risk factors for thrombosis N (%)	53 (22)

TABLE 2 Major bleeding and thrombotic complications

Event	Site/Type of event	N
Bleeding	Central nervous system	2
	Gastrointestinal tract	3
	Locomotor system	6
	Parenchymal organs	4
	Gynaecological tract	3
Thrombosis	Venous thromboembolism	18
	Stroke	10

Conclusions: In patients with APS the extended anticoagulant therapy with VKA is safe and efficient in a dedicated anticoagulation clinic setting. In this high-risk group of patients the rate of adverse events of anticoagulant therapy is comparable to general VKA-treated population.

PB 1457 | Elevated Oxidized Low Density Lipoprotein Levels in Antiphospholipid Syndrome is Related to the Autoantibodies against High Density Lipoprotein and its Associated Proteins

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Background: In antiphospholipid syndrome(APS), oxidized low-density lipoprotein (oxLDL), a key molecule in atherogenesis, serves as the source of anionic particles that bind to beta2glycoprotein I and reinforces procoagulant activity or concomitant atherosclerosis. High density lipoprotein (HDL) is the major molecule that not only blocks function of oxLDL but helps prevent its production with the assistance of HDL-associated proteins such as paraoxonase type 1 (PON-1) and apolipoprotein A-1 (ApoA-1). In systemic lupus erythematosus (SLE), autoantibodies against HDL are prevalent.

Aims: To clarify the contributions of autoantibodies against HDL or its associated proteins on oxLDL production in APS.

Methods: Total of consecutive patients with primary APS (PAPS) (n=26), SLE (n= 48: SLE with APS 24) and non-lupus connective tissue diseases (others)(n=19) who visited Hokkaido University Hospital during 2009 and 2012 were comprised. ELISA were performed to detect aHDL, aApo-A1,aPON-1 and oxLDL levels.

Results: Serum levels of oxLDL were significantly elevated in PAPS (PAPS vs. SLE without APS vs. SLE with APS vs. others: 3.27+/-6.89 vs. 1.06+/- 2.32 vs. 2.51 +/- 4.12 vs. 0.8 +/- 3.72 mg/ml, p< 0.05). Titers of aHDL were similar among patient groups, while titers of aApo-A1 and aPON-1 were significantly elevated in PAPS. Multiple autoantibodies positivity were highly observed in PAPS(17/26) and SLE with APS(20/24) while low in others (SLE without APS 10/24, control 8/19). None of the autoantibodies independently correlated with the serum level of ox-LDL, however, patients with multiple antibodies showed higher titers of serum oxLDL(multi-positive vs less than one antibody positive: 2.54 +/-1.82 vs. 1.87+/- 2.21 p< 0.0001). None of the aPL or aPL scores correlated with serum oxLDL levels.

Conclusions: Elevated oxLDL in APS may be one of the causal mechanisms of the disease and might be consequence of the impairment of the antioxidant function of HDL induced by the presence of antibodies against HDL and its associated proteins.

PB 1458 | Platelet Activation by Antiphospholipid Antibodies through the IgG Receptor FcγRIIa: Possible Role in Thrombosis Associated with Antiphospholipid Syndrome?

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Background: Antiphospholipid antibodies (aPLAbs) targeting beta-2 glycoprotein I (B2GPI) are of primary importance in thrombosis associated with antiphospholipid syndrome (APS). The predominance of the IgG isotype in APS is conspicuously linked with increased risk of thrombosis, raising the question whether the platelet IgG receptor, FcγRIIa, may play a role in thrombosis caused by aPLAbs, as is the case in heparin-induced thrombocytopenia in which heparin Abs directly activate platelets through FcγRIIa. We have previously shown that:

(1) goat anti-human B2GPI-Abs ± human B2GPI strongly activate human platelets in vitro, and that this activity is abolished by the anti-FcγRIIa antibody IV.3;

(2) anti-B2GPI immune complexes are thrombotic in mice transgenic for human FcγRIIa but not in wild type mice.

Aims: We sought to investigate if IgG from patients with aPLAbs can activate platelets in a manner dependent on FcγRIIa.

Methods: IgG was purified from plasma of 46 patients with aPLAbs and/or lupus anticoagulant. The capacity of the IgG to activate platelets (±B2GPI) was evaluated by serotonin release assay (SRA) and washed platelet aggregation (WPA). With WPA, platelets ± IV.3 were either: (a) primed with ADP followed by IgG introduction or (b) incubated with IgG (30 min) followed by introduction of low thrombin concentrations.

Results: With or without B2GPI, IgG from 10 of 46 (22%) patients caused platelet dense granule release. In all cases this was abolished by IV.3, indicating the dependence of FcγRIIa. Aggregation of ADP-primed platelets was observed with 1 of 2 of the above 10 IgGs. Preincubation of platelets with aPL IgG from 2 patients, sensitized platelets to aggregate in response to otherwise

subaggregetory thrombin stimuli. This effect was also abolished by IV.3.

Conclusions: These findings suggest that aPLAbs from patients can directly activate and/or sensitize platelets in a FcγRIIa-dependent manner. This mechanism may contribute to thrombosis in patients with aPLAbs.

PB 1459 | Prognostic Impact of Antiphospholipid Antibodies on the Clinical Course of Primary Immune Thrombocytopenia or Autoimmune Hemolytic Anemia

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Background: It is controversial if antiphospholipid (aPL)-related primary immune thrombocytopenic purpura (ITP) or autoimmune hemolytic anemia (AHA) should be defined as antiphospholipid syndrome (APS).

Aims: To evaluate if aPLs influence the clinical course of primary ITP or AHA.

Methods: This is a prospective cohort study, participants had newly diagnosed primary ITP or AHA, they were enrolled between January 2013 and January 2016 and followed up until December 2016. Lupus anticoagulant (LAC), IgG/IgM anticardiolipin and anti-beta2-glycoprotein1 (aB2GP-1) assays were performed at diagnosis. The primary endpoint was the response to first-line treatment and the secondary endpoints were multiple treatment lines, splenectomy, thrombosis and bleeding events.

Results: Eighty-four patients (72 ITP, 11 AHA, 1 Evans syndrome) were included and followed for 17 months (3.5 - 38 months). Twenty-two patients (26.2%) were positive for aPL (10 LAC, 7aB2GP-1 and 5 double-positive). aPL-positive patients were predominantly female (77.3% vs. 53.2%,P=0.04) and 90.9% met the criteria for initial treatment (versus 85.5% of aPL-negative patients, P=0.5). The response to first line-treatment was similar between groups (75% in aPL-positive vs. 70.5% in aPL-negative;P=0.5). Cox regression analysis revealed that aPL positivity was not a risk factor for first-line treatment failure (HR= 1.38 95%CI=0.69-2.75, P=0.3), multiple treatment lines (HR= 1.30 95%CI=0.66-2.59, P=0.4) and splenectomy (HR=0.88 95%CI=0.26-3.08, P=0.8). Four patients had thrombosis and aPL positivity was not a risk factor for thrombosis (HR=0.65 95%CI=0.09-4.69, P=0.6). The risk for bleeding events was also similar between positive and negative aPL patients (HR=1.55 95%CI=0.82-2.93, P=0.2).

TABLE 1 Demographic and clinical features of the cohort patients

	aPL positive n=22	aPL negative n=62	P value
ITP	18 (81,8%)	54 (87,1%)	
AHA	4 (18,2%)	7 (11,3%)	
Follow-up duration in months, media (IQ)	22.3 (15.2-31.5)	16.2 (8.5-25.2)	0.76
Male:Female	1:1.14	1:3.4	0.04
Age in years media (IQ)	33.9 (21.6-58.1)	39.5 (28.4 - 61.4)	0.20
P value was calculated using Fisher test for categorical data and Mann Whitney test for continuous data			

TABLE 2 Patients clinical course

	aPL positive n=22	aPL negative n=62	P
Patients requiring treatment, number (%)	20 (90.9%)	53 (85.5%)	0.72
First-line therapy wt corticosteroids	72.7%	77.4%	0.9
Patients with sustained remission after first-line therapy, number (%)	15 (75%)	32 (71%)	0.2
Patients requiring two or more treatment lines, number (%)	13 (59%)	27 (44%)	0.31
Splenectomy, number (%)	5 (22.7%)	7 (11.3%)	0.28
Anual incidence of thrombosis during the follow-up	4.5%	2.1%	0.28
Anual incidence of bleeding events during the follow-up	31.8%	40.8%	0.84
P values were calculated using Fisher test for categorical data and Mann Whitney test for continuous data			

Conclusions: The results demonstrated that aPLs do not influence the clinical course of primary ITP or AHA. The study suggests that aPL-positive ITP or AHA are not distinct entities, not supporting the inclusion of these aPL-related manifestations as clinical criteria for APS diagnosis.

PB 1460 | Identifying "Second Hit" Risk Factors Associated with Thrombosis and Pregnancy Morbidity in aPLs Positive Patients

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Background: The evaluation of thrombotic and pregnancy risks associated with antiphospholipid antibodies (aPL) in individual patients without APS clinical manifestation is challenging.

Aims: To identify potential predictors of thrombosis and pregnancy morbidities among aPL positive patients.

Methods: This study included 112 consecutive persistent aPL positive patients who attended clinic at University of Texas Southwestern Medical Center. All patients had persistent positive moderate to high titer aPL. The aPL profiles were assessed with commercial assay. Hypertension (HTN) was classified based on 8th Joint National Committee guidelines. Hyperlipidemia (HLD) was defined as fasting total cholesterol >200 mg/dl. When assessing risk factors associated with pregnancy morbidities, only reproductive age (age< 45) female controls were used. Pearson Chi-squared analysis and multivariable logistic regression were used to evaluate correlation between different risk factors and clinical manifestations.

Results: Of the 112 aPL positive patients, 69 (61.6%) patients had criteria APS clinical manifestations and 43 patients did not. Among APS patients, 57 (82.6%) patients had primary APS. When comparing APS patients to asymptomatic aPL positive patients, HTN (OR=5.357, 95%CI 1.343 - 19.06, P=0.0116) was significantly associated with arterial thrombosis (Fig 1) and the presence of lupus anticoagulant (OR=3.075, 95%CI 1.289- 7.346, P=0.0114) was significantly associated with venous thrombosis (Fig 2). Age, HLD, smoking, raynaud, livedoid reticularis, presence of IgA aPL or triple positive aPL did not demonstrate significant correlation with either arterial or venous thrombosis. None of the analyzed clinical characteristics or aPL profiles showed significant correlation with obstetric manifestations.

Conclusions: HTN is a potential predictor of arterial thrombosis and the presence of LA is a potential predictor of venous thrombosis in aPL positive patients.

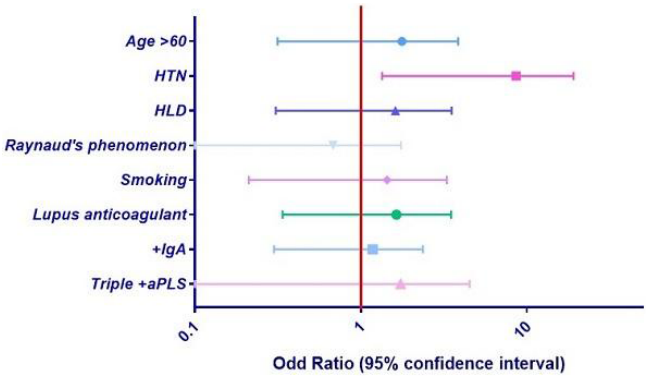

FIGURE 1 Arterial thrombosis risk factors

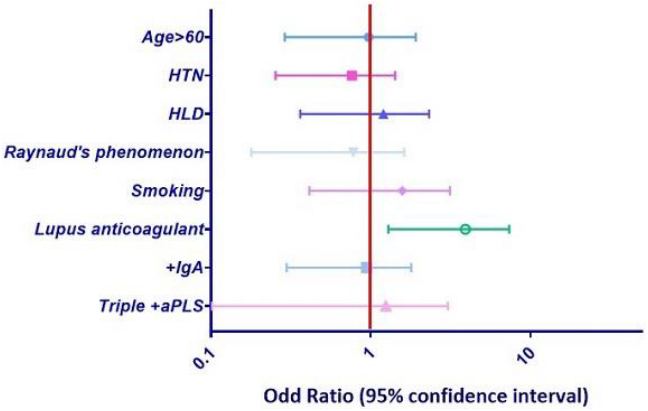

FIGURE 2 Venous thrombosis risk factors

PB 1462 | Are Antiβ2-glycoprotein I IgG Domain I a Useful Tool to Predict Thrombosis?

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Background: Antiphospholipid syndrome (APS) is a high-risk acquired thrombophilia characterized by thrombosis and the presence of antiphopholipid antibodies (APL). Thrombotic risk assessment is still a challenge and, for this purpose, the sensitivity and specificity of some antibodies have been tested (lupus anticoagulant (LAC), anticardiolipin and anti ß2-glycoprotein I (Aß2GPI)). Aß2GPI are an independent risk factor for thrombosis in APS. Recently, Aß2GPI binding a limited epitope of domain I (Gly40-Arg43) have been particularly associated with thrombosis.

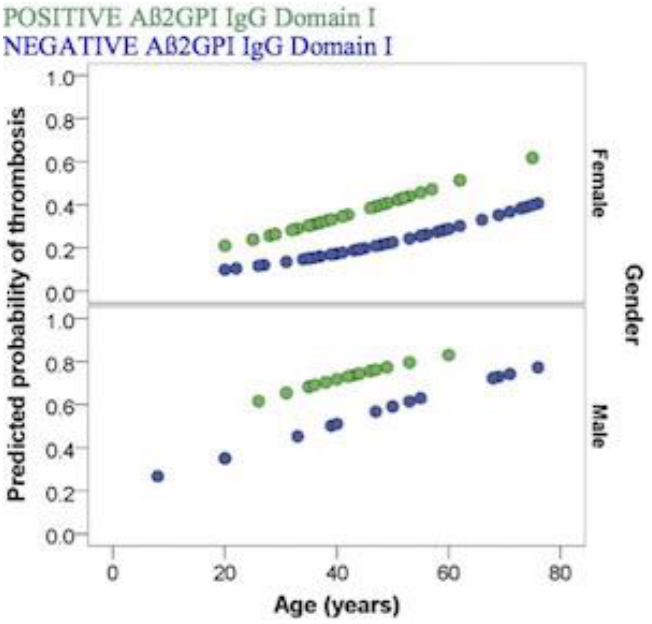

FIGURE 1 Aß2GPI IgG Domain I and probability of thrombosis in LAC negative patients

Aims: The aims of this study was to evaluate the clinical association of Aß2GPI-domain I and thrombosis in Aß2GPI IgG positive patients.

Methods: We have performed a cross-sectional observational study including 114 Aß2GPI IgG positive patients (44 thrombotic APS). We have studied LAC and the APL recommended in the Sydney criteria as well. All APL including Aß2GPI-domain I IgG have been performed by chemoluminiscent immunoassay and LAC has been confirmed by dilute Russell viper venom time coagulation test. We have also considered the variables age and gender. The statistical analysis (logistic regression- multivariate analysis) was carried out using the IBM SPSS ($p< 0.05$).

Results: In thrombotic APS, 68% of patients were LAC and Aß2GPI IgG positive, not showing Aß2GPI IgG Domain I a statistical association (p:0.09). In contrast, 32% were LAC negative and Aß2GPI IgG positive. In this cohort, we have observed a statistical association between Aß2GPI IgG Domain I and thrombosis (p: 0.042), with an odds ratio of 2.429 (1.311-5.733), enhanced by age and male gender (see figure 1).

Conclusions: According to our results, older patients and male gender with Aß2GPI IgG Domain I positivity but LAC negativity have a high risk of developing thrombosis.

PB 1463 | Oral Anticoagulation Cost in Primary Antiphospholipid Syndrome: Comparison between Warfarin and Hypothetical Rivaroxaban

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Background: The novel direct oral anticoagulants are challenging warfarin in several clinical settings including antiphospholipid syndrome with the incentive of lower cost than warfarin.

Aims: To perform a cost comparison of one year of oral anticoagulation management with warfarin versus hypothetical rivaroxaban in twenty patients whose average international normalised ratio (INR) time in therapeutic range was 69±11%.

Methods: For twenty consecutive patients with thrombotic primary APS attending a dedicated anticoagulant clinic we assessed average individual weekly dose of warfarin, average INR, average time in therapeutic range, average yearly attendance to calculate the average yearly cost of warfarin management and compared with hypothetical daily fixed dose rivaroxaban at 20 mgs. The averages of individual patients were summed and divided by 20 to gain a total avarage. Paired T-test was used for cost comparison

Results: Each attendance costs the Italian Health Service 17.85 euros that includes clinic attendance, venepuncture and INR cost; the average patient attendance was 18.3 times a year and average yearly warfarin consumption was 10 boxes (300 tablets of 5 mgs). Average yearly individual warfarin cost was 22±6 euros and total yearly warfarin cost for 20 patients was 446.36 euros; average yearly individual attendance and laboratory cost was 334±56 euros and total cost for 20 patients was 6,693.75 euros. This the total cost of managing 20 patients was 7,140.11 euros. The same costs for managing patients with rivaroxaban 20 mg daily would be 663.66 euros per patient per year that for 20 patients would be **13,273.20** euros per year compared to 7,140.11 ($p< 0.0001$).

Conclusions: The management of 20 thrombotic APS patients with rivaroxaban 20 mg day for one year is 46% more expensive than warfarin; with a time in therapeutic range of almost 70% we cannot justify the use of rivaroxaban on the basis of cost alone that would be a major economic burden to the Italian Health Service.

PB 1464 | Comprehensive Characterization of Platelet Parameters for Thrombosis Risk Prediction in Patients with the Lupus Anticoagulant

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Background: Lupus anticoagulant (LA) positive patients are at high risk for arterial and/or venous thromboembolic events.

Aims: To investigate the association of platelet parameters, namely platelet count (PLT), mean platelet volume (MPV), and the MPV/PLT ratio with thrombosis risk in patients with the LA.

Methods: In this prospective cohort study, we followed 165 persistently LA positive patients (female=136, 82%) over a median follow-up (FU) of 9.2 years.

TABLE 1 Baseline characteristics of the study population (n=165)

Variable	Median [25th-75th percentile], or absolute count (percent)
Age at entry (years)	41 [30-60]
Platelet count (G/L)	222 [175-258]
MPV (fL)	10.5 [9.9-11.2]
MPV/Platelet ratio	0.5 [0.4-0.6]
aPTT-LA (seconds)	87.3 [69.9-116.5]
aβ2GPI IgM (MPL)	5.6 [2.5-15.2]
aβ2GPI IgG (GPL)	9.4 [2.1-48.6]
aCL IgM (MPL)	9.1 [3.5-23.0]
aCL IgG (GPL)	18.6 [6.3-71.7]

Platelet parameters were investigated at baseline, and at each of the 1,047 FU visits. During FU, 41 thrombotic events (arterial n=21, venous n=20) occurred.

Results: At baseline, the median PLT and MPV levels were 222G/L [25^{th}-75^{th} percentile: 175-258] and 10.5fL [9.9-11.2], respectively.

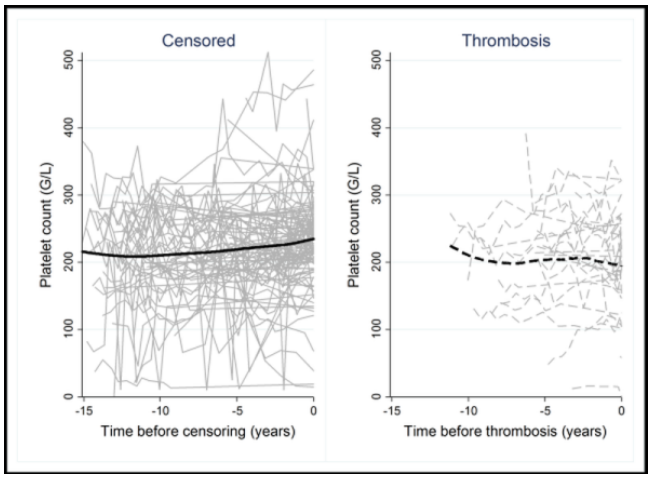

FIGURE 1 Platelet count trajectory in LA-positive patients with and without thrombotic complications during follow-up

In univariable time-to-thrombosis competing risk regression, a higher MPV (SHR per 1fL increase=1.13, 1.05-1.21, p=0.001), and higher MPV/PLT ratio (SHR per 1 unit increase=2.45, 1.75-3.42, p< 0.0001) predicted for a higher risk of thrombosis. However, these associations did not prevail after multivariable adjustment for diabetes, active smoking, and the lupus-sensitive aPTT (Adjusted SHR=1.04, 0.96-1.13, p=0.33 and 1.43, 0.84-1.42, p=0.19). In longitudinal analysis of 1,047 follow-up visits, PLT count slightly increased over time (change/year=+1.4G/L, 0.0-2.94, p=0.06), whereas the other two parameters remained constant throughout FU (not shown). In joint modeling of longitudinal platelet characteristics and time-to-thrombosis, longitudinal changes of platelet characteristics were not associated with thrombosis risk (Hazard ratio for a 5G/L increase in PLT=0.98, 0.96-1.00, p=0.12, Figure 1, others not shown).

Conclusions: In the current analysis, the three investigated platelet parameters were not prognostic for thrombosis risk in patients with the LA. However, high MPV might generally express a cardiovascular phenotype at high thrombotic risk.

PB 1465 | Severe Vitamin D Deficiency a Potentially Risk Factor for Recurrent Miscarriages in Women with Positive Antiphospholipid Antibodies

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Background: Vitamin D levels are low in patients with antiphospholipid syndrome (APS), especially on thrombotic APS, and also related to the recurrence of thrombosis. In vitro studies showed that vitamin D exerts an antithrombotic and immunomodulator function by inhibiting anti-β2GPI antibody-mediated tissue factor expression. There are no enough data between the relation of vitamin D levels and miscarriages/ infertility in women with positive antiphospholipid antibodies (APL).

Aims: We aimed to investigate if Vitamin D deficiency in women with APL is related with a risk of recurrent miscarriage or repeated (>three) in vitro fertilization (IVF) failures.

Methods: A prospective observational study was conducted in women positive antiphospholipid antibodies (at least one antiphospholipid antibody, twice positive with an interval >12 weeks), between -2013 2016. Primary outcome of the study was to correlate the presence of APL antibodies with blood levels of vitamin D. Linear regression model was used and level of statistical significance was defined at P< 0.05.

Results: There were 268 women enrolled in the study. Mean levels of vitamin D were 9.2 ± 18.6ng/ml (normal >30ng/ml).122 women had APL antibodies but incomplete APS diagnosis (only one miscarriage and/or repeated IVF failures) and 146 classical obstetric APS. Rate of vitamin D deficiency was %88.8, of which %28.7 mild (30-20ng/ml), %41.4 moderate (20-10ng/ml) and %18.7 severe deficiency < 10ng.ml. No significant correlation was detected regarding infertility (P=.47) and miscarriage (P=.58). Vitamin D levels were 9.8 ± 17.9 in patients with recurrent miscarriage vs. 8.9 ± 18.8 in women with infertility. Rate of recurrent miscarriages was significantly higher in the category of women with severe deficiency of vitamin D (%42.0 vs. %28 in the rest of women, P< 0.05).

Conclusions: Women with APS, or APL antibodies have low levels of vitamin D. Severe deficiency of vitamin D is associated with recurrent miscarriages in women with antiphospholipid antibodies.

PB 1466 | Antibodies against Domain I of Beta2-glycoprotein I Have No Added Value for Prediction of Antiphospholipid Syndrome Compared to Classical Antiphospholipid Antibodies

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Background: The diagnosis of antiphospholipid syndrome (APS) is made by the combination of vascular thrombosis and/or obstetric complications together with antiphospholipid antibodies (aPLs). Beta 2-glycoprotein I (ß2GPI) is the major antigen for aPLs. Recently, antibodies directed to the first domain of ß2GPI have been suggested to have added value above total ß2GPI for diagnosis of APS.

Aims: To evaluate whether ß2GPI-domain I antibodies (anti-ß2GPI DI) are a better predictor of APS.

Methods: In two hospitals blood samples from patients referred for suspicion of APS and with one positive classical antibody test, were collected. Lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), anti-ß2GPI antibodies (anti-ß2GPI) and anti-ß2GPI-DI were measured. Anti-ß2GPI DI, anti-ß2GPI and aCL were determined using EIA (chemiluminescent or fluoro-enzyme immunoassay). Final diagnosis of APS was made by the combination of clinical features and the positivity of one of the three accepted aPLs. Results were correlated with diagnosis of APS.

Results: 62 patients were included. Finally, 25 patients were classified having an APS while 37 patients did not fulfill the criteria. Odds ratio for APS were as follow: LAC 2.2 (95% CI 0.7-7.0), aCL 2.6 (95% CI 0.9-7.7), anti-ß2GPI 3.8 (95% CI 1.3-11.5) and anti-ß2GPI DI 2.4 (95% CI 0.8-7.0). Anti-ß2GPI and anti-ß2GPI DI as aCL and anti-ß2GPI DI correlated well (Spearman's correlation coefficient respectively ρ:0.3 , $p< 0.05$; ρ:0.6 , $p< 0.01$).

Conclusions: In this patient population, anti-ß2GPI DI have no added value in predicting APS compared to anti-ß2GPI, aCL or LAC.

PB 1467 | Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION): Comparison of Real World and Core Laboratory Lupus Anticoagulant Results

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Background: APS ACTION is conducting a 10-year international study of disease course in antiphospholipid (aPL) positive patients. The five Core Laboratories (CL) perform lupus anticoagulant (LA) and other aPL assays to confirm aPL positivity. A previous exercise showed good agreement between the CL by using the same methodology, analyser type, and reagents.

Aims: To examine agreement in LA status between Core and hospital laboratories of centres entering patients. APS patients were included if they were tested for LA within one year prior to enrolment and fulfilled International consensus criteria.

Methods: LA in non-anticoagulated (AC) samples by DRVVT (Screen/Confirm) and silica clotting time using an ACL TOP500 analyser (IL) was assessed by all CL. LA status of patients on vitamin K antagonists (VKA) was assessed at a single CL (UK) by DRVVT (using equal volume of patient/normal plasma mixtures) and Taipan/Ecarin time ratio.

Results: In this ongoing study, 355 samples were analysed. Results are shown for 239 samples (105 non-AC and 134 VKA). 116 samples were excluded due to incomplete clinical information, insufficient plasma, or on other anticoagulants. Of the included samples, hospitals submitted LA results on >3 occasions in 154 patients, 2 occasions in 61 patients, and 1 occasion in 24 patients. Results judged as equivocal at the CL (abnormal results and lack of evidence for an inhibitor) were considered as "LA not detected". Agreement for LA results between the original hospital and CL was 88.8% and 78.1% for non-AC and VKA patients, respectively. Inclusion of LA equivocal samples as LA positive would have decreased the agreement to 75.4% for the non-AC samples but increased it to 84.8% for the VKA samples.

Conclusions: There was very good agreement on LA status between the original hospital and CL, particularly for the non-AC samples. This is encouraging both for international consensus on LA testing and the validity of aPL data accumulating in the APS ACTION database.

PB 1469 | Thrombocytopenia is Uncommon in High-risk Patients with Antiphospholipid Syndrome (APS)

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Background: Thrombocytopenia is the most common non-criteria hematologic manifestation in patients with the antiphospholipid syndrome (APS). It has been often reported with a prevalence beetween20 to 53%. In the Euro-Phospholipid project,29,6% of patients had thrombocytopenia (< $100x10^9$/L). The rate in patients with primary APS (PAPS) was 21% and 41,9% in those with secondary APS (SAPS).Prevalence reported in the literature depends on the different cut-off (< $100x10^9$/L or < $150x10^9$/L) used.

Aims: To assess the prevalence of thrombocytopenia in a cohort of high-risk APS patients.

Methods: We analyzed 116 high-risk triple positive APS patients (41 males and75 females, aged from 22 to 80 years, median 48,6 years). Among these patients, 82 were PAPS and 34 SAPS (21 LES, 13 lupus-like disease).

Results: Using a cut-off of $150x10^9$/L, the rate of thrombocytopenia is 15% (mean $208,6x10^9$/L± 72, minimum $6x10^9$/L - maximum $541x10^9$/L). The prevalence is 16% in PAPS and 12% in SAPS. Considering a cut-off of $100x10^9$/L, the rate of thrombocytopenia decreases to 6% (7% in PAPS and 3% in SAPS). Two patients with very low platelet count had associated immune thrombocytopenia and underwent splenectomy.

Five of these patients, with normal platelet count, underwent a catastrophic phase of the disease. During this period, the rate of thrombocytopenia was 100%.

Conclusions: This study shows that in high-risk APS patients the rate of thrombocytopenia is lower than that reported in the literature. A

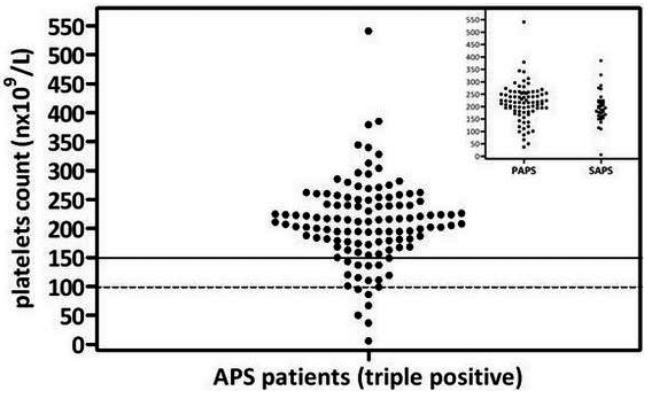

FIGURE 1 Platelets count in all triple APS patients, and in subgroups PAPS (Primary APS) and SAPS (Secondary APS).

drop in platelet count was observed in all patients who deteriorate into the catastrophic form of the disease.

No significative difference between PAPS and SAPS platelet counts has been found.

PB 1470 | Circulating Neutrophil-Extracellular Traps (NETs) in Patients with the Antiphospholipid Syndrome

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies (APLs), arterial and venous thrombosis, and fetal losses. Neutrophil-extracellular traps (NETs) are related with inflammatory and autoimmune diseases and it has been suggested that autoantibodies can induce NETs and enhance thrombotic risk.

Aims: To evaluate plasma circulating NETs levels in a cohort of patients with APS as a model of autoimmune disease associated with hypercoagulability.

Methods: In this cross-sectional study plasma circulating NETs levels were measured in 60 patients with APS [48 primary APS (PAPS), and 12 APS associated with systemic lupus erythematous (SLE) (SAPS)], in 32 patients with SLE [10 with APLs, and 22 without APLs], and in 50 healthy individuals (control group). No statistically significant differences in age and sex were found among groups. A total of 33 APS patients had history of arterial thrombosis (26 in PAPS and 7 in SAPS groups) and 17 history of venous thrombosis (12 in PAPS and 5 in SAPS groups). Samples were obtained outside of acute active phase of the disease. Plasma circulating NETs levels were measured as histone-DNA complexes by a quantitative sandwich-enzyme-linked immunosorbent assay (ELISA). Results were expressed as a ratio between mU of patient/ mU of standard sample. Results are reported as mean± SD. Statistical significance was defined as a p-value < 0.05.

Results: Plasma circulating NETs levels were 0.86± 0.33 in PAPS patients, 0.81± 0.37 in SAPS patients, 0.86± 0.26 in SLE patients with APLs, and 0.71± 0.10 in SLE patients without APLs, and 1.06± 0.28 in controls. There were no significant differences in the levels of NETs among the groups. In addition, in the patients with APS no statistically significant differences were found between patients with or without history of venous or arterial thrombosis.

Conclusions: Patients with APS in a non-active phase of the disease have not significantly elevated plasma circulating NETs levels.

PB 1471 | Catastrophic Antiphospholipid Syndrome: Onset and Outcome

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Background: Multiple vascular occlusions in three or more organs associated with high titer antiphospholipid antibodies (aPL) is known as catastrophic antiphospholipid syndrome (CAPS)

Aims: To assess retrospectively the prevalence of CAPS in pts with APS and to determine the long-term outcome of CAPS pts who survived after CAPS.

Methods: Data of 162 pts. with systemic lupus erythematosus (SLE) and with aPL and 94 pts. with primary APS (PAPS) were analyzed. Mean (±SD) follow-up was 10.7 (4.6) years and mean age at time of including in the study was 33.0 (11.0) and 35.4 (10.1) years respectively. IgG/M anticardiolipin antibodies (aCL), lupus anticoagulant were measured in all pts.

Results: The development of CAPS was found in 43 pts: 33/162 (20.4%) pts with SLE and aPL (23F; 10M) and 10/94 (10.6%) with PAPS (8F; 2M) respectively. Thirty two (25/33 (75.5%) and 7/10 (70.0%) patients were died. Two of 11 survived CAPS pts. developed thrombosis in a year with death event. The analyses of the concomitant factors which may initiate CAPS were assessed. There were SLE flare (n=12), initial menopause (n=2), infections (n=12) including pneumonia (n=7), acute respiratory disease (n=3), food poisoning (n=1), abscess (n=1) as provoking factors in SLE+APS pts. Cancer was revealed in 1 SLE pts and trauma after road accident in 1 SLE pts. Thrombotic microangiopathy as cause of CAPS was found in 3 pts (1 with SLE, 2 with PAPS). Fulminant purpura was at onset of CAPS in all 3 cases. Two of them had recurrent severe skin necrosis with multiple organ failure and died due to severe sepsis in 1 and 2 years. Triggering factor in PAPS pts. was pneumonia (n=2) and abscess (n=1), in 7 pts these factors were not detected. Treatment comprised high dose of steroids, full anticoagulation and immunosuppressant in most of the cases, antibiotics administered when infection was evident.

Conclusions: Triggering factors of CAPS were flare of SLE and infection. Significant functional impairment due to initial CAPS had all 9 survived CAPS patients.

PB 1472 | Effect of Hydroxychloroquine on Circulating Levels of Inflammatory Cytokines in Patients with Primary Antiphospholipid Syndrome

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Background: Hydroxychloroquine (HCQ) has immunologic and antithrombotic effects in systemic lupus erythematous (SLE). However, the effect of HCQ in primary antiphospholipid syndrome (PAPS) with thrombosis is not determined.

Aims: The aim of this study was to evaluate the impact of HCQ on the immunologic profile of PAPS patients.

Methods: This is a prospective study that included 23 patients with PAPS and thrombosis, we quantified the circulation levels of cytokines before treatment (BT) and after 6 months of HCQ 400mg/day. We also included 28 healthy controls, matched by age and gender, for reference values. Serum levels of tumor necrosis factor-alpha (TNF-a), interleukin (IL) -2, -4, -8, -10, -17, CD40L and interferon-gama (INF-g) were performed by multiplex assays (Milliplex,Millipore).

Results: Patients median age was 44 years-old and 87% were female. The antibody profiles were: 6 triple positive, 6 double positive (lupus anticoagulant -LAC plus anti-beta2glycoprotein1) and 11 isolated LAC. Before treatment, circulating levels of TNF-a were similar to controls (median 3.5 vs. 0.2pg/mL; P=0.4), but decreased after-HCQ (median 0.01pg/mL; P=0.05). Levels of IL-8 were higher BT compared to controls (median 6.4 vs. 4.7 pg/mL; P=0.04) and did not change after-HCQ (median=7.5pg/mL; P=0.1). Conversely, levels of CD40L were lower BT than in controls (median 2598 vs. 3562 pg/mL; P=0.01) and remained low after-HCQ (median=2297 pg/mL; P=0.6). Before treatment, levels of IL-2 were similar to controls, but had a trend to decrease after-HCQ (median 0.46 pre-HCQ and 0.01 pg/mL after-HCQ, P=0.09). Levels of INF-g and IL-10 were similar between controls and patients and did not alter after-HCQ, however the amount of cytokines detected was variable. IL-4 and IL-17 were not detected.

Conclusions: In conclusion, HCQ may inhibit TNF-a, an inflammatory cytokine released mainly by monocytes, which is the main inflammatory cell involved in APS pathogenesis. However, the drug has minimal effect on the levels of other interleukins.

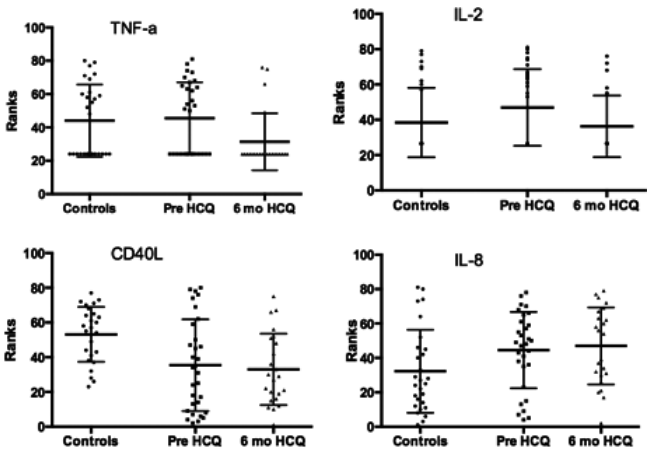

Figure 1. Distribution of the levels of TNFa, IL-2, CD40L and IL-8 in controls and in patients, before and during the treatment with HCQ.

FIGURE 1

PB 1473 | A High Molecular Weigh Protein Is Responsible of Anticoagulant Activity in Isolated Lupus Anticoagulant

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Background: The term „lupus anticoagulant“ (LAC) refers to an acquired *in vitro* inhibitor blood coagulation. This activity is usually associated to the presence heterogeneous group of immunoglobulins directed towards βeta2 glycoprotein I (β2GPI). However, in the case of isolated LAC (negative anticardiolipin and anti-β2GPI antibodies), the substance conferring anticoagulant activity to the plasma is unknown.

Aims: To analyze the plasma of patients with isolated LAC to identify the substance responsible for this activity.

Methods: We analyzed the plasma of 14 patients with isolated LAC by affinity purifying IgG using a Protein G column. We then tested the anticoagulant activity of eluted IgG and that of flow through (FT) both with dRVVT and aPTT. Plasma of strongest isolated LAC was poured into a Sephacryl High Resolution S-300 column from which 43 1mL fractions were collected and tested for LAC activity and the presence of IgM, IgG and β2GPI in specific ELISA assays. Finally, we precipitated the euglobulin fraction from plasma of positive LAC patients with PEG 8.000 at 5% to isolate the globulin plasma fraction.

Results: IgG isolated from 14 patients with only LAC positivity didn't prolong dRVVT test (37"±0.33 control time vs a mean of 37.46"±0.55 for IgG fractions). Anticoagulant activity was in the FT (dRVVT= 56.58"±12.46 SD). Using the plasma of the strongest LAC patient, LAC activity was in the first peak coming from the S-300 column . The dRVVT in these fractions was 45.9"±1.9 (control 37"±0.2). In this peak we found a high concentration of IgM, and very low concentration of IgG and β2GPI. LAC activity was also present in globulin fraction isolated with PEG 8.000 precipitation at first and then with S-300 column (dRVVT=52.1"±2.33, control 35.85"±0.85). This peak analyzed by SDS-page showed the several proteins with high molecular weigh.

Conclusions: We can hypothesize that the responsible of LAC activity in isolated LAC is a high molecular weight protein that needs more studies for its identification.

PB 1474 | DNA Methylation Pattern Analyses in Patients with Antiphospholipid Syndrome: Screening Study

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Background: Epigenetic modification has been studied in many immune disorders to elucidate its pathophysiologic correlation, along with genomic, epigenomic profiling. Aberrant DNA methylation and its impact on gene expression have been implicated.

Aims: This study is to investigate a global DNA methylation status of antiphospholipid syndrome (APS) patients with CpG microarray, and compared it with healthy controls.

Methods: Human peripheral whole blood were obtained from 6 APS (2 primary and 4 secondary APS) and from 6 age, sex-matched healthy controls. Total genomic DNA extracted using the QIAamp DNA mini and blood kit protocol. To discover aberrantly methylated genes in APS by genome-wide search, we introduce Illumina Infinium Human Methylation 450K BeadChip for directly identifying differentially methylated regions of the genomes in each pooled whole blood between APS patients and healthy controls. Immunologic profiles of the patient group including aPL, aCL, LA, anti-b2GP1 were obtained.

Results: The fluorescent signal intensities were extracted using GenomeStudio Methylation module software. One-hundred and thirty two CpG sites were filtered by criteria of delta mean >0.2, and p value < 0.05 after the quantile normalization to reliably compare data from multiple samples by minimize non-biologic differences. Seventy nine CpG sites (KNDC1, ABHD8, CDK2AP1, MX1, IL16, ROCK2, SNTG2, etc) were hypermethylated and 53 sites (CYP2E1, BTNL2, DTX2, GCC2, SLC9A3, etc) were hypomethylated. All differentially methylated CpG sites were located in 5'UTR, TSS1500, TSS200, Exon, intergenic body, 3'UTR. Hierachical clustering (figure1) and the functional classification analysis using DAVID was done.

Conclusions: Hundreds of candidate genes were screened by BeadChip microarray of peripheral blood in APS patients. High-throughput, next generation sequencing of the individual patients for validation will be underwent and the correlation with immunologic profile and thrombotic features will be investigated.

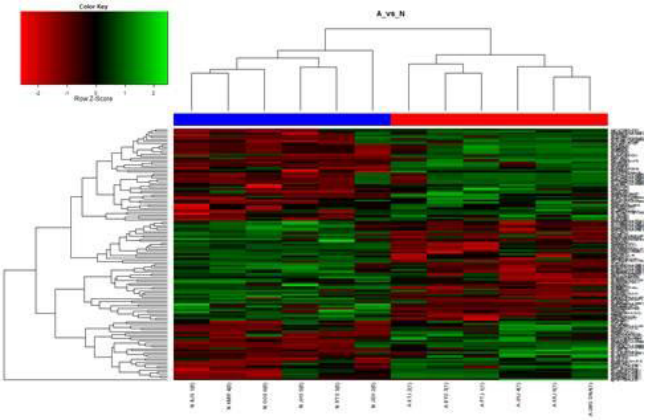

FIGURE 1 Hierachical clustering of the most significantly different CpG sites.

PB 1475 | What Is the Risk of First Thrombosis in Asymptomatic Antiphospholipid Antibodies Carriers?

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Background: Antiphospholipid Syndrome (APS) is defined by the presence of antiphospholipid antibodies (aPL) plus thrombotic events (TE) or obstetric morbidity (OM). Persistent aPL are occasionally found in individuals without previous TE. However, the risk of first TE in asymptomatic aPL carriers is unknown. Recently, triple positivity (Lupus Anticoagulant (LA), Anticardiolipin Antibodies (ACA) and anti-ß2-glicoprotein I (aß2GPI) positivity is claimed to increase the risk of APS.

Aims: Evaluate the incidence of TE in a group of asymptomatic aPL carriers.

Methods: We evaluated retrospectively and prospectively 49 individuals (38 women, 11 men), with a median age at diagnosis of 45 years-old (yo) (7-80 yo) and a laboratorial diagnosis of aPL positivity. Individuals were excluded if it was not possible to confirm aPL in a second sample or if follow-up was less than 2 years.

Results: The studied population's characteristics are depicted on Table 1. Different aPL profiles are described in Table 2. Our follow-up was of 416 individuals/year with an average duration of 8.5 years (2-27 years). During this period, it was reported only one TE in a 75 yo woman with isolated LA+ and essential hypertension who had stroke symptoms, but without imaging confirmation.

TABLE 1 Characterization of the studied population

Sex (Female/ Male)	38 / 11
Median age at diagnosis (years)	45 (7-80)
Autoimmune diseases (n=12)	
SLE / others	10 / 2
aPL profile	
Isolated LA positivity	27
Triple positivity	16
ACA and/ or aß2GP positivity*	6
Mean follow-up (years)	8.5 (2-27)

*ACA IgG / ACA IgM / aβ2GPI IgM / ACA IgM and β2GPI IgM (3/1/1/1); SLE: Systemic Lupus Erythematosus

TABLE 2 Characterization of the different aPL profiles

	Isolated LA positivity (n=27)	Triple positivity (n=16)	ACA and/ or aß2GP positivity (n=6)
Sex (Female/ Male)	19/ 8	13/ 3	6/ 0
Median age (years)	56 (8-80)	28.5 (7-75)	38 (30-61)
Context of aPL detection	19 aPTT E / 4 AI / 2 OM	3 aPTT E / 7 AI / 3 OM	1 AI / 4 OM
AAS prophylaxis (Yes/ No)	9/ 13	6/ 9	6/ 0
AI diagnosed	5	6	1
LA intensity	1.68	2.1	-
Mean follow-up (years)	8.3	8.4	8.0

AAS: Acetylsalicylic Acid; AI: Autoimmune disease; E: Extended; SLE: Systemic Lupus Erythematous

Conclusions: Among aPL, LA is considered the most import risk predictor for TE. However, when it is present alone, it seems not to relate with an increased thrombotic risk. In our population, considering that episode as a TE (even without confirmation), the incidence of TE was of 0.45%/year, not different of the same age Caucasian population (approximately 0.4%/year). On the other side, triple positivity has been related with an increased risk of TE, with an incidence of 5.3%/year[1]. However, in our population, we didn´t find such association. Our data differs from previous studies that suggest triple positivity as an important risk factor for thrombosis.

[1]Pengo V et al., Blood 2015

PB 1476 | Clinical Relevance of Anti Phospholipid Antibodies in Deep Venous Thrombosis

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Background: Diagnosis of antiphospholipid syndrome (APS) is quite difficult and challenging. Our study deduced the sensitivity & specificity for each test to antiphospholipid antibodies (APA) in patients with deep venous thrombosis (DVT) and the relation of increasing antibody titer to recurrent thrombosis.

Aims: We aimed to determine the frequency of positive APS detected by laboratory investigations in patients with unexplained DVT.

Methods: Eighty patients with DVT diagnosed by Doppler ultrasonograpy were screened for lupus anticoagulant by (APTT-LA) and dilute russel viper venom time (dRVVT) screen ratio then positive patients were confirmed by mixing study and (dRVVT) confirm test respectively. Anticardiolipin (ACL) IgG and beta2 glycoprotein I (β2GPI) IgG were done for all patients using enzyme linked immune sorbent assay.

Results: APS showed positivity in (68/80) 85% of our patients using APTT-LA and dRVVT screen ratio that was confirmed by mixing studies, Rosner index and dRVVT correction ratio. Twelve patients out of all patients (12/80) were positive by either ACL or β2GPI . Both ACL and β2GPI showed perfect agreement with Rosner index and dRVVT correction ratio (Kappa index=1.000).Our results showed a significant correlation between increasing age and number of DVT attacks. Rosner index and ACL IgG were significantly correlated with age and increased number of attacks. Accordingly patients with recurrent attacks of DVT showed significantly higher median age and higher median ACL IgG titer in our study. The most striking result was the high sensitivity & specificity of APTT-LA and dRVVT screen ratio (100%) for each of them in comparison to other tests.

Conclusions: Positivity in this study highlighted the importance of using both APTT-LA and dRVVT tests together in the detection of LA. However, further studies on larger number of patients using the four tests (APTT-LA, dRVVT, ACL and β2GPI) are recommended to be performed to explore the best diagnostic assays for APS.

PB 1477 | Results of Lupus Anticoagulant Investigation in Unselective Population of Patients Referred for Testing

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Background: Laboratory evaluation of lupus anticoagulant (LA) antiphospholipid antibodies is an integral part of the diagnostic criteria for antiphospholipid syndrome (APS).

Aims: The aim of this study was to analyze the results of laboratory evaluation of LA antibodies in unselected patients referred for testing during a period of two consecutive years (from January 2015 to December 2016).

Methods: LA antibodies were evaluated by a panel of commercial coagulation assays (Siemens, Germany) including three screening tests: activated partial thromboplastin time (APTT), APTT mixing test, dilute Russell´s viper venom time (dRVVT) screen test (LA1) and confirmation test (LA2).

Results: The prevalences of positive LA results in unselected consecutive patients referred for testing were: 26/810 (3.2%) in 2015 and 24/878 (2.7%) in 2016 with the overall prevalence of 50/1688 (3.0%) for the period of two years. There were 26 female and 24 male patients with positive LA results, with a median age of 40 years (95%CI for median 37-48 yrs; interquartile range 29-59 yrs). Among all LA positives, venous thromboembolism (VTE) accounted for more than a half of all cases (28/50), including positive LA result in 25 cases of deep venous thrombosis (DVT) and in 3 cases of pulmonary embolism (PE). The prevalences of other clinical diagnoses among all LA positive results were as followed: cerebrovascular insult (3), varicose veins (2), cardiovascular disease (2), transient ichemic attack (2), miscarriage (2), infertility (1), autoimune haemolytic anemia (2), systemic lupus

erythematodes (2), rheumatoid arthritis (2), epilepsy (1), vasculitis (1), hemiplegia (1) and urticaria (1).

Conclusions: Laboratory investigation of LA antibodies among unselected consecutive patients referred for testing has shown an overall prevalence of 3.0%. As expected, VTE accounted for most of the positive LA results. However, beside of VTE patients, there were a number of other clinical diagnoses with positive LA results.

PB 1479 | Detection of Thrombogenicity Positive Antiphospholipid Antibodies in Antiphospholipid Syndrome Diagnosis

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Background: Anti-cardiolipin antibodies (aCL) and anti-β-2-glycoprotein I antibodies (ab2GPI) represent two out of three laboratory criteria for detection of antiphospholipid syndrome (APS). The domain I in anti-β-2-glycoprotein I is a new target for better identification of antibodies and are associated to thrombotic risk in antiphospholipid syndrome.

Aims: The aids of our study was to determine the significance of Domain I in anti-β-2-glycoprotein I as new biomarker for determining thrombotic risk in antiphospholipid syndrome and detection their thrombogenicity

Methods: We detect Domain I ab2GPI and diluted prothrombin time on a group of 74 patients with antiphospholipid syndrome diagnosis. All patients has positive antibodies in at least one class aCL and ab-2GPI antibodies. The determination of ACL and β-2-GPI IgG and IgM antibodies was performed by the chemiluminescent assay (HemosIL AcuStar®) in singlet.

Results: We detect Domain I ab2GPI positivity at 21 samples in our group. The thrombotic complications was been observed at 21 from 74 patients. The incidence of thrombotic complications in the total group was established as 28,4 %, in comparison to group positive for DI anti-β2GPI with the incidence of thrombotic complications 57 %. Performing of assay improved positive predictive value from 25% pretest to 68% for patients with positive test.

Conclusions: The new chemiluminescent method for detection of Domain I ab2GPI with detection of thrombogenicity of antibodies shows better compliance with clinical outcome than the actual examination.

Supported by grant LF-2017-001 and MH CZ - DRO (FNOl, 00098892)

PB 1480 | Tissue Factor-bearing Microvesicles and Acquired Protein S Deficiency Contribute to DIC in a Patient with Elevated Antiphospholipid Antibodies

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Background: Acquired (i.e. autoimmune) protein S (PS) deficiency is a rare and potentially life-threatening disorder.

Aims: We report the case of a 76-year-old woman with a thrombohemorrhagic syndrome due to DIC in the context of elevated antiphospholipid antibodies and acquired PS deficiency.

Methods: We used standard laboratory tests to measure PC, PS, activated PC resistance (APC-R), factor V gene mutation Leiden, lupus anticoagulant (LA), and antibodies to cardiolipin (aCL) and beta2-glycoprotein I (anti-β2-GPI). Mixing studies with normal human plasma (NHP) were used to screen for a functional PS inhibitor. Tissue factor (TF)-specific procoagulant activity (PCA) of plasma microvesicles (MVs) was assessed by single-stage clotting assay.

Results: The patient presented with a painful reticular erythema indicating microvascular thrombosis and a consumptive coagulopathy: prothrombin time 45.5% (normal: 80-130%), fibrinogen 0.5 g/L (1.8-4.0 g/L), D-dimer 34 mg/L (< 0.5 mg/L). Surgical and antibiotic treatment of concurrent sigmoid diverticulitis did not resolve DIC. Overt malignancy was excluded. The coagulopathy was only controlled by continuous systemic anticoagulation. Titers of IgM-aCL were elevated to 90 U/mL
(< 10 U/mL). Antinuclear antibodies and tests for LA and anti-β2GPI were negative. There was APC-R with a ratio of 1.7 (> 2.0) in the absence of factor V gene mutation Leiden. Free and total PS antigen was normal, but PS activity was severely reduced to 14% (55-125%) with no correction upon mixing with NHP. Significant MV-associated TF PCA was detected. A short-term course of oral glucocorticoids was ineffective.

Conclusions: In this case, generation of MV-associated TF PCA, possibly due to monocyte activation by antiphospholipid antibodies, in combination with acquired APC-R and depletion of protein S activity caused a systemic coagulopathy characterized by microvascular thrombosis, which could only be controlled by uninterrupted systemic anticoagulation.

PB 1481 | Use of Direct Oral Anticoagulant in Antiphospholipid Syndrome: Systematic Review of Literature

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Background: Direct oral anticoagulants (DOACs) are approved for prevent thrombotic recurrence in venous thromboembolism disease but their clinical efficacy and safety are not studied in APS patients. The RAPS trial demonstrated an increased thrombin potential in APS patients with rivaroxaban versus warfarin, suggesting a higher thrombotic risk. Authors concluded that rivaroxaban is safe in APS patients

because there was not thrombotic recurrence during the follow up of 7 months. Several reports of APS patients treated with DOACs have raised safety issues with thrombotic recurrence during treatment.

Aims: We want clarify the safety of DOACs in APS patient and if there are factors associated with thrombosis recurrence.

Methods: We summarized available literature on DOACs use in APS patients through a systematic review and we used Wilcoxon and Fisher's exact test. Missing data were excluded from analyses.

Results: Our systematic review identified 299 published APS patients treated with DOACs; among them, 31 experienced a recurrent thrombosis while on treatment. The three DOACs used was rivaroxaban (N=206), dabigatran etexilate (N=89) and apixaban (N=4) ; there was no difference between them. Triple positivity (positivity of all three laboratory criteria for APS) was associated with a 3-fold increased risk for recurrent thrombosis (19% versus 58%; p< 0,05). There was no difference between previous APS manifestation. Comparisons of a part of the patients' characteristics according to the presence of a thrombotic recurrence while on DOACs are available in Table 1.

TABLE 1 Comparisons of a part of the patients' characteristics according to the presence of a thrombotic recurrence while on DOACs

	APS without recurrence thrombosis (n= 268)	APS with recurrence thrombosis (n= 31)	p value
aPL profile :			
Lupus anticoagulant	112 (75)	19 (79)	
Anticardiolipin	76 (51)	21 (88)	< 0.05
Antibeta2glycoprotein 1	58 (39)	18 (75)	< 0.05
Triple positivity	31 (19)	14 (58)	< 0.05
Oral anticoagulant :			
Rivaroxaban	180 (67)	26 (84)	
Dabigatran	84 (31)	5 (16)	
Apixaban	4 (2)	0 (0)	

Conclusions: In conclusion, DOACs should be used with caution in APS patients and randomized control trials with clinical primary endpoints assessing clinical efficacy and safety are awaited to establish whether the prescription of DOACs could be a safe alternative to warfarin.

PB 1482 | Plasma Thrombogenic Potential in Patients with Granulomatosis with Polyangiitis in Comparison to Antiphospholipid Syndrome

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Background: Granulomatosis with polyngiitis (GPA) is a multisystem inflammatory disease characterized by the antineutrophil cytoplasmic antibody (ANCA)-associated necrotizing vasculitis of small vessels. To the varying degree GPA may be associated with thrombotic complications. Antiphospholipid syndrome (APS) is an autoimmune disorder where venous and arterial thrombosis dominate.

Aims: We assessed *in vitro* endogenous thrombin generation potential in GPA patients in comparison to APS in order to elucidate possible mechanisms responsible for thrombotic manifestations in this disease.

Methods: *In vitro* endogenous thrombin generation potential was assessed using a calibrated automated thrombogram (CAT, Thrombinoscope BV, Maastricht, the Netherlands) and thrombin-antithrombin complexes were determined using ELISA (TAT, Siemens, Germany). All test were performed in 8 patients with GPA, 7 patients with APS and 13 healthy controls. All APS patients received enoxaparine at the time of blood drawing.

Results: Peak thrombin generation was highest in GPA patients compared to APS and control subjects: 498.5 vs 183.2 and 298.1 nM (p=0.0006 and p< 0.0001, respectively). Similar observation was made for endogenous thrombin potential (ETP): 2322.6 vs 1511.3 and 1281.4 nM/min (p=0.009 and p=0.0002, respectively) and TAT concentrations: 11.15 vs 3.6 and 2.99 µg/L (p=0.06 and p=0.0045, respectively). The longest lag time and time to peak thrombin generation were observed in APS compared to GPA and controls: 10.6 vs 3.3 and 2.3 min (p=0.0005 and p< 0.0001, respectively) and 14.9 vs 5.3 and 4.8 min (p=0.0003 and p< 0.0001, respectively).

Conclusions: To our knowledge, this is the first study showing an increased thrombin generation potential in patients with GPA, which can at least in part explain the mechanism responsible for a higher tendency to thrombotic incidents in this disease. It may also indicate an unmet need in terms of GPA therapy strategy.

This study was supported by the Polish National Science Centre UMO-2015/17/B/NZ6/03459.

PB 1483 | Evaluation of Antiphospholipid Antibodies Level in Patients with Type 2 Diabetes mellitus in Enugu South East Nigeria

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Background: Antiphospholipid antibodies (APLs) are implicated in ischaemic thrombotic phenomena. DM, in particular type 2 is often complicated with ischaemic thrombotic events. This study therefore seeks to investigate the role of APLs in the pathogenesis of thrombotic events in type 2 DM. The detection of APLs may predict the risk of developing ischaemic thrombotic events.

Aims: To detect and assess the prevalence of antiphospholipid antibodies (APLS) in subjects with type 2 diabetes mellitus (T2DM).

Methods: This is a consecutive cross sectional study of subjects with T2DM attending diabetic clinic of UNTH. A total of 210 subjects, age and sex matched, were randomly recruited; 70 with complicated T2DM,70 with uncomplicated T2DM and 70 healthy controls. Ethical

clearance was obtained from UNTH ethical committee. The lupus anticoagulant(LA) was assayed using commercial dilute Russells viper venom time (DRVVT) by Technoclone GMbH Austria,Vienna. IgG-B_2 GPI-dependent ACA was assayed using ELISA test kit (anticardiolipin IgG ELISA kit GWB-521211 by Genway Biotech San Diego USA), platelet count was done using haematology analyser-mythic 22.

Results: The prevalence of lupus anticoagulant (LA) showed a prevalence of 7.1%in complicated T2DM, 4.3%in uncomplicated T2DM and healthy control subjects respectively, while the prevalence of IgG-B_2 GPI-dependent ACA was 4.3% in all the groups. Analysis of variance (ANOVA) showed significant statistical difference in mean platelet count for complicted T2DM 208.5(52.30) and healthy control277.5(23.90), (F=77.993,P< 0.001). Pearsons statistical analysis showed significant correlation for APLS and platelet count in complicated T2DM subjects (r=0.316, p=0.008).

Conclusions: The study didnot find any causal or other casual association between T2DM and the occurrence of APLS positivity however APLS may be simply an aggravating factor for thrombosis in T2DM where the two consitions co exist in the same patient.

PB 1484 | Antiphospholipid Antibodies Related with Thrombosis

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Background: Antiphospholipid antibodies (APA) are a family of antibodies directed against proteins bound to negatively charged phospholipids. The presence of APA is a biological marker for antiphospholipid syndrome (APS).

Aims: To evaluate the correlation between APA and thrombosis in patients with CVI (cerebrovascular infarct), AMI (acute myocardial infarct), and DVT (deep venous thrombosis) and pregnancy.

Methods: It is a retrospective study on patients from the Outpatient Department. We used micro ELISA kit to compare with commercial micro ELISA kits (QUANTA Lite TM b_2GP I).

Results: We made a clinical investigation for three classes of immunoglobulin - b_2GP I antibodies of 833 patients in four groups with different diagnoses. 218 were diagnosed with CVI, 232 with AMI, 123 with DVT and 260 were pregnant women. All patients have augmented concentration of all three classes of immunoglobulin and there is high correlation of APA and clinical manifestation of the different diseases. At highest risk were patients with DVT, because 30% had augmented IgG (59, 2 IU) and IgA (5, 63 IU) antibodies. The largest portion of them (32%) had augmented IgM (4, 47 IU).

From 218 patients with CVI - 19% had augmented concentration of IgG (40,91IU), but mostly augmented concentration were antibody from IgG classes.

From 232 patients with AIM -17% were with high concentration of IgM (3,56 IU) immunoglobulin classes the value of other classes of immunoglobulin were less.

From 260 pregnant women with pathological pregnancy, (29%) have augmented concentration of IgG (35, 76 IU). Concentration of IgG is the biggest.

Conclusions: CVI and pathological pregnant women were augmented concentration of IgG class,in patients with AMI and DVT were augmented concentration of IgM class Augmented concentration of all APA classes is in correlation with thrombosis, or a very elevated risk for thrombosis. It is very important to devise a protocol or algorithm to follow up and treat .

PB 1485 | Outcomes in Patients with Antiphospholipid Antibodies at a Tertiary Care Center

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Background: The diagnosis of "definite antiphospholipid syndrome" (APS) requires one clinical manifestation and confirmed laboratory criterion. Patients (pts) may be suspected to have APS without meeting strict criteria and others are referred for an incidental prolonged aPTT, or a falsely positive VDRL. Management of these patients remains a challenge.

Aims: To identify scenarios that prompt APS work-up and to evaluate the course of pts with aPLAs without strict clinical criteria for APS.

Methods: Retrospective review of records of pts referred to the Hematology clinic for APS evaluation from January 2006 to December 2016. We collected demographics, reason for referral, type of aPLs, clinical manifestations, treatment and outcomes. The institutional review board approved the study.

Results: Eighty-four pts were identified. The median age was 36 (range 19-85), and 63 (75%) were female. Reason for referral was identified for all pts (table 1).

TABLE 1 Reasons for referral for APS work-up

		Count
Sex	Female	63 (75%)
	Male	21 (25%)
Reason to work-up	Prolonged aptt	21 (25%)
	First or single event	37 (44%)
	Recurrent events	16 (20%)
	Other (SLE, positive VDRL, Family history, Mitral valve vegetation and neurologic manifestations)	10 (12%)

APS work-up was initiated in 20 (24%) pts for incidental prolonged aPTT, in 38 (45%) after a single APS-defining event, in 16 (19%) due to

>1 prior event, and in 12% for other reasons. The diagnoses of primary, secondary APS or CAPS were made in 51% of patients. Asymptomatic positivity for aPLs was found in 49%. Amongst the asymptomatic pts only 5/30 (17%) later diagnosed with APS, while the majority 23 (77%) remained asymptomatic during a median follow-up of 64 months. Amongst these 30 pts, 15 (50%) received no prophylaxis while other 15 were on long-term ASA, or ASA +/- LMWH during pregnancy. The 5 pts in this subgroup who were diagnosed with APS were all on thromboprophylaxis when the event occurred.

Conclusions: Incidental finding of aPLS, specifically aPTT prolongation in asymptomatic individuals appears to be associated with benign outcomes. Clinical prediction tools may be useful in selecting pts from this subgroup who may benefit from thromboprophylaxis.

PB 1486 | Recurrent Arteriovenous Fistula Thrombosis in Female Patient with Antiphospholipid Syndrome (APS), Factor V Leiden Mutation and MTHFR C677 T Mutation

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Background: Vascular access-related complications are an important cause of morbidity which accounts for 14-17% of hospitalizations for regular dialyses. Some studies imply anastomatic stenosis as the major predisposing factor for thrombotic complications. Some recent reports suggest antiphospholipid antibodies as the likely cause of such complications.

Aims: We describe the case of a 43 year old female with LA, factor V Leiden and MTHFR C 677T gene mutations who experienced 3 episodes of recurrent arteriovenous fistula thrombosis.

Methods: Her recurrent thrombotic complications were related to PTFE grafts. In the perioperative period she was monitored for anastomotic stenosis and evaluation of hypercoagulable states.

Results: The patient was hospitalized for 3 severe recurrent arteriovenous fistula thrombosis episodes following 22 years of hemodialysis and was treated with enoxoparin. No lymphadenopathy or hepatosplenomegaly were determined. Laboratory findings included: factor V Leiden and MTHFR C 677T gene mutations-heterozygotes, elevated level of plasma : homocysteine (43,9 umol/l ref. range 5-14umol/l), LDL and HDL cholesterol (298,6mg/dl) and triglicerydes (278,9ng/dl) and factor VIII activity-268.22%. Weak lupus anticoagulant was found, Therapy with enoxoparin at 1.0 mg/kg dose for 4/7 no hemodialysis days and 0.7mg/kg dose for 3/7 hemodialysis days s.c. daily dose resulted in clinical improvement: elimination of severe recurrent arteriovenous fistula thrombosis.

Conclusions: In the female patient with factor V Leiden and MTHFR C 677T gene mutations recurrent episodes of arteriovenous fistula thrombosis occurred only when lupus anticoagulant was recognized. Long-term enoxoparin therapy was effective in prevention subsequent thrombotic events.

PB 1487 | Comparison of Genetic Risk Score Models to Predict First Venous Thromboembolism in a Population-based Case-cohort. The Tromsø Study

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Background: The predictive capability of genetic risk score models for first venous thromboembolism (VTE) has not been extensively studied in prospective population-based studies.

Aims: To evaluate and compare the capability of three different genetic risk score models to predict first VTE in a case-cohort recruited from the general population.

Methods: Cases with a first VTE (n=660) and an age-weighted subcohort (n=1803) were sampled from three surveys of the Tromsø study (inclusions in 1994-95, 2001-02 and 2007-08, and follow-up until 31.12.2012). Single nucleotide polymorphisms (SNPs) previously implicated in VTE were genotyped in DNA isolated from whole blood (ABO: rs8176719, rs7853989, rs8176743 and rs8176750; F2: rs1799963; F5:rs6025, rs11823905 and rs118203906; F11: rs2036914; F12: rs1801020; F13: rs5985; FGG: rs2066865; SERPINC1: rs121909548; SERPINA10: rs2232698). Individual genetic risk scores were calculated using three genetic risk score models: *Factor V Leiden + Prothrombin (FVL+PT)*; *De Haan 5-SNP score(de Haan)* and *Thrombo inCode(TiC)*. The models were evaluated in terms of discrimination (C-statistics) and reclassification (continuous net reclassification improvement, NRI).

Results: The C-statistic was 0.551 (95% 0.539-0.573) for FVL+PT, 0.611 (95% 0.590-0.643) for TiC (p=0.0002 versus FVL+PT), and 0.594 (95% 0.573-0.634) for de Haan (p=0.017 versus FVL+PT). The NRIs considering FVL+PT as standard procedure were 0.67% (95% CI -1.78-2.42) for de Haan and 17.2% (95% CI 6.3-25.0) for TiC (p=0.0007).

Conclusions: Both the TiC and de Haan genetic risk score models performed better than FVL+PT, and overall, TiC appeared to be better than the de Haan score in predicting first VTE in a general population. Future research should focus on the predictive ability of these risk score models in high-risk situations of VTE and combine genetic risk scores with clinical information.

PB 1488 | Next-Generation DNA Sequencing Approach to Study Burden of Rare Variants in Loci Susceptible to Cerebral Vein Thrombosis

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Background: Cerebral vein thrombosis (CVT) is a rare life-threatening disease annually affecting 4 adults/million. Genetic risk factors are deficiencies of the natural anticoagulant proteins antithrombin, protein C, protein S, factor V Leiden and prothrombin 20210A mutation. In 20% of patients, the cause of CVT remains unknown.

Aims: To identify genetic loci with an increased burden of rare variants as putative risk factors for CVT.

Methods: We investigated 171 Italian CVT patients and 298 healthy controls. Patients were selected using the following criteria: objective diagnosis of CVT, Caucasian decent, no active cancer. Targeted sequencing of the protein-coding regions of 737 candidate genes related to hemostasis and inflammation, 150 ancestry informative markers and 28 thrombosis-associated variants with a final target size of approximately 4 Mb was performed on Illumina HiSeq2000.

Results: Sequencing and data analysis revealed 13,161 rare variants (MAF< 1%) in 733 genes, of which 13,107 single nucleotide variants and 54 insertions/deletions. Gene-based association analysis of these rare variants using Burden test revealed 33 loci with $P< 0.05$, of which the top associations were found in the *ANO6* ($P=0.002$), *HABP2* ($P=0.004$) and *NQO1* ($P=0.004$). In the Sequence Kernel Association Test (SKAT), we identified 17 loci with $P< 0.05$. The top association was found in the *USF1* locus ($P=0.003$). However, none of these loci passed the 20% Bonferroni-Hochberg threshold for multiple testing. In addition, we performed separate analysis for *F5*, *F8* and *VWF* loci, for which the entire genomic region was sequenced. We found non-significant association of variants located in intron 2 ($P=0.02$) and introns 15-20 ($P=0.01$) of the *F8* locus with CVT.

Conclusions: Gene-based tests of association using Burden and SKAT did not yield definitive evidence for rare variants in loci related to hemostasis and inflammation associated with CVT.

PB 1489 | Impact of Age and Prothrombotic Genotypes on the Risk of Incident Venous Thromboembolism

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Background: Venous thromboembolism (VTE) is a mulitcausal disease with genetic and acquired risk factors. The incidence increases exponentially with increasing age. It is, however, uncertain how prothrombotic genotypes affects the risk of VTE in different age-groups.

Aims: To investigate the age-specific effect of prothrombotic genotypes on the hazard ratio (HR), incidence rate difference (IRD) and population attributable risk (%PAR) of incident VTE in subjects below and above 60 years in a general population with a wide age range.

Methods: Cases with a first VTE (n=692) and an age-weighted randomly selected sub-cohort (n=2016) sampled from the Tromsø 4-6 surveys (1994-2012) were included. DNA isolated from blood was genotyped for ABO (rs8176719), FV-Leiden (rs6025), prothrombin 20210A (rs1799963) and KNG1 (rs710446). Cox regression models adjusted for age, sex and BMI were used to calculate age-stratified HR across gene variants. IRDs and %PAR were calculated.

Results: In subjects < 60 years of age, prothrombotic genotypes were generally associated with equal or higher HRs, equal or lower IRDs, and higher %PARs for VTE than in subjects ≥60 years of age. For ABO, HRs were 1.47 (95% CI 1.17-1.86) and 1.22 (95% CI 0.97-1.53), IRDs 0.57 (95% CI 0.29-0.85) and 0.43 (95% CI 0.04-0.82), and %PARs 26% and 11% in subjects below and above 60 years, respectively. For FV-Leiden, HRs were 2.20 (95% CI 1.66-2.92) and 2.21 (95% CI 1.60-3.07), IRDs 2.02 (95% CI 1.18-2.88) and 2.41 (95% CI 1.19-3.64), and %PARs 10% and 7% in subjects below and above 60 years, respectively.

Conclusions: Our findings show that, even though the relative risk of VTE according to prothrombotic genotypes is highest in the young, the absolute risk difference is similar in young and elderly due to a higher incidence of VTE in the elderly. The proportion of VTEs that can be attributed to the individual prothrombotic genotypes was, however, substantially higher in the young population.

PB 1490 | Joint Effects of Ischemic Stroke and Prothrombotic Genotypes on the Risk of Venous Thromboembolism. The Tromsø Study

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Background: Previous studies have reported that patients with ischemic stroke are at transient increased risk of venous thromboembolism (VTE), particularly provoked VTE during the first 3 months following the stroke. Prothrombotic genotypes may augment the risk of VTE under conditions of high thrombosis risk related to the stroke (e.g. hospitalization, immobilization).

Aims: To investigate the joint effect of prothrombotic genotypes and ischemic stroke on risk of VTE in a case-cohort recruited from a general population.

Methods: Cases with incident VTE (n=660) and a randomly selected age-weighted sub-cohort (n=1803) were sampled from 3 surveys of the Tromsø study. DNA isolated from blood was genotyped for rs6025 (factor V Leiden [FVL]), rs1799963 (F2), rs8176719 (ABO), rs2066865 (FGG) and rs2036914 (F11). Cox regression models were used to calculate hazard ratio (HR) for incident VTE by individual- and categories of risk alleles (de Haan 5-SNP score; 0-1, 2, 3-4 and ≥5) and ischemic stroke.

Results: There were 264 incident ischemic strokes of which 60 had an incident VTE. The risk of VTE increased linearly across categories of total number of risk alleles in subjects without and with stroke (p-values < 0.001). A joint effect was observed for the total number of risk alleles and stroke on VTE risk. Subjects with ≥5 risk alleles without stroke had a 2.6-fold (HR 2.58, 95% CI 1.52-4.41) higher VTE risk, whereas subjects with ≥5 risk alleles and stroke had a 6.3-fold (HR 6.31, 95% CI 2.57-15.48) higher VTE risk compared to stroke-free subjects with 0-1 risk alleles. Similar effects were displayed for stroke combined with individual risk alleles of ABO, FVL and FGG on the risk of VTE.

Conclusions: We showed an allele-dependent risk of VTE in ischemic stroke and a joint effect of increasing number of risk alleles and stroke on VTE risk. Our findings may suggest that the number of risk alleles in the de Haan score should be considered when assessing thrombosis risk in patients with ischemic stroke.

PB 1491 | Joint Effects of Myocardial Infarction and Prothrombotic Genotypes on the Risk of Venous Thromboembolism. The Tromsø Study

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Background: Previous studies have shown that myocardial infarction (MI) is associated with a transient increased risk of venous thromboembolism (VTE). Some prothrombotic genotypes, associated with VTE risk, are also shown to increase the risk of arterial thrombosis. However, it is not known whether prothrombotic genotypes in subject with acute MI increases the future risk of VTE.

Aims: To investigate the joint effect of prothrombotic genotypes and MI on risk of VTE in a case-cohort recruited from a general population.

Methods: Cases with a first venous thromboembolism (n=643) and a randomly selected age-weighted cohort (n=1768) were sampled from 3 surveys of the Tromsø study (1994-95, 2001-02 and 2007-08). DNA isolated from blood was genotyped for rs6025 (F5, factor V Leiden [FVL]), rs1799963 (Prothrombin 20210A), rs8176719 (ABO), rs2066865 (FGG) and rs2036914 (F11). Cox regression models were used to calculate hazard ratios (HR) with 95% confidence interval (CI) for incident VTE by single and categories of risk alleles (de Haan 5-SNP score; 0-1, 2, 3 and ≥4 risk alleles) and MI.

Results: There were 272 incident MIs and of which 47 had an incident VTE. In subjects without MI, the risk of VTE increased linearly (p< 0.001) across increasing categories of risk alleles with HR 1.64 (95% CI 1.24-2.18) for subjects with ≥4 compared to those with 0-1 risk alleles. In contrast, there was no linear increase in VTE risk across categories of risk alleles in MI patients, and no joint effect of MI and increasing risk alleles on VTE risk. Among the individual SNPs, a joint effect on VTE risk was found for two risk alleles at FGG and MI (HR 4.87, 95% CI 2.30-10.32) compared to those with no risk alleles and no MI (HR 1.35, 95% CI 1.01-1.80).

Conclusions: Our findings showed that, except for FGG, no combined effects were observed for prothrombotic genotypes and MI on risk of VTE. This suggests that the number of risk alleles in the de Haan score should not be considered when assessing thrombosis risk in patients with MI.

PB 1492 | Population-based Incidence of Venous Thromboembolism in Oklahoma County, OK April 1, 2012-March 31, 2014

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Background: Although published venous thromboembolism (VTE) incidences range from 1-2 per 1,000 population in the US, there is uncertainty in differentiating event-based from patient-based estimates, especially by race/ethnicity.

Aims: We aimed to estimate the incidence of patient-based and event-based VTE events in Oklahoma County by race/ethnicity.

Methods: In collaboration with the Centers for Disease Control and Prevention, we established a population-based surveillance system in Oklahoma County, OK for April 1, 2012-March 31, 2014 to estimate the incidence of VTE events. The Health Commissioner

TABLE 1 Age-adjusted overall and race/ethnicity-stratified patient-based VTE incidence rates (IR) per 1,000 by disease presentation

Demographic characteristic	VTE		DVT Only		PE±DVT	
	IR	95% CI	IR	95% CI	IR	95% CI
Age-Adjusted Overall	2.35	2.27, 2.43	1.47	1.41, 1.54	0.87	0.82, 0.92
Age-Adjusted Race/Ethnicity						
American Indian/Alaska Native	1.09	0.81, 1.37	0.69	0.47, 0.91	0.40	0.23, 0.57
Asian/Pacific Islander	0.63	0.40, 0.86	0.41	0.23, 0.60	0.22	0.08, 0.35
Black	3.14	2.90, 3.37	1.97	1.79, 2.16	1.16	1.02, 1.30
Hispanic	0.63	0.50, 0.77	0.39	0.29, 0.50	0.24	0.16, .032
White	2.57	2.46, 2.67	1.59	1.50, 1.67	0.98	0.92, 1.05

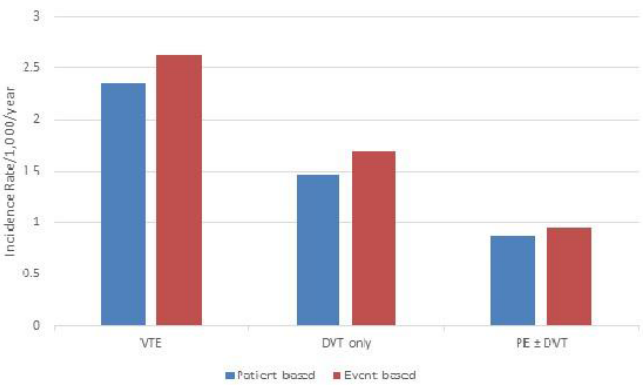

FIGURE 1 Age-adjusted IRs for VTE, DVT, and PE with or without DVT for patient-based and event-based events

made VTE reportable and delegated surveillance responsibilities to the University of Oklahoma, College of Public Health, making IRB unnecessary under Federal statute. Active surveillance involved reviewing imaging studies (e.g., chest computed tomography and compression ultrasound) from all inpatient and eligible outpatient facilities. Passive surveillance included obtaining hospital discharge data for VTE-related conditions. We merged datasets from active and passive methods and reviewed discordant patients. We used Census Bureau estimates to define the Oklahoma County population at risk. All crude and age-adjusted incidence rates (IR) are reported per 1,000 population per year with 95% confidence intervals (CI).

Results: We identified 3255 unique patients and 3650 VTE events. The crude and age-adjusted patient-based VTE IRs were 2.16 (2.08, 2.23) and 2.35 (2.27, 2.43), respectively. Overall age-adjusted event-based IR was 2.63 (2.54, 2.71). Overall and race-stratified patient-based IRs are reported in Table. The figure compares age-adjusted patient- and event-based IRs.

Conclusions: Patient-based VTE IR indicates a significant burden of VTE. Event-based IR estimates are 12% higher than patient-based. VTE IRs differ significantly by race/ethnicity.

PB 1493 | Differential Impact of Surgery as a Trigger Factor for Venous Thromboembolism in Patients with and without Prothrombotic Genotypes

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Background: Surgery is widely known to increase the risk of venous thromboembolism (VTE), but limited knowledge exists on the differential impact of surgery as a trigger for VTE in patients with and without prothrombotic genotypes.

Aims: To explore the influence of surgery as a trigger for VTE in patients with and without common prothrombotic genotypes, using a case-crossover design.

Methods: The study included 531 cancer-free patients with an objectively confirmed first-lifetime VTE. DNA isolated from whole blood was genotyped for rs6025 (FV Leiden) and rs8176719 (ABO). All surgical procedures were registered within each subject during the last 90-day period before the VTE diagnosis (risk period), and in four preceding 90-day comparison periods. To avoid potential carry-over effects, a 90-day washout period was implemented between the risk and control periods. Statistical analysis was performed using conditional logistic regression models.

Results: Overall, 85 (16%) patients underwent surgery in the risk period, and the OR of surgery was 11.4 (95% CI: 7.4-17.5). When we stratified the analysis according to blood type, the risk of VTE by surgery was 13-fold (OR: 13.1, 95% CI: 7.4-23.4) and 7.5-fold (OR: 7.5, 95% CI: 3.9-14.7) among patients with non-O and O, respectively. The OR of surgery in patients with and without FV Leiden, was 12.9 (95% CI: 2.7-62.5) and 10.9 (95% CI: 7.0-17.1), respectively.

Conclusions: Surgery is a major trigger factor for VTE independent of the presence of prothrombotic genotypes. However, surgery may have greater impact as a trigger for VTE in patients with non-O blood type, than those with blood type O.

PB 1494 | Hospitalization as a Trigger for Venous Thromboembolism - Results from a Large Population-based Case-crossover Study

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Background: Previous studies have reported that up to 50% of patients with venous thromboembolism (VTE) has undergone recent hospitalization. However, studies on the impact of hospitalization as a trigger factor for VTE are limited.

Aims: To investigate the impact of hospitalization as a trigger factor for VTE.

Methods: We conducted a population-based case-crossover study of 531 cancer-free subjects with an objectively confirmed first-lifetime VTE. All hospitalizations were registered within each subject during the last 90-day period before the VTE diagnosis (risk period), and in four preceding 90-day comparison periods. A 90-day washout period between the control- and risk periods was implemented to avoid potential carry-over effects. Conditional logistic regression was used to calculate odds ratios (OR) of VTE according to hospitalization.

Results: In total, 249 (47%) of the VTE-patients had been hospitalized in the risk period. The OR of hospitalization was 21.3 (95% CI: 15.1-30.0). Moreover, the OR increased according to the number of hospitalizations within each period from 18.7 (95% CI: 13.1-26.6) in those with one hospitalization to 45.7 (95% CI 23.7-87.9) in those with ≥2 hospitalizations. The OR of hospitalization for reasons other than surgery, was 19.7 (95% CI: 13.2-29.3), and increased from 18.1 (95% CI: 12.1-27.2) in those with one hospitalization to 37.2 (95% CI: 16.0-86.5) in those with ≥2 hospitalizations. Overall, the risk increased slightly with increasing number of total days spent in hospital (OR per one day increase: 1.08, 95% CI: 1.04-1.13), and the OR for hospitalization ≥5 days was 5.7 (95% CI: 2.4-13.7).

Conclusions: Hospitalization is a major trigger factor for VTE, regardless of the reason for hospitalization. The risk increases with increasing number of hospitalizations and length of hospital stay.

PB 1495 | The Rare Allele of the K858R Variant of Factor V Protects from the Risk of Venous Thrombosis but Only in Non Carriers of the FV Leiden Mutation

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Background: Activated factor V (FV) is an important cofactor of the coagulation cascade, which acts mainly by facilitating the conversion of prothrombin to thrombin by activated factor X. Two genetic variations within the F5 gene are now known to influence the risk of venous thrombosis (VT): the FV Leiden (rs6025, R534Q), associated with a resistance to activated protein C (PCa), and the more recently identified polymorphism rs4524 (K858R). While the rare allele Q534 is associated with an increased risk of VT, the rare R858 allele is associated with a decreased risk.

Aims: However, no study has examined the cumulative impact of these two variations on VT risk.

Methods: In this work, we studied the association between these 2 genetic variations and the risk of VT in 4 French case-control samples gathering 4173 patients and 5970 controls.

Results: We first confirmed that the Q534 allele (frequency ~ 2%) is associated with an increased risk of VT (OR = 3.41, p = 6.97 10^{-41}) while the R858 (frequency ~ 26%) is associated with a decrease in risk (OR = 0.83, p = 6.11 10^{-6}). Since the linkage disequilibrium between these two variations was complete and negative (D ´= -1), only 3 haplotypes were observed (R534K858, R534R858 and Q534K858), the Q534R858 haplotype not being present in the French population. In addition, an haplotype analysis demonstrated that the mutation of FV Leiden has a dominant effect on that of R858 since individuals mutated for FV Leiden are at the same risk of disease whenever they are composite heterozygotes or not for the rare allele R858 (ie diplotype R534K858 / Q534K858 vs R534R858 / Q534K858) OR :3.37 vs 2.52 (p=0.15).

Conclusions: In conclusion, our study demonstrated that the allele R858 of F5 rs4524 variant protects from the risk of VT but that its presence does not reduce the risk of VT in individuals mutated for FV Leiden. The mechanisms involved in protection on VT deserve to be studied more precisely since this polymorphism is located at distance from the sites of cleavage of the PCa.

PB 1496 | Joint Effects of Prothrombotic Genotypes and Body Height on Risk of Venous Thromboembolism: The Tromsø Study

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Background: Previous studies have reported synergistic effects of several thrombosis-associated single nucleotide polymorphisms (SNPs) and obesity on the risk of venous thromboembolism (VTE). Tall stature is associated with increased VTE risk, but the joint effect of prothrombotic genotypes and tall stature on VTE risk is unknown.

Aims: To investigate the joint effects of prothrombotic genotypes and tall stature on the risk of VTE.

Methods: Cases with incident VTE (n=675) and a randomly selected age-weighted sub-cohort (n=1845) were sampled from 3 surveys of the Tromsø study. DNA isolated from blood was genotyped for rs6025 (factor V Leiden [FVL]), rs1799963 (F2), rs8176719 (ABO), rs2066865 (FGG) and rs2036914 (F11). Cox regression models were used to calculate age- and sex-adjusted hazard ratios (HR) for incident VTE by categories of risk alleles (de Haan 5-SNP score; 0-1, 2, 3-4 and ≥5) and body height, and for the combined exposures (tall stature and ≥5 risk alleles).
Results: There was a linear increase in VTE risk by increasing categories of body height where subjects in the upper quintile (≥ 178 cm) had 1.6-fold higher VTE risk (HR 1.60; 95% CI 1.19-2.16) than those in the two lowest quintiles (40 percentile,< 166 cm). The risk of VTE increased across categories of risk alleles (p< 0.001) to HR 2.14; 95% CI 1.45-3.16 in those with ≥ 5 compared to those with 0-1 risk alleles. Subjects with ≥5 risk alleles and tall stature (≥ 178 cm) had a 2.6 -fold (HR 2.57, 95% CI 1.15-5.76) higher VTE risk than subjects < 166 cm with 0-1 risk alleles. The relative excess risk caused by interaction (RERI) was -0.54, suggesting that the combined effect of the exposures was less than each factor alone.
Conclusions: We found that the combined effect of risk alleles and body height was essential similar to each factor alone. In contrast to obesity, tall stature and the number of risk alleles did not show a synergistic effect on the risk of VTE.

PB 1497 | Factor XIII Val34Leu Polymorphism Reduces Whole Blood Clot Weight in a Fibrinogen-dependent Manner

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Background: The common FXIII-A polymorphism, Val34Leu (V34L) results in 2.5-fold faster FXIII activation, but paradoxically, protection against venous thrombosis (VT). This effect is hypothesized to result from fibrinogen concentration-dependent changes in fibrin clot structure; at high (prothrombotic) fibrinogen levels, presence of the Leu allele decreases fibrin network density. Notably, during VT, both FXIII activity and fibrin network density are positively associated with red blood cell (RBC) retention in contracted clots and consequently, clot weight. The effect of the V34L polymorphism during whole blood clot formation has not been investigated.
Aims: Determine the effect of the FXIII-A V34L polymorphism on whole blood clot composition and weight.
Methods: Platelet-poor plasmas from 86 healthy human donors (40 FXIII-$A^{Val/Val}$, 28 FXIII-$A^{Val/Leu}$, and 18 FXIII-$A^{Leu/Leu}$) were reconstituted with freshly-isolated platelets and O-negative RBCs. Clotting was triggered with tissue factor and re-calcification, and contracted clots were weighed. Clot weights were correlated with donor sex, age, FXIII activity, presence of major FXIII polymorphisms, factor V Leiden mutation, and fibrinogen level.
Results: Univariate analysis showed mean clot weight did not differ between reconstituted whole blood clots generated from $FXIII^{Val/Val}$, $FXIII^{Val/Leu}$, or $FXIII^{Leu/Leu}$ plasmas. Neither sex or FXIII activity, nor presence of the FXIII-B intron K polymorphism or factor V Leiden mutation, correlated with whole blood clot weight for any genotype. In a multiple linear regression analysis adjusting for fibrinogen level and age, compared to $FXIII^{Val/Val}$ and $FXIII^{Val/Leu}$, $FXIII^{Leu/Leu}$ was significantly associated with reduced clot weight.
Conclusions: In plasmas with high fibrinogen levels, homozygous presence of the Leu34 allele results in the production of smaller whole blood clots. The V34L polymorphism may protect against VT by decreasing fibrin network density and RBC retention in clots and consequently, reducing thrombus size.

PB 1498 | Impact of Prothrombotic Genotypes on the Association Between Red Cell Distribution Width and Risk of Venous Thromboembolism. The Tromsø Study

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Background: Red cell distribution width (RDW), a measure of the variability in size of circulating erythrocytes, is associated with incident venous thromboembolism (VTE). The mechanism underlying the association remains unclear.
Aims: To investigate the joint effects of prothrombotic genotypes and RDW on the risk of VTE in a case-cohort recruited from the general population.
Methods: The study included 666 cases with a first-ever VTE and a randomly selected age-weighted sub-cohort (n=1937) sampled from the fourth survey of the Tromsø Study. Baseline characteristics, including RDW, were obtained in 1994/95, and subjects were followed until December 31st 2012. DNA isolated from blood was genotyped for rs6025 (factor V Leiden (FVL)), rs179963 (F2), rs8176719 (ABO), rs2066865 (FGG) and rs2036914 (F11). Cox regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) for incident VTE by categories of RDW and risk alleles (de Haan 5-SNP score; 0-1, 2, 3-4, and ≥5), and for the combined exposures (high RDW and ≥5 risk alleles).
Results: Subjects with RDW values above the 80th percentile had a 26% increased risk of VTE compared to those with RDW values below the 40th percentile (HR 1.26, 95% CI: 1.01-1.56). The risk of VTE increased across categories of risk alleles (p< 0.001) to HR 2.20 (95% CI 1.50-3.23) in those with ≥5 compared to those with 0-1 risk alleles. Subjects within the highest RDW quintile and with ≥5 risk alleles had a 3.9-fold (HR 3.95, 95% CI: 2.15-7.26) higher risk of VTE than those in the lowest RDW category and with ≤1 risk allele. The relative

excess risk caused by interaction (RERI) was 1.62, suggesting that the combined effect of the exposures was higher than the sum of the individual exposures.

Conclusions: We found that a high number of risk alleles in combination with a high RDW yielded a modest synergistic effect on the risk of VTE.

PB 1499 | Homozygous and Heterozygous Factor V Leiden Mutation (FVL) Is Associated with an Increased Risk for Recurrent Venous Thromboembolism (VTE) after First VTE

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Background: A better risk stratification for recurrent VTE in patients with a first episode of VTE is urgently needed.

Aims: To evaluate the relative risk (hazard ratio, HR) of spontaneous recurrent VTE associated with FVL and prothrombin G20210A mutation (PTM).

Methods: This is a retrospective study covering more than 20 years after a first VTE event in a group of 1,440 patients with first VTE. For statistical analysis, we used a multivariate proportional hazard model adjusted for age, sex, and type of first VTE (spontaneous vs. non-spontaneous).

Results: The adjusted hazard ratios for recurrent spontaneous VTE were as follows: PTM heterozygous 1.30 (95% CI 0.81-2.1), FVL heterozygous 1.33 (95% CI 1.03-1.74), FVL homozygous 2.27 (95% CI 1.19-4.36), combined heterozygous FVL and PTM 1.40 (95% CI 0.64-3.06). Detailed results on absolute risks per year stratified according to spontaneous and non-spontaneous first VTE will be presented.

Conclusions: In contrast to previous smaller studies we now could demonstrate a significantly increased risk of VTE recurrence associated with heterozygous and homozygous FVL. This is in opposition to previous guideline statements. Our results may influence future treatment recommendations.

PB 1500 | Genotype-phenotype Correlations in a Large Cohort of Subjects with Suspicion of Inherited Protein C or Protein S Deficiency

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Background: Hereditary deficiencies of natural anticoagulant proteins including protein C (PC) and protein S (PS) are known causes of inherited thrombophilia.

Aims: We aimed to assess the performance of genetic analysis of *PROC* and *PROS1* with relation to PC and PS assay data.

Methods: In a retrospective cohort study we genotyped 314 subjects with PC and 460 subjects with PS deficiency. Mutations were detected by direct sequencing of the coding regions including splice sites of *PROC* and *PROS1*. We performed MLPA in order to reveal large copy number variations. The characterisation of the variants was performed by in silico evaluation tools, including Polyhen-2, SIFT, multiple sequence alignment, splicing prediction and molecular graphic imaging. PC and PS assay data were provided by external centres. Statistic methods of logistic regression and ROC curve analysis were applied to evaluate the correlation between laboratory status and inherited deficiencies and to propose cut-off values for the indication of genetic testing.

Results: 101 (36 previously unknown) different mutations were identified in 226 subjects of the PC and 81 (36 previously unknown) in 197 subjects of the PS deficiency cohort, correlating with an overall mutation detection rate (MDR) for *PROC* of 72% and *PROS1* of 43%, respectively. MDR correlated negatively with PC and PS levels. In addition, we observed a clear correlation between laboratory data and the type of mutation. For PC activity we determined a cut-off value of 61% with a sensitivity of 81% and a specificity of 64% for revealing a causal gene mutation. Within the PS cohort factor V Leiden (FVL) showed a significant influence on MDR. For individuals without FVL the proposed cut-off value for PS activity is 45% associated with a sensitivity of 70% and a specificity of 65%.

Conclusions: Our findings suggest that in patients with PC activity below 61% and PS levels (activity or free antigen) below 45% genetic analysis represents a useful diagnostic tool to confirm inherited PC and PS deficiency.

PB 1501 | Identification and Characterization of a Novel Mutation at the C-terminal Region of Antithrombin Leading to *in vivo* Polymerization and Venous Thrombosis

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Background: Antithrombin (AT) belongs to serpin superfamily and identification of natural variants in AT improves our understanding of the molecular mechanisms involved in its interaction with target proteases and heparin.

Aims: Identification and characterization of genetic basis of low AT levels.

Methods: DNA sequencing was used for identification of genetic variations in AT deficient Indian DVT patients followed by purification

of wild type and variant AT using hi-trap heparin affinity chromatography. Variant AT was characterized using *in silico* studies and techniques like electrophoretic mobility, fluorescence, circular-dichroism (CD) and transmission electron microscopy (TEM). Informed consent was obtained from the patients.

Results: In an Indian patient with type II AT deficiency (activity: 55%, antigen: 82%), a novel point mutation, g.13397A>G (Ala427Thr) was identified. Previously reported polymorphisms in Indian DVT patients like rs2227589, *Dde*I and C-4X were found to be absent. High molecular weight bands on SDS-PAGE elution profile and reduced fluorescence emission intensities of both tryptophan and bis-ANS were observed for Ala427Thr. Far-UV CD and thermal denaturation studies showed increased α-helical content, decreased β-strands and a high melting temperature (T_m= 77.06 ± 0.15°C) indicating variation in secondary structure and a change in conformation. Visualization of wild type and Ala427Thr AT through TEM incubated at 60°C for 0, 30 and 90 minutes showed the presence of polymeric variant AT even at 0 minute which were otherwise absent from the wild type AT. ASA analysis of variant AT revealed decreased stability and post mutation, Ala427Thr formed additional hydrogen bonds with Asp72 and Asp74 in addition to Asn75.

Conclusions: Substitutions at tail of AT have global effects, altering the conformation and affecting functions elsewhere. Study of AT mutations in Indian patients will help not only in mechanistic understanding of AT but also in diagnosis and treatment of thrombotic patients in India.

PB 1502 | Genetic Characterization of Antithrombin, Protein C and Protein S Deficiencies in Slavic Thromboembolic Patients

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Background: Inherited deficiencies of natural anticoagulants such as antithrombin (AT), protein C (PC) and protein S (PS), with the prevalence of 0.02-0.17%, 0.2-0.3% and 0.5%, respectively, in the general population of Europe, are associated with an increased risk of thromboembolic events.

Aims: We sought to assess the prevalence of *SERPINC1*, *PROC* and *PROS1* gene mutations and their impact on the thromboembolic manifestations in Central-Eastern European patients with natural anticoagulants deficiencies.

Methods: A total of 91 unrelated patients aged 40.1±13.6 years with AT (n=39), PC (n=26) and PS (n=26) deficiencies were screened for mutations by Sanger sequencing and multiplex ligation-dependent probe amplification.

Results: The overall mutation detection rate for *SERPINC1* and *PROC* genes was 97 and 92%, respectively. For AT and PC activities close to the normal range (83 and 70%, respectively), the mutation detection rate remained high (66 and 90%, respectively). For *PROS1* gene, mutation detection rate reached 90% only when the PS activities were below 55%. Above this value the mutation detection rate decreased to 40%.Missense mutations accounted for 83% of all mutations in *PROS1* gene and up to 56% in the *SERPINC1* and *PROC* genes. The profile of nonsense and splice-site mutations was 8-23% and 8-15%, respectively, for all three genes. Small deletions were present in *SERPINC1* and *PROC* genes (7% for both) but gross deletions only in *SERPINC1* gene (11%). Approximately one-third of mutations reported here are novel. Missense mutations occurred more frequently in patients with venous thrombosis compared to those with arterial thrombosis (30 vs. 10%, p=0.04).

Conclusions: To our knowledge, this is the largest cohort of Slavic patients deficient in natural anticoagulants evaluated for genetic background. Selection of patients for genetic screening of inherited thrombophilia, including mainly PS deficiency, should be based on activity of this protein.

PB 1503 | The Association between Genetic Variants in *Cholesteryl* Ester Transfer Protein (CETP) Gene and Hemostatic Factor Levels in Healthy Controls and the Associated Risk of a First Venous Thrombosis

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Background: Cholesteryl ester transfer protein (CETP) plays an important role in lipoprotein metabolism. Previous studies have suggested that the *CETPTaqI* B1/B2 allele is associated with the risk of venous thrombosis (VT), and the potential mechanism of CETP procoagulant activity was related to the direct binding of CETP to FXa.

Aims: To investigate the association between genetic variants located in *CETP* gene and haemostatic factors in healthy individuals and to assess the associated risk of a first event of VT with these *CETP* genetic variants.

Methods: Analyses were performed in the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis (MEGA) case-control study, with ethical approval obtained. Three independent SNPs identified from a large genome-wide association study (GWAS) in the Netherlands Epidemiology of Obesity (NEO) study (unpublished data) explain >16% of the variance of serum CETP concentrations. CETP unweighted/weighted genetic risk scores (GRS) were derived from these three SNPs to reflect genetically determined CETP concentrations. The association between CETP and 22 haemostatic factors (pro-/anti-coagulant and fibrinolytic factors) was assessed by linear regression from an additive model in controls

only (n=2,853), adjusted for age and sex. 3,960 VT cases and 4,787 controls were included to investigate the association between genetically determined CETP concentrations and VT risk by logistic regression analysis.

Results: In the controls (median age, 50 years; 52.8% women), both unweighted and weighted GRSs indicated that only factor VII activity was (negatively) associated with genetically determined CETP concentrations (weighted GRS β -2.81%, 95% CI: -5.44 to -0.17). However, no association was observed with the risk of a first VT.

Conclusions: Genetically determined CETP concentrations showed a weak (negative) association to factor VII activity. However, this did not affect the associated risk of a first VT.

PB 1504 | Molecular Genetic Diagnostics of Protein S and Protein C Deficiency

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Background: Deficiency of the natural anticoagulants protein S or protein C are important risk factors for venous thromboembolism. Genetic testing for protein S and C variants may improve diagnostics of protein S and C deficiency.

Aims: To investigate for disease-causing variants in the *PROS1* or *PROC* genes in patients with suspected inherited protein S or protein C deficiency and their first-degree relatives.

Methods: The study was approved by a recognized medical ethics committee. Following informed consent, patients with suspected protein S or C deficiency based on specialised coagulation tests and their first-degree relatives were investigated. In total, 83 individuals from 40 families with protein S deficiency and 46 individuals from 17 families with protein C deficiency were genotyped using direct sequencing of exons and flanking intronic regions of the *PROS1* or *PROC* genes. Patient samples with no causative point mutation were further investigated using Multiplex ligation-dependent probe amplification (MLPA) in order to identify large structural rearrangements. History of thrombosis and use of anticoagulation was recorded.

Results: Variants likely to be disease-causing were identified in 62% of the protein S deficiency families whereas variants were discovered in more than 64% of the families with protein C deficiency. The findings included several not previously reported variants such as the nonsense variants in *PROS1*: c.1467_1468delA; p.(Ile490Leufs*6) and c.1351C>T; p.Arg451* and the missense variant *PROC*: c.503T>C; p.Leu168Pro.

Conclusions: Genetic testing provides a useful tool to validate inherited protein S or protein C deficiencies and facilitate diagnostics in affected families. Although no causative variants could be identified in a significant number of families, we propose genetic testing in all patients when specialised coagulation tests indicate protein S or C deficiency.

PB 1505 | GPX1 Pro198LEU Polymorphism as a Risk Factor for Venous Thromboembolism in Young Men from North-Western Russia

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Background: Oxidative stress is often involved in pathogenesis of endothelial dysfunction which, in turn, could be frequently seen in patients with thrombotic complications, in particular, venous thromboembolism (VT). Glutathione peroxidase encoded by GPX1 gene has an important role in prevention of oxidative damage since it catalyzes the reduction of organic hydroperoxides and hydrogen peroxide by glutathione. GPX1 Pro198Leu polymorphism may affect enzymatic activity and, thus, the risk of VT development.

Aims: To evaluate the role of GPX1 Pro198Leu polymorphism in pathogenesis of VT in young men from North-Western Russia (NWR).

Methods: Retrospective study involved 172 men suffered from episode(s) of VT before 45 years old (mean age 33.7±8.4 years) and 129 age-matched healthy men (HM). All individuals originated from NWR. GPX1 Pro198Leu polymorphism was discriminated by PCR-RFLP technique. The differences in genotype distributions between the groups were estimated by Fisher`s exact test. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated by using GraphPad Prism software.

Results: Homozygosity for the GPX1 Leu198 variant was more frequently seen in VT patients compared to HM (15.7% vs. 4.7%; OR=3.8; 95%CI: 1.5-9.5; p=0.003). In young men with isolated deep-vein thrombosis (DVT), DVT complicated by pulmonary embolism (PE) or isolated PE GPX1 198 Leu/Leu genotype was seen in 16.0%, 15.4% and 16.0% of cases, respectively. In the group of 126 VT patients having neither FV Leiden nor FII G20210A mutation, the GPX1 198 Leu/Leu genotype was present in 21 (16.7%) cases (OR=4.1; 95%CI: 1.6-10.5; p=0.002, compared to HM). Moreover, the frequency of homozygous 198 Leu/Leu variant was almost 2-fold higher in men with recurrent VT (20.9% vs. 11.6% in patients with non-recurrent VTE; OR=2.0; 95%CI: 0.9-4.8; p=0.12).

Conclusions: We suggest that GPX1 Pro198Leu polymorphism is an independent risk factor for VT in young men from North-Western Russia and could have an impact on recurrence of disease.

PB 1506 | Genetic Association Study between Candidate SNPs and Brazilian Patients with Venous Thromboembolism (VTE)

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Background: VTE is a multifactorial disease, caused by the interaction of genetic and environmental factors. Genome-wide association studies (GWAS) have been performed in the last years, and genetic variants associated to VTE susceptibility were identified mainly in Caucasian populations.

Aims: The aim of this study was to validate 29 SNPs associated to VTE in a Brazilian population.

Methods: The VTE group included 436 patients with a history of unprovoked VTE without acquired or inherited thrombophilia. The control group included 430 healthy individuals. This sample presented 94.76% of statistical power to detect genetic association, estimated by GPOWER v.3.1 software. Among both groups, we genotyped 29 SNPs within 22 genes related to VTE and additional 90 SNPs to evaluate population structure between cases and controls. We estimated allele frequency and Hardy-Weinberg disequilibrium of the SNPs by PLINK v.1.07 software. Association analysis and odds ratio estimation were performed by logistic regression in PLINK software either. The statistical results were adjusted by Bonferroni correction to avoid bias due to multiple comparisons. In order to evaluate whether VTE and control sample present similar genetic structure, we performed the Analysis of Molecular Variance (AMOVA) by ARLEQUIN v3.5.1.2 software

Results: AMOVA results showed a Fst < 0.05 over all loci, and a between-groups variance component of 0.0%, indicating that the two groups did not present differences in genetic structure between each other. Among the 29 SNPs genotyped in association study, after Bonferroni correction of logistic regression none was found associated with VTE ($p>0.05$ in all SNPs).

Conclusions: We compared VTE and control populations, who presented genetically similar, and found different results in SNPs association study with VTE when compared to literature. Brazilian´s population genetic heterogeneity probably explains this result, highlighting the influence of population´s genetic structure in association studies.

TABLE 1 Single Nucleotide Polymorphisms associated with VTE and the logistic regression results

GENE	SNP	Odds Ratio Literature	Odds Ratio Study	Associated Phenotype	GENE	SNP	Odds Ratio Literature	Odds Ratio Study	Associated Phenotype
CYP4V2	rs13146272	1.22	0.86	+ FXI levels	PROCR	rs6088735	1.35	1.06	- Protein C levels
SERPINC1	rs2227589	1.29	1.04	- Antithrombin levels	EDEM2	rs6120849	Not available	1.05	- Protein C levels
GP6	rs1613662	1.15	1.00	+ Platelet activation and aggregation	GCKR	rs1260326	Not available	1.18	- Protein C levels
F11	rs2036914	1.49	1.01	+ FXI levels	BAZ1B	rs17145713	Not available	0.93	- Protein C levels
F11	rs2289252	1.33	0.96	+ FXI levels	DNAJC6	rs1413885	Not available	0.97	- Protein C levels
HIVEP1	rs169713	1.20	0.98	Strongly associated with VT risk	TC2N	rs1884841	1.27	Excluded from analysis by Hardy-Weinberg equilibrium test	+ VW Factor levels
KNG1	rs710446	1.19	0.88	- aPTT levels	FVW	rs1063857	1.15	1.08	+ VW Factor levels
C4BPB	rs3813948	1.24	1.06	+ C4BP levels	STXBP5	rs1039084	1.11	1.03	+ VW Factor levels
PROCR	rs867186	1.22	1.07	- Protein C levels	CLEC4M	rs868875	1.07	Excluded from analysis by Hardy-Weinberg equilibrium test	- VW Factor levels

TABLE 2 Single Nucleotide Polymorphisms associated with VTE and the logistic regression results

Gene	SNP	Odds Ratio literature	Odds Ratio Study	Associated Phenotype	Gene	SNP	Odds Ratio literature	Odds Ratio Study	Associated Phenotype
SCARA 5	rs2726953	0.98	Excluded from analysis by Hardy-Weinberg equilibrium test	VW Factor levels alteration	NME7	rs16861990	1.79	0.97	FV related
SCARA 5	rs9644133	1.12	0.90	- FVIII levels	SLC44A2	rs2288904	1.19	1.11	+ SLCC44A2
STAB2	rs4981022	0.97	0.95	- VW Factor levels					
STAB2	rs4981021	1.10	0.96	+ FVIII levels					
STX2	rs7978987	1.01	0.94	VW Factor levels alteration					
BAI3	rs9363864	0.58	1.25	- FVIII and FVW Factor levels					
FV	rs2420371	2.27	0.97	Strongly associated with VTE risk					
ABO	rs687621	1.52	Excluded from analysis by Hardy-Weinberg equilibrium test	Strongly associated with VTE risk					
SERPINF2	rs8074026	Not Available	1.01	Serpine protease inhibitor related					

PB 1507 | Absolute Quantification of Jak2 V617F in Patients with Splanchnic Venous Thrombosis by Droplet Digital PCR

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Background: Myeloproliferative disorders (MPDs) represent a risk factor for thrombosis in the portal, mesenteric, and hepatic districts (SVT). The acquired somatic V617F mutation on Janus kinase 2 (JAK2), containing the G-to-T transvertion in exon 14, can occur in MPD patients, and is a risk factor for SVT independently of the presence of overt MPDs.

Aims: This study was aimed to test the utility and efficiency of JAK2 V617F quantification in SVT patients tested by Droplet Digital PCR (ddPCR).

Methods: A cohort of 132 patients with documented SVT, prospectively enrolled at our center between 1997 and 2015, were re-evaluated for JAK2 V617F mutation by using ddPCR to absolutely quantify the mutate allele in each patient.

Results: The JAK2 V617F mutation was detected in 42 out of 132 patients (31.8%). In all patients the mutation was in heterozygous state. The ddPCR allowed us to quantify the mutate allele in all the 42 positive patients at the diagnosis and during the clinical follow-up. It was possible to evaluate JAK2 V617F mutation changes over time in each positive patient and to uncover the mutation at low percentage in patients negative at the diagnosis.

Conclusions: The ddPCR is a suitable, precise, and sensitive method for absolute quantification of the JAK 2 V617F mutation in patients with SVT, and may be useful for early disease detection and clinical management of patients without the need for a standard curve or comparison to a reference gene
(limit of detention was 0.01% for both qPCR and ddPCR).

PB 1508 | Screening of Deletions in Protein S Deficient Families

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Background: Inherited protein S (PS) deficiency is known to be associated with increased thrombotic risk in deficient families. This autosomally dominant disorder is usually caused by mutations in the *PROS1* gene, but there is an increasing evidence of large deletions/duplications, which are relatively common in point mutation negative PS deficient families.

Aims: The aim of our study was to examine variations in the *PROS1* gene in 13 unrelated Czech families with manifested PS deficiency and a history of thrombosis.

Methods: We used the direct sequencing of all exons with intron flanking regions of *PROS1* (Castoldi E., 2010) to search for point mutations and other variations. We also used multiplex ligation-dependent probe amplification (MLPA) (MRC HOLLAND, NL) to check for deletions/duplications. All index patients from 13 families were screened for protein S gene mutation both by direct sequencing and by MLPA.
Results: We identified one large deletion spanning the whole gene and we also found 12 different point mutations (9 missense, 2 single nucleotide insertions and 1 splice site mutation). Two of the unrelated families had the same mutation. We identified six novel mutations. We also found possible deletion in exon 2 by MLPA in one patient. However, this was later explained by a point mutation present at the ligation site of the MLPA probe for exon 2, resulting in false positive deletion signal. All patients except two were heterozygous for one mutation. Second mutation in these two families is under causality screening.
Conclusions: We found mutations in all protein S deficient families, six of them were novel. One large deletion (1 of 13) was proven by MLPA. Although MLPA is a useful tool for the detection of large deletions or duplications, it is susceptible to error. Patients who have positive deletion output should be checked by sequencing. Moreover, the possibility of two or more mutations in a single patient should be considered in the mutation screening schedule.

PB 1509 | microRNA gene (miR -146aC>G, miR -149T>C, miR -196a2T>C and miR -499A>G) Polymorphisms in Korean Patients with Venous Thromboembolism

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Background: MicroRNAs are well known to short non-coding RNAs that play a role in post-transcriptional regulation of gene expression. Recent studies were reported microRNAs are potential biomarkers in different cardiovascular diseases. So, we focused that Venous thromboembolism (VTE), one of the cardiovascular diseases, may be associated with microRNA gene.
Aims: Aim of this study was four microRNA polymorphisms that miR -146aC>G, -149T>C, -196a2T>C and -499A>G association with venous thromboembolism susceptibility.
Methods: We investigated the associations with microRNA polymorphisms in 203 venous thromboembolism patients and 300 controls. The patients were enrolled between March 1999 and February 2010 at the Department of CHA Bundang Medical Center (Seongnam, South Korea). DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer´s instructions.
Results: Genotyping was performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The haplotype combination analysis of microRNA was shown that microRNA (miR -146a, miR -149, miR -196a2, miR -499) in G-T-C-G haplotypes (OR, 51.68; 95% CI, 3.028-881.9, P=0.0001) and microRNA (miR -146a, miR -196a2, miR -499) in G-T-A haplotypes (OR, 1.750; 95% CI, 1.194-2.566, P=0.005) were increased risk of venous thromboembolism. Whereas microRNA (miR -146a, miR -196a2, miR -499) in C-C-G haplotypes (OR, 0.318; 95% CI, 0.129-0.784, P=0.011) were decreased risk of venous thromboembolism. Especially, miR -196a2T>C in male (TC: AOR, 0.498; 95% CI, 0.260-0.956, P=0.036; TC+CC: AOR, 0.528; 95% CI, 0.290-0.961, P=0.037) was significantly associated with primary venous thromboembolism.
Conclusions: Our finding suggests that miR -196a2T>C polymorphism associated with venous thromboembolism susceptibility.

PB 1510 | The Association of Arg485Lys Polymorphism with FV Levels, APC Ratio and its Role in DVT: A Study from India

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Background: Arg485Lys polymorphism, located in A2 domain of FV, has been associated with increased risk of thrombosis. However, association of this polymorphism with FV levels and APC ratio is controversial and its role in DVT is not fully elucidated in India.
Aims: To study the effect of Arg485Lys polymorphism on FV levels, APC ratio and its association with DVT in India.
Methods: 75 Doppler proven DVT patients and 75 age and sex matched healthy controls were studied. FV Leiden and Arg485Lys were detected by PCR-RFLP and allele specific PCR respectively. Patients with acquired factors like malignancy, pregnancy and acute thrombotic phase were excluded. FV activity levels were determined using FV deficient plasma kits. APC ratio was calculated by dividing of clotting time in the presence and absence of APC.
Results: FV Leiden was seen in 13.3% (10/75) of patients and absent in controls. 20 (26.6%) patients and 8 (10.6%) controls carried Arg485Lys polymorphism and difference was statistically significant (p=0.024, χ^2=6.35). FV levels in patients and controls were significantly reduced in carriers of 485Lys than noncarriers (Patients: carriers 97.7±11.0%, noncarriers 107.4±13.4%, p=0.008, Controls: carriers 95.2±8.0%, noncarriers 103.1±10.9%, p=0.043). As compared to APC ratio in noncarriers of 485Lys (2.94±0.468), values were lower in carriers of 485Lys in patients (2.68±0.460, p=0.042) and controls (2.71±0.290, p=0.192). 5 patients carried both FV Leiden and Arg485Lys polymorphism. APC ratio was further reduced in carriers

of both FV Leiden and Arg485Lys (1.52±0.178) than FV Leiden alone (1.82 ±0.198, p=0.014).

Conclusions: Carriership of the 485Lys allele reduces FV levels and APC ratio. In combination with FV Leiden, it significantly decreases APC ratio as compared to FV Leiden alone. Significantly higher prevalence of this polymorphism in patients is suggestive of its association with DVT, however study on larger sample size is required to confirm these findings.

PB 1511 | A Novel Splice Site Mutation Leading to AT Deficiency

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Background: Antithrombin (AT) is an important inhibitor of blood coagulation, which regulates the amount of thrombin in the bloodstream. AT deficiency is associated with a high risk of venous thromboembolism and correlates with a genetic defect of AT encoding the gene *SERPINC1*. However, in 18% no mutation is being found.

Aims: To identify a mutation in the patient from the AT deficiency family where no mutation was detected in *SERPINC1* in previous screening.

Methods: We investigated an 18-years old boy with a severe AT deficiency and a severe thromboembolic event. His father and brother had low levels of AT as well. We evaluated AT:AT(Xa) activity (Biophen AT anti(h)Xa LRT, Hyphen Biomed), AT(FIIa) activity (Biophen AT ANTI-IIa, Hyphen Biomed), AT:Ag (LIAtest ATIII, Stago). Human genomic DNA was isolated from the whole blood. All 7 exons of *SERPINC1* including the intron-exon boundaries were PCR-amplified and analysed using corresponding intronic primers, AmpliTaq Gold DNA Polymerase, BigDye Terminator v3.1 Cycle Sequencing kit and 3500 Series Genetic Analyzer (all ThermoFisher Scientific).

Results: We confirmed a severe form of AT deficiency: AT(FXa)- 47%, AT(FIIa)- 53%, AT:Ag- 65%. We found no mutation repeatedly from 2 different DNA in *SERPINC1*. So, we analysed DNA of proband`s brother and father. We detected no mutation in brother`s, but we found a mutation in father`s DNA in the front of exon 6 c.1154-1G>C. Boys also had a known polymorphism rs2759328 in intron 5 inherited from their mother. It was located in the forward primer of exon 6, which then worked as an allele specific primer and amplified the healthy allele inherited from the mother predominantly. When, we designed a new primer located out of the area of the polymorphism, we found the mutation in both boys.

Conclusions: We realized that location of primers could sometimes be a cause of not detecting a mutation. Finally, we identified a novel familial splice site mutation c.1154-1G>C of *SERPINC1* that causes AT deficiency.

PB 1512 | Gender Differences in the Incidence of Venous Thromboembolism among Chronic Lung Diseases and Rheumatoid Arthritis in Korea

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Background: The effect of gender on the incidence of venous thromboembolism (VTE) has not been well studied among chronic lung diseases and rheumatoid arthritis in Korea.

Aims: We evaluated the venous thromboembolism incidence associated with chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), rheumatoid arthritis (RA), and general population.

Methods: We used Korean Health Insurance Review and Assessment Service (HIRA) data from January 2013 to December 2013 among 51,448,491 Korean residents. Patients with COPD (n = 222,130), ILD (n = 20,946), or RA (89,562) were identified using the International Classification of Disease-10 diagnostic codes.

Results: The women with VTE incidence rates per 100,000 persons for the study population with COPD, ILD, RA, and the general population were 1642, 1551, 299, and 48 respectively, while men with the VTE incidence for each group was 1033, 1280, 396, and 38, respectively.

Conclusions: VTE incidence was significantly higher in women with COPD, ILD, general population than that of men, while the women with an incidence of VTE was lower than that for men in patients with RA in Korea.

PB 1513 | Application of Massive Parallel Sequencing Searching for Rare Genetic Variants of Protein C and S Associated with Thrombophilia - Implementation to Clinical Practice

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Background: Thromboembolism have multifactorial etiology but one of the main causes is thrombophilia. Really common and severe cause of thrombophilia is deficiency of protein C, S or antithrombin III (PC, PS, AT III). The genetic cause are mutations without mutational hotspots areas in the studied genes. Therefore it is appropriate to use methodology of massive parallel sequencing (MPS) and expanded molecular genetic analysis for detection of genetic cause of deficiency of those proteins. Selected proteins are encoded by genes: PROS, PROC, SERPINC1. In these genes have been described more than 800 mutations whose clinical manifestation may be a lack or loss of function of the gene product, which are involved in the inhibition of coagulation factors. Pathogenic mutations in these genes exhibit similar

risk of thrombosis as routinely tested mutations of FV Leiden and FII Prothrombin (G20210A).

Aims: Apply the massive parallel sequencing (MPS) and expanded molecular genetic analysis for detection of genetic cause of deficiency of protein C and S.

Methods: MPS was carried out by Ion Torrent PGM platform. We used Ampliseq Designer for design of multiplex with 100% coverage of coding sequences and exon / intron border areas. The data were processed by Torrent Suite programs - Ion Reporter and Next Gene - available database of clinical variants (ClinVar, HGMD).

Results: In the first run there were examined 10 patients. They were selected for repeatedly detected reduced levels of protein C or S, at the same time they were excluded for secondary etiology of that condition. We have revealed six missense mutations in PROS1(2) and PROC (4) so far.

Conclusions: MPS offers complex tool for identification genetic causes of severe thrombophilia states applicable to laboratory and clinical praxis. Further it will be useful to add MLPA analysis for patients where MPS doesn´t reveal any genetic cause. MLPA should improve testing for better detection of large rearrangements in examined genes.

PB 1514 | Combined Protein C / Protein S Deficiency: Genotype-Phenotype Relationship in an Italian Family Carrying Two Novel Mutations

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Background: Protein C (PC) and Protein S (PS) defects may lead to an increased risk of venous thromboembolism and arterial disease. Individuals with inherited PC/PS deficiency have about a 2-to 11-fold increased risk for venous thromboembolism.

Aims: We describe a case of PC/PS deficiency. Two novel mutations in PC and PS genes were identified.

Methods: We observed a 50-years-old man who had suffered from idiopathic superficial vein thrombosis at right leg at 49-year-old and a myocardial infarction at 45-year-old in presence of modifiable risk factors (BMI 33, smoking, diabetes mellitus). Laboratory investigations evidenced low plasma levels of functionally active PC (56.7%; n.v.:70-140%) and free PS (25%; n.v.: 60-150%). Family history documented that: his father died at 54-year-old because of a myocardial infarction and his daughter (25-year-old, BMI 21.4, smoking) had low plasma levels of functionally active PC (57%) and of free PS (22%), and no noteworthy clinical event. Functionally active PC was determined by a chromogenic method. Quantitative free PS was determined by an ELISA method. Direct sequencing analysis of the PC and PS genes was performed to identify the causative mutations. Multiple alignments of PC and PS proteins was created by the HomoloGene system.

Results: Both the patient and his daughter were found double heterozygotes for two novel mutations, p. Met255Lys in the PC gene and p.Ala525Asp in the PS gene. HomoloGene indicated that the Met255 in PC gene represents a poor conserved residue across species while the Ala525 in PS gene seems to be a more conserved residue. However, findings from SIFT predictions suggested a possible damaging effect of both mutations on PC and PS structures. At variance with the SIFT, Polyphen-2 predictions revealed the only PC mutation could have a damaging effect on protein structure.

Conclusions: The PC/PS deficiency of our case is likely due to the p.Met255Lys and p.Ala525Asp variants identified in PC and PS genes, respectively.

PB 1515 | Impact of Factor V-Leiden, Prothrombin G20210A and MTHFR C677T Mutations on Egyptian Patients with Sickle Cell Disease

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Background: Sickle cell disease (SCD) shows activation of the blood coagulation, fibrinolytic system, increased platelet activity and consumption of coagulation inhibitors. Many thrombophilic genes polymorphisms are suggested as potential modifiers of vascular complications pathophysiology.

Aims: is to study the impact of factor V Leiden (FVL), prothrombin (PTH) gene mutation, and methylenetetrahydrofolate reductase (MTHFR) polymorphism as risk factors for vascular complications in patients with SCD.

Methods: This case-control study was carried on 40 SCD patients, and 40 healthy control subjects of matched age and sex attending Ain Shams University Hospitals after obtaining an informed consent. All the studied individuals were subjected to: complete blood picture, blood film examination, reticulocyte count, hemoglobin electrophoresis, prothrombin time, activated partial thromboplastin time, polymerase chain reaction detection of FVL (G1691A), PTH (G20210A) and MTHFR (C677T) genes mutations. Data were analyzed statistically using SPSS version 24.

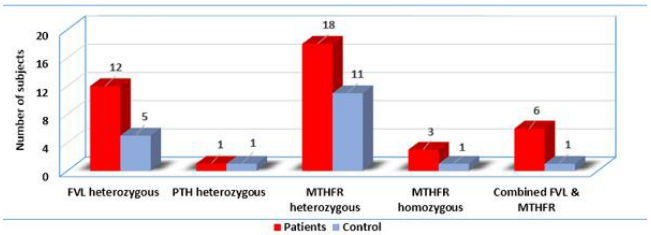

FIGURE 1 Prevalence of genetic mutations in patient and control groups

TABLE 1 Association between FVL; MTHFR genotypes and demographic, clinical & laboratory data

Parameter	Wild FVL No=28	Hetero FVL No=12	Test of Significance	P value	Hetero MTHFR No=19	Wild MTHFR No=18	Homo MTHFR No=3	Test of Significance	P value
Disease onset: Median, months	21	15	Z=1.434	0.1	24	18	6	Z=0.787	0.4
Sex:			Fisher´s Exact Test	0.03				X2=1.367	0.5
girls (16)	8	8			6	9	1		
boys (24)	20	4			13	9	2		
BMI: mean± SD	19.5±5.1	18.1±4.5	t=0.82	0.4	21.1±5.8	16.9±2.7	19.3±4.5	t=4.019	0.02
SCD type:			Fisher´s Exact Test	1.0				X2=2.426	0.2
SCA (13)	9	4			8	5	0		
S. Thalassemia (27)	19	8			11	13	3		
Dactylitis:			X2=5.199	0.02				X2=0.932	0.6
Present (21)	18	3			11	8	2		
Absent (19)	10	9			8	10	1		
VOC:			Fisher´s Exact Test	1.0				X2=1.787	0.4
Present (13)	9	4			6	5	2		
Absent (27)	19	8			13	13	1		
Stroke:			Fisher´s Exact Test	0.006				X2=6.793	0.03
Present (6)	1	5			2	2	2		
Absent (34)	27	7			17	16	1		
HBF%: Median	9.2	8.5	Z=1.348	0.1	9.1	9	15.1	Z=3.221	0.052
HBS%: Median	56.5	50.4	Z=0.215	0.8	60	39.5	53.6	Z=0.677	0.5

Results: Significant difference was found between patients and control subjects as regards the thrombophilic genes mutation rate (70% and 42.5% respectively; P=0.01), however insignificant difference was found when each genotype prevalence of FVL, PTH and MTHFR were compared separately(P=0.056, 1.0, 0.1 respectively) (figure1).

FVL and MTHFR mutations were significantly associated with stroke (table1).

Logistic regression analysis (including age, disease duration, hemoglobin (Hb) level, HbF%, HbS%, FVL, MTHFR) showed significant association between decreased HbF and occurrence of vascular complications (P=0.03).

Conclusions: Despite of low impact of these genes mutation in the occurrence of vaso-occlusive crises in SCD patients, FVL and MTHFR mutations are found to be associated with occurrence of stroke. The early detection of these mutations may be useful for better management. Moreover, patients with low HbF or high HbS should be candidates for extensive therapy.

PB 1516 | Heightened Hypercoagulability in Sickle Cell Anemia Patients with Chronic Leg Ulcers

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Background: Sickle Cell Anemia (SCA) is associated with an increase in pro-thrombotic factors and a decrease in physiologic anticoagulants, resulting in predisposition to venous thrombo-embolism. SCA patients suffer from chronic, recurrent, painful, slow-to-heal chronic leg ulcers (CLU); which are associated with aesthetic deformities and reduced quality of life.

Aims: This study was designed to compare the coagulation profile of Ghanaian SCA patients with CLU to controls.

Methods: The coagulation profile consisting of prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration along with complete blood count (CBC) was determined in 50 SCA patients with CLU, compared with 50 SCA patients without CLU and 45 HbAA participants. SCA patients were recruited from Ghana Institute of Clinical Genetics, Accra, while the HbAA participants were voluntary blood donors. All participants gave written informed consent and the study was approved by our ethical and protocol review committee. ANOVA was used to analyse mean difference across the 3 groups.

Results: SCA patients with CLU had the lowest mean Hb concentration of 7.30±1.42g/dl, followed by SCA without CLU (7.99±1.39g/dl), with controls having the highest (14.01±1.29 g/dl) P=0.000. As shown in table 1.SCA patients with and without CLU had increased mean platelet counts (p=0.000); shorter mean APTT (p=0.007) and marginally prolonged mean PT (p=0.008) compared to HbAA controls. Mean fibrinogen concentration was higher in SCA patients with CLU and without CLU compared to HbAA controls, however this was not statistically significant (P = 0.127).

TABLE 1 Markers of Coagulation in Study Subjects (mean ± standard deviation)

Parameter	SCA With CLU (n = 50)	SCA Without LU (n = 50)	Control (n = 45)	P-value	Reference Range
Platelet Count(×10^9/L)	478.1±177.3	424.2±169.0	226.3±53.2	0.000	150 - 400
PT (Seconds)	16.0±2.6	16.0±1.7	14.9±1.2	0.008	12 - 16
APTT (Seconds)	31.3±6.2	34.5±6.8	35.2±6.1	0.007	26 - 36
Fibrinogen (mg/dl)	314.3±109.8	284.9±83.5	276.9±88.0	0.127	150 - 350

Conclusions: Our findings suggest heightened hypercoagulability in SCD patients with CLU. It is however uncertain if this increase plays a role in the pathogenesis of chronic leg ulcers in SCA.

PB 1517 | Chronic Thromboembolic Pulmonary Hypertension (CTEPH) and Trombophilia: What Should We Know?

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Background: Venous thromboembolism (VTE) is a chronic disease. Recurrence can be prevented by anticoagulants. Numerous laboratory risk factors of VTE have been identified, which has lead to a practice called laboratory thrombophilia screening. The knowledge of these factors should improve counseling patients regarding their duration of anticoagulation.

Aims: To study the frequency of thrombophilia in patients with CTEPH.

Methods: We examined 36 patients with different degree of pulmonary hypertension (PH) by echo-cardiography: 20 people (55,6%) with the first degree (SPAP 30 - 50 mmHg), 9 people (25%) with the second degree (SPAP 51 - 80 mmHg), 7 people (19.4%) with the third degree (SPAP over 80 mmHg). Blood was testing for the presence of a lupus anticoagulant (LA), antibodies (Ab) to cardiolipin classes IgM and IgG, Ab to β2 glycoprotein 1 (Ab to β2GP1).

Results: In 16 of the 36 patients revealed a high titer of anticardiolipin Ab/LA/Ab to β2GP1. In 7 patients of the main group (4 men and 3 women) was diagnosed antiphospholipid syndrome (APS). The feature of APS in patients with CTEPH was the primary character APS (71,4%), whereas secondary APS detected only in 28,6%. Patients with identified high titer of Ab to phospholipids in most cases, there had been multiple lesions in both lungs, recurrent pulmonary embolism (PE). Increase in titer anticardiolipin Ab/LA/Ab to β2GP1 detected in 44,4% of patients and the comparison of clinical markers APS and increase resistance of specified Ab allowed to diagnose APS in 19,4% of cases.

Conclusions: In 33,3% of cases there were associated forms of thrombophilia in patients with CTEPH, which helped to explain the recurrent nature of venous thrombosis/thromboembolism in this subgroup of patients. These results can be the basis of the strategy of patients with CTEPH, forming groups of risk of recurrent venous thrombosis and CTEPH, which help us to preventive incidents of PE.

PB 1518 | Apolipoprotein E Gene Polymorphisms: An Indian Study Showing Lateral Association with Thrombophilic Risk Factors in Patients with Deep Vein Thrombosis

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Background: Deep vein thrombosis (DVT) is a multifactorial disease; many acquired and genetic factors being implicated in its etiology. The degree of association for these factors may vary from strong to only modest for some. Polymorphisms in the *APOE* gene encoding for three common isoforms (*ε2*, *ε3*, *ε4*) is an upcoming potential pathogenic factor whose role is unclear in DVT.

Aims: To investigate the association of *APOE* gene polymorphisms in Indian DVT cases and to understand its interplay with the known risk factors.

Methods: Equal number of DVT cases and healthy controls (N=100, each) were studied for known acquired and hereditable thrombophilic risk factors. *APOE* gene polymorphisms were studied using polymerase chain reaction (Figure 1A). Odds ratio and χ^2-test were applied to study the association of the *APOE* genotypes/alleles with DVT and its risk factors.

Results: The known factors showing significant difference for cases *versus* controls are summarized in Table 1.

TABLE 1 Risk factors showing significant association with DVT cases

Risk factor	Cases (N)	Controls (N)	Odds ratio (95% confidence interval)	p-value
Median weight, kg	70 (100)	67 (100)	-	0.008
History of fracture	12 (100)	2 (100)	6.7 (1.4-30.7)	0.006
Smoking	20 (100)	8 (100)	2.9 (1.2-6.9)	0.014
Pregnancy	11 (29)	0 (34)	42.9 (2.4-769.6)	0.011
Recurrent pregnancy loss	10 (29)	1 (34)	17.4 (2.1-146.4)	<0.001
Lupus anticoagulant positivity	5 (62)	1 (100)	8.7 (1-76.2)	0.021
β2-glycoprotein1 antibody, IgG isotype positivity	9 (100)	0 (100)	9.8 (1.2-78.8)	0.009
Factor V Leiden mutation	16 (100)	2 (100)	9.3 (2.1-41.8)	0.004

The *ε2/ε3* and *ε3/ε4APOE* genotypes were commoner in DVT cases than controls (for *ε2/ε3*⇒10% *vs.* 9%, OR=1.123, CI=0.436-2.895; for *ε3/ε4* ⇒18% *vs.* 11%, OR=1.776, CI=0.792-3.984) but not statistically significant (Figure 1B).

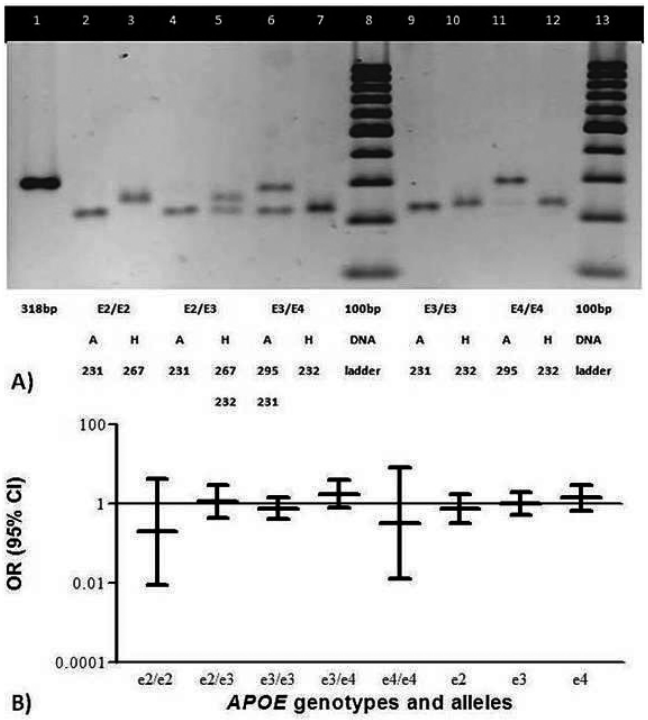

FIGURE 1 A) APOE digestion products with RE AflII (A) and HaeII (H). B) Odds ratio and 95% confidence interval for various APOE genotypes and alleles

[A) APOE digestion products with RE AflII (A) and HaeII (H). B) Odds ratio and 95% confidence interval for various APOE genotypes and alleles.]

The chance of developing DVT during/after pregnancy was more in a female with $\varepsilon 2/\varepsilon 3$ genotype (N=29; p= 0.019). The $\varepsilon 3/\varepsilon 3$ genotype offered a protective effect from DVT in a female with recurrent pregnancy loss (N=29; p= 0.016). Normal antithrombin levels in DVT cases were seen significantly frequent with a $\varepsilon 3/\varepsilon 3$ genotype (N=62; p=0.03) than those with low levels. Non-O blood group DVT individuals showed a significantly frequent $\varepsilon 3/\varepsilon 4$ genotype (N=100; p=0.023) compared to O blood group.

Conclusions: The study showed that the distribution of *APOE* genotypes was not statistically significant in DVT and has only modest association. A lateral association of these genotypes with thrombophilic risk factors was observed which may have prophylactic and therapeutic implications in the future by targeting the pathways of ApoE action.

PB 1520 | The Factor V Leiden Mutation Is Associated with Increased Sperm Count

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Background: The coagulation factor V Leiden mutation G1691A (FVL) has a high prevalence in Caucasians despite its association with venous thromboembolism. Male carriers have higher fecundity, which might partly explain this apparent evolutionary paradox. We previously observed a statistically non-significant increase in sperm count in FVL carriers.

Aims: To test whether FVL was associated with increased total sperm count and to find an underlying biological mechanism. We also tested the association with the prothrombin G20210A mutation (PGM).

Methods: We determined the FVL and PGM genotype in two cohorts and aggregated the results with a previously published cohort. We accounted for baseline differences and study level clustering using a mixed effects model. We explored possible biological underpinnings by investigations of genetic linkage and a FVL mouse model (R504Q mutation). In public haplotype data from subjects of European descent, we tested linkage disequilibrium of FVL with all SNPs in a 1.5 MB region around the *F5* gene. We sequenced exons of four genes hypothesized to be linked to FVL in carriers with extreme sperm counts.

Results: The cohorts are summarized in Table 1. Carriers of FVL, but not of PGM, had a higher total sperm count than non-carriers (Figure 1), with an adjusted mean difference of 31x 10^6 (p = 0.048). There were no differences in normalized internal genitalia weights, epididymis sperm content and sperm motility between FVL (n=14) and wild type mice (n=7). No polymorphisms were found to be in linkage disequilibrium, neither in the public databases nor in a subgroup of FVL carriers with extreme sperm counts.

Conclusions: FVL is associated with higher sperm count. Although the effect seemed present in all three cohorts, it had a borderline statistical significance and would thus benefit from further confirmation. Absence of the effect in PGM carriers and in mice might suggest the cause is indirect, but we found no evidence of genetic linkage.

TABLE 1 Baseline characteristics and total sperm count.

	Amsterdam 1* Jan 2000 - Jul 2007	Amsterdam 2 Jul 2007 - Dec 2014	Copenhagen Jan 2007 - Dec 2009
Population	Consecutive male partners of subfertile couples without known causes for spermatogenic failure	Consecutive male partners of subfertile couples without known causes for spermato-genic failure	General Danish population
n	908	627	854
FVL	Carrier 4.0 %; Non carrier 95.0 %; Unknown 1.0 %	Carrier 4.6 %; Non carrier 94.9 %; Unknown 0.5 %	Carrier 7.3 %; Non carrier 92.7 %; Unknown 0.0 %
PGM	Carrier 1.7 %; Non carrier 98.0 %; Unknown 0.3 %	Carrier 2.2 %; Non carrier 97.4 %; Unknown 0.3 %	Carrier 1.5 %; Non carrier 98.5 %; Unknown 0.0 %
Age - years (median (IQR))	36.3 (32.6-40.7)	37.0 (32.6-41.7)	19.1 (18.8-19.7)
Abstinence - days (mean ± SD)	4.3 ± 2.3	4.2 ± 2.2	3.1 ± 2.2
Current smoking	29.6%	24.1%	58.4%
Main outcome Total sperm count - 106/ ejaculation (median)	Overall 161; FVL carrier 236; FVL non carrier 160; PGM carrier 159; PGM non carrier 161	Overall 150; FVL carrier 233; FVL non carrier 149; PGM carrier 218; PGM non carrier 149	Overall 145; FVL carrier 157; FVL non carrier 144; PGM carrier 134; PGM non carrier 145.

* Previously published. FVL, Factor V Leiden mutation; PGM, Prothrombin G20210A mutation; IQR inter quartile range; SD standard deviation.

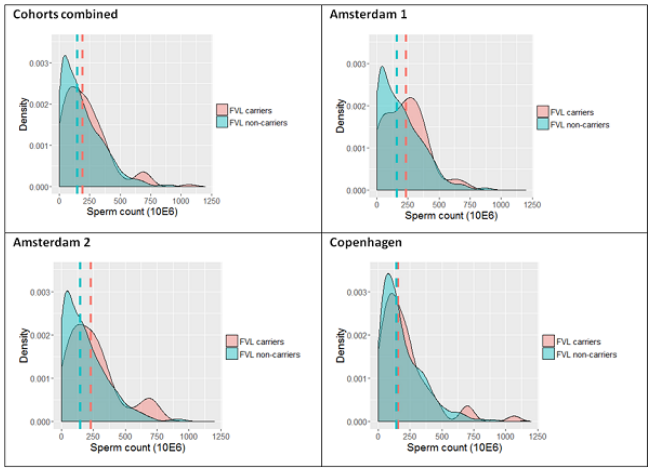

FIGURE 1. Density estimations of total sperm count in FVL carriers versus non-carriers. Dashed lines indicate group median

PB 1521 | Risk Factors of Cerebral Venous Sinus Thrombosis - Single Center Experience

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Background: Cerebral venous sinus thrombosis (CVST) is a rare manifestation of venous thromboembolism (VTE). The estimated CVST incidence for adults is 3-4 per million cases. The potential risk factors for CVST are congenital and acquired thrombophilia.

Aims: Evaluation of the prevalence of congenital thrombophilia, antiphospholipid syndrome (APS) and other acquired risk factors for VTE in patients with a history of CVST.

Methods: patients with recognized CVST are referred to IHTM for identification of hematological and other risk factors for this disease. Our study comprised 204 fully diagnosed CVST patients from the register data-base: 154 women and 50 men, mean age - 39 years (range 14-78). Medical interview included age at the time of the CVST diagnosis and coexistence of VTE acquired risk factors (pregnancy, delivery, oral contraceptives (OCPs), infection, cancer). Each patient was examined for congenital thrombophilia (antithrombin, protein C and S deficiency, factor V Leiden and prothrombin gene mutation G20210A). Antiphospholipid antibodies were also determined.

Results: Thirty-five (17,2%) patients had congenital thrombophilia, eleven (5,4%) were diagnosed with APS. In 9/12 cases (4,4% of all patients) JAK-2 positive myeloproliferative neoplasm was confirmed. At the time of CVST diagnosis sixty-eight/154 (44,2%) women ingested OCPs, while in twenty-five (16,2%) CVST occurred during pregnancy or at delivery.

Conclusions: In our population of patients the ingestion of oral contraceptives, pregnancy and delivery were the main risk factors for CVST.

PB 1523 | Platelet to Lymphocyte Ratio and Neutrophil to Lymphocyte Ratio as Risk Factors for Venous Thromboembolism

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Background: High Platelet to Lymphocyte Ratio (PLR) and Neutrophils to Lymphocyte Ratio (NLR) are markers of subclinical inflammation associated with arterial thrombosis. The association between high PLR and NLR and the risk of venous thromboembolism has not been investigated yet.

Aims: To investigate whether high PLR or NLR are associated with an increased risk of venous thromboembolism (VTE) or cerebral venous thrombosis (CVT).

Methods: Case-control study of patients with VTE or CVT referred to Milan Thrombosis Center for thrombophilia screening from Jan 2007 and Dec 2013. Patients had already discontinued anticoagulant therapies and healthy individuals referred in the same time period were used as control. Patients were investigated at least after three months from the acute event. Multivariable logistic regression was used to calculate odds ratios (OR) for PLR and NLR values >95th percentile. The interaction of high values of PLR and NLR with thrombophilia in determining the risk of VTE or CVT was also investigated. 486 VTE patients, 100 CVT patients and 299 controls were included in the study.

Results: Patients with high PLR or NLR were not at increased risk of VTE or CVT. Subgroups analysis showed that high PLR increased the risk of CVT associated with transient risk factors (OR 2.65, 95%CI 1.02-6.92). A synergistic effect on the risk of CVT for the combination of high values of PLR and thrombophilia abnormalities was observed (OR 7.67, 95%CI 1.67-35.27).

Conclusions: PLR and NLR are not associated with VTE or CVT. Perhaps, high PLR increases the risk of CVT associated with transient risk factors.

PB 1524 | Quantification of the Effect of NET-derived Cell-free DNA on Thrombosis and Haemostasis Phenotypes

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Background: Cell-free DNA (cfDNA) is a surrogate marker of neutrophil extracellular traps (NETs). It has been shown that cfDNA provide scaffolding for platelet adhesion and promote fibrin deposition in the vasculature by enhancing the activity of coagulation factors, thus contributing to thrombosis.

Aims: Evaluate the effect of cfDNA on quantitative intermediate phenotypes related to thrombosis, haemostasis and inflammation in Spanish families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT2) Project.

Methods: cfDNA was quantified in platelet poor plasma of 935 subjects belonging to 35 families with thrombosis of GAIT2. Sytox Green was used to label DNA. Fluorescence was measured at 485 excitation and 538 emission wavelengths. cfDNA concentrations were calculated through a standard curve. Associations between cfDNA and phenotypes were quantified using mixed effects models, and the significance using likelihood ratio test. All the models used take into account the family structure and have the proband correction. This ascertainment scheme was used to minimize bias and allow the best estimation for general population parameters.

Results: Significant positive correlations with cfDNA were observed for some blood coagulation factors. The most significant one was for functional fibrinogen (FIBc b=0.151; p-val=6.44e-07), indicating that extracellular DNA from NETs could mainly enhance fibrinogen deposition and fibrin formation. Functional clotting FVIII (FVIIIc b=0.083; p-val=3.91e-03), FIXc (b=0.059; p-val=3.31e-02) and FXIIc (b=0.056; p-val=3.30e-02) also exhibited positive correlations with cfDNA.

Conclusions: Haemostasis-related phenotypes such as FIBc, FVIIIc, FIXc and FXIIc might be involved in the interaction between NET-derived cfDNA and thrombosis. In addition, the quantification of the effect of cfDNA on these coagulation factors showed that extracellular DNA could promote their activation and contribute to a prothrombotic phenotype.

Grants: PI12/00612, RD12/0042/0032, SGR-01068, SGR-1240, PI15/00269.

PB 1525 | Iron Deficiency Alters Red Blood Cell and Neutrophil Adhesion and Arterial Thrombosis

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Background: Clinical studies describe a link between iron deficiency and thrombosis. The pathophysiological basis is poorly understood.

Aims: We generated a mouse model of iron deficiency to examine the cellular and molecular determinants of thrombosis in this setting.

Methods: C57BL6J mice were initiated on an iron poor or iron sufficient diet (Takeda) starting at 3 weeks of age for a total of 5 weeks. To examine thrombosis, we used the carotid artery thrombosis photochemical injury model. Microfluidic channels coated with endothelial proteins laminin, fibronectin or VCAM-1 were flowed with citrated whole mouse blood and examined for RBC adhesion. E-selectin immobilized microfluidic channels were perfused with neutrophils.

Results: Mice on the iron deficient diet developed significant microcytic anemia and mild thrombocytosis and lower plasma ferritin levels as compared to controls. No difference was noted in total white cell or differential count. Iron deficient mice had significantly shorter times to occlusive carotid artery thrombosis. Coagulation assays (PT and aPTT) were not different between the two groups. Significantly larger number of iron deficient RBCs adhered to all three endothelial proteins (laminin, fibronectin, and VACM-1) and neutrophils to E-selectin as compared to controls on microfluidic studies.

Conclusions: These studies demonstrate that iron deficiency affects arterial thrombosis. Our studies are the first to identify cellular elements that may be involved in the generation of the increased risk to thrombosis in iron deficiency, i.e. red blood cells and neutrophils as demonstrated by our microfluidic assays. Presently, studies are examining endothelial and platelet activation, venous thrombosis, and molecular mechanisms. Iron deficiency has a high prevalence worldwide. Understanding the molecular underpinnings offers insight into thrombotic mechanisms affecting a large group of people, especially in women of the reproductive age.

PB 1526 | Complete Protein S Deficiency and Pregnancy Outcome in Murine Model

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Background: Complete protein S (PS) deficiency is a rare fatal thrombophilia associating purpura fulminans (PF) and disseminated intravascular coagulation (DIC). We obtained a full rescue of *Pros1*$^{-/-}$ lethality by targeting *F8*. *F8*$^{-/-}$*Pros1*$^{-/-}$ mice did not show PF or DIC.

Aims: Because thrombophilias are associated with pregnancy loss, we investigated complete PS deficiency on pregnancy outcome.

Methods: Pregnancy monitoring, blood cells count, coagulation tests and histology in *F8*$^{-/-}$*Pros1*$^{-/-}$, *F8*$^{-/-}$*Pros1*$^{+/+}$ and *F8*$^{+/+}$*Pros1*$^{+/+}$ pregnant mice and embryos.

Results: Vaginal plugs were observed in all 19 *F8*$^{-/-}$*Pros1*$^{-/-}$ females but, independently of the male breeder genotype, no litters were produced. Relevant embryonic mortality was found at E11.5-12.5 (58%): embryos were mostly macerated but some of them showed hemorrhages and thrombosis with no PF. *F8*$^{-/-}$*Pros1*$^{-/-}$ genotype was underrepresented among embryos (33*vs*50%) at E9.5-10.5 and suppressed after E12.5. Differently all embryos collected from *F8*$^{-/-}$*Pros1*$^{+/+}$ and *F8*$^{+/+}$*Pros1*$^{+/+}$ pregnant mice were alive. Recurrent pregnancy loss never affected *F8*$^{-/-}$*Pros1*$^{-/-}$ mice survival. In comparison to E12-16 *F8*$^{-/-}$*Pros1*$^{+/+}$ gravid mice, *F8*$^{-/-}$*Pros1*$^{-/-}$ pregnant mice had reduced platelet count (829±92*vs*559±79G/L), fibrinogen (2.4±0.3*vs*1.0±0.1g/L), increased TAT (12.6±4.3*vs*25.4±2.8ng/L) whereas PT was normal. In addition, placenta but not lung and liver sections showed fibrin clots only in *F8*$^{-/-}$*Pros1*$^{-/-}$. Treatment of *F8*$^{-/-}$*Pros1*$^{-/-}$ pregnant mice with enoxaparin or low-dose aspirin prevented pregnancy loss. However, litter size was slightly reduced in the aspirin compared to the enoxaparin group (2-4 *vs* 5-6).

Conclusions: Targeting F8 did not prevent pregnancy loss due to placental thrombosis in gravid *Pros1*$^{-/-}$ mice. *F8*$^{-/-}$*Pros1*$^{-/-}$ pregnant mice displayed coagulation activation with no overt DIC. Aspirin or better enoxaparin treatment prevented pregnancy loss, indicating that thromboprophylaxis might apply to pregnancy in severe inherited thrombophilias.

PB 1527 | Thrombophilic Phenotype in a Neonate and Five Young Siblings of a Family with Homozygous Antithrombin Type 2 (Budapest III) Deficiency

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Background: Identification of a neonate with inferior vena cava (IVC) and right renal vein thrombosis, related with discrepant antithrombin (AT) laboratory findings and low efficacy of enoxaparin (ENOX) treatment.

Aims: To identify the nature of the AT deficiency and subsequently modification of treatment. Furthermore examination of family members was performed to clarify the mode of inheritance and establish a phenotype genotype correlation.

Methods: IVC and renal vein thromboses of the neonate were diagnosed on the 12th day of life. AT activity was first determined by chromogenic factor Xa-based assay (AT-Xa). For control AT was examined by chromogenic thrombin-based assay (AT-T) and AT antigen concentration by AT LIA assay (AT ag). ENOX was monitored by anti factor Xa activity (AXa) assay. Molecular genetic analysis was performed by direct sequencing. For treatment ENOX 3 mg/kg per day i.v., AT was supplied i.v. for 30 days. For long-term treatment the child was switched to warfarin.

Results: The initial AT-Xa level was 18%, age-related other clotting parameters were normal. AT-T of 60% and AT-Ag of 59% indicated an AT deficiency type 2. Low AXa levels under ENOX indicated an impaired interaction of AT, heparin and FXa. Moreover, AT supplementation improved ENOX AXa effect from 0 to 1.8 AXa-IU/ml, and the IVC thrombus was dissolved. Molecular genetic analysis revealed a homozygous (+/+) mutation in exon 2 of *SERPINC1*-gene (c.391C>T p.Leu131Phe) identified also in his parents and siblings (Table 1). All family members except the father (+/-) were found to be homozygous for the AT mutation. Interestingly, even under warfarin treatment the propositus showed elevated d-dimer.

TABLE 1 Patient and family data of the antithrombin phenotype, genotype and d-dimer as a molecular activation marker (adult reference ranges)

Table 1	Patient	Mother	Father	Brother	Sister 1	Sister 2	Sister 3	Sister 4
Age (Y, days)	12 d	34.9	37.2	13.8	12.4	10.4	4.2	2.8
AT-Xa (%) (ref. 83-118)	18	55	56	14	13	14	22	16
AT-T (%) (ref. 85-120)	59	98	93	60	71	65	73	74
AT ag (%) (ref. 80-120)	51	87	85	62	69	64	65	67
d-dimer (mg/l) (ref. <0.5)	0.79	0.18	0.42	0.65	0.81	0.55	1.08	1.04
p.Leu131Phe	+/+	+/+	+/-	+/+	+/+	+/+	+/+	+/+

Conclusions: The p.L131F mutation (Budapest III) is well-known to be associated with a defective heparin binding. However, our data indicate that predominantly the interaction with FXa is impaired while the interaction with thrombin is obviously not affected. Increased d-dimer levels indicated a prethrombotic state in all children of the family.

PB 1528 | Venous Thromboembolism in Women Using Hormonal Contraception and the Risk of Recurrence. Findings from a Single Center

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Background: Venous thrombosis including deep-vein thrombosis (DVT) and pulmonary embolism (PE) is considered a multifactorial disease associated with genetic and acquired risk factors. In women of reproductive age, the main cause of venous thromboembolic disease (VTD) is hormonal contraception. However, other risk factors interact to produce VTD. Identifying these factors may allow intervene in risk situations and limit the occurrence of VTD with its potential morbidity and mortality.

Aims: Identified risk factors in VTD.

Methods: We reviewed the characteristics of our series of 120 women with objectively confirmed VTD associated with hormonal contraception. We analyzed its clinical data and thrombophilia studies which were performed in more than 80% of them. Recurrent VTE was recorded in 12 of the 120 female patients (10%) and we analyzed the principal risks factors. All statistical analysis was performed using R statistical software version 3.1.1 (2014-07-10).

Results:

TABLE 1 Results

Patients (N)	Total (120)	Recurrence (12)	P-Value
Clinical Features			
Mean age (years +- SD)	29 (+/- 7.5)	33 (±8.9)	0.1
Body mass Index (Kg/m2 +-SD)	26 (+/-6)	26 (±3.2)	0.69
Risk factors for VTD			
Immobility	32 (26.67%)	5 (41.67%)	0.12
Family history	29 (24.19%)	3 (25.00%)	0.42
Venous thromboembolism characteristics			
Pulmonary embolism	53 (44.17%)	3 (25.00%)	0.08
Proximal deep vein thrombosis	63 (52.50%)	9 (75.00%)	0.72

TABLE 2 Results

Patients	Total (120)	Recurrence (12)	P-Value
Type of combined oral contraceptive			
Antiandrogen	31 (25.83%)		NA
Third generation progestogen	26 (21.67%)		NA
Thrombophilia tests			
Factor V Leiden	11 (9.17%)	1 (8.33%)	1.00
Prothrombin mutation	19 (15.83%)	3 (25.00%)	0.44
Increased factor VIII (>195%)	20 (16.67%)	8 (66.67%)	0.06
Combined trombophilia	4 (3.33%)	2 (16.67%)	NA
Time to recurrence >= 10 years		7 (58.00%)	

Conclusions: Contraceptive use remains the most important risk factor for VTD in women of reproductive age. Additional risk factors such as obesity, immobilization, family history of VTD and certain thrombophilic defects, as well as elevated factor VIII, are frequent and should be part of thrombophilia study. Defining high risk patients, may improve interventions in risk situations (e.g. administering antithrombotic prophylaxis or discontinuing contraceptive use after lower limb trauma) in order to prevent VTD.

Recurrence is low in this population, once contraceptive use is discontinued and thrombophylaxis is offered in subsequent pregnancies. In our subgroup, high levels of factor VIII was a prominent thrombophilic feature and though it has been implicated in recurrence in idiopathic VTD, is less recognized in this situation. However larger studies are needed to confirm this.

PB 1529 | Thrombophilia Screening Practices in a Tertiary Care Hospital: An Assessment of Appropriateness and Financial Impact

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Background: Despite the limited value of routine thrombophilia testing in determining etiology of venous thromboembolic events (VTE), thrombophilia screening is increasing, often for inappropriate clinical indications.

Aims: To assess appropriateness of current thrombophilia testing practices in a tertiary care hospital in Calgary, Alberta, financial impact and patient morbidity.

Methods: We retrospectively obtained a random sample of 500 patients from the list of patients aged >18 years who underwent thrombophilia testing (n=3578) in 2015. Demographics, test

indication, requesting specialty, results and cost incurred were collected. Appropriateness of testing was determined using major published guidelines with input from clinical experts. Statistical analysis was performed using descriptive summary measures.

Results: Out of 500 patients who underwent testing, 469 were included in the study. 74% of patients were women (mean age 41 years, range 18-87 years) and 26% were men (mean age 52 years, age range 18- 83 years). Among 2691 tests performed, 78% were deemed inappropriate, 15% appropriate and 7% lacked sufficient clinical information. Inappropriate testing was mainly ordered by Family Medicine (47%) and Rheumatology (21%). Autoimmune disease (excluding workup for lupus) (51%) provoked VTE (14%), early pregnancy loss (not meeting clinical criteria for testing) (13%) were main indications for inappropriate testing. The cost of inappropriate testing was $42,699 (80% total testing cost). This translates to a total cost of $358,797 for inappropriate testing annually.

Conclusions: Our study indicates a higher incidence of indiscriminate and inappropriate testing resulting in a significant financial impact on the health care system. There is potential for substantial health care cost savings with implementation of stringent criteria for ordering thrombophilia screens. Service specific educational intervention remains key to reducing inappropriate testing.

PB 1530 | Retrospective Analysis of the Size of Recurrent Pulmonary Embolus: Single Centre Study

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Background: There are currently three different models that predict risk of recurrence for patients with unprovoked venous thromboembolism; HERDOO2 score, Vienna prediction model and DASH score. As far as we know, no study has addressed whether the size of a first pulmonary embolism (PE) correlates with size of PE at recurrence. If a correlation were to be found, then this could be used, along with the current risk prediction models, to assess the balance of risks of long-term anti-coagulation.

Aims: Our aim was to determine, through a retrospective cohort of patients with PE, whether radiological size of a first PE predicted radiological size at recurrence.

Methods: Retrospective analysis of all patients with recurrent PE over 25 months (1st November 2014-30th November 2016) at our teaching hospital. PE was defined as radiologically massive (RMPE) if it was bilateral main pulmonary artery, saddle PE or there was evidence of right heart strain. All other PE´s were radiologically non-massive (RNMPE). Patient exclusions: on anti-coagulation at recurrence, if CT pulmonary angiogram not used to diagnose first and recurrent PE or if historical information unavailable.

Results: 837 patients had PE in the study period at our hospital. 100 patients had recurrent PE and 37 were excluded (n=63). Patients whose first PE was RNMPE (46/63, 73% of patients) had a 15.2% chance of RMPE at recurrence where as those who presented first with a RMPE (17/63, 27% of patients), had a 17.6% chance of RMPE at recurrence (odds ratio 1.19, 95% confidence interval 0.27-5.27, p=0.81).

Conclusions: The chances of having a radiologically massive PE at recurrence are similarly low irrespective of the size of the first PE (15.2% v 17.6% for first RNMPE or RMPE respectively). Currently, the size of a first PE should not be considered as a major factor in whether long term anti-coagulation is continued after an initial treatment period. Further prospective multi-centre studies with clinical endpoints are needed to further expand on this study.

PB 1531 | Hyperaggregability of Platelets with Low Dose of ADP and Epinephrin in Patients with Venous Thrombosis: Preliminary Results from the RETROVE Project (Risk of Enfermedad TROmboembólica VEnosa)

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Background: Aggregability of platelets (PLT) with low doses of epinephrine (EPI) and/or ADP in normal people is manifested in two states. One is where PLT aggregation is greater than 60 % (PLT hyperreactivity) and the other is where aggregation is less than 40% (PLT hyporreactivity). This PTL hyperreactivity has been associated with thrombosis.

Aims: To determine if this aggregability is associated with venous thrombosis in the Spanish population.

Methods: A total of 397 controls with a mean age of 49±18 years (190 men and 207 women) and 397 patients with thrombosis with a mean age of 64±18 years (193 men and 204 women) were included in our study. None of the individuals had taken aspirin in the 10 days before blood extraction.

Platelet Aggregation in PRP: we use ADP and EPI at 0.5µM to separate hyper and hyporreactivity populations. We used Aggregation analyzer to generate the curves of aggregation. It measured % of maximum aggregation and % of area under curve (AUC). *Statistics Analyses:* Frequencies were compared using the Chi squared test. p values < 0.05 were considered statistically significant. 50th percentile of % AUC were calculated and analyzed.

Results:

Aggregability of 0.5µM of EPI (AUC) showed statistically significant differences with 50th percentile (>22.83 %): which was a higher percentage in patients than in controls (57.9% vs 50.0%; difference of 7.9%; CI95%:1.03-1.83) (p=0.03). Individuals in the upper 22.83% AUC corresponded to individuals with PLT hyperreactivity.

Aggregability of 0.5µM of ADP(AUC) showed also statistically significant differences with 50th percentile (>13.13 %): a higher percentage in patients than in controls was observed (62.9% vs 50.0%; difference of 12.9%; CI95%:1.27-2.27) (p=0.0001).

Conclusions: Our results suggest that hyper- and hypoaggregability is significantly different in the control population than in the patients. It is notable that PLT hyperreactivity is associated with an increased risk of venous thrombosis.

RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269.

PB 1532 | The Association of D-dimer Level with Common Inherited and Acquired Risk Factors of Thrombosis

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Background: D-dimer is a sensitive laboratory marker of intravascular fibrin formation and data of prospective studies showed that elevated D-d levels might be associated with poor outcomes in patients with different diseases.

Aims: The objective of this study was to identify hemostatic, biochemical and clinical determinants of D-d level in a population-based cohort of 1104 dwellers of Tomsk aged 25-64 years, which were evaluated withing the frame of the multicenter epidemiological survey of cardiovascular diseases in different regions of the Russian Federation (ESSE-RF study).

Methods: In addition to routine tests, we measured D-dimer by an ELISA (Asserachrom, Stago) and screened for prothrombotic mutation (FV G1691A, FII G20210A, FG-β -G455A) by RT PCR (Applied Biosystems). The functionality of the protein C pathway was assessed with a chromogenic assay (ThromboPath, Instrumentation Laboratory) and presented as Protac-induced Coagulation inhibition Percent (PiCi%). Categorical variables are presented as number and percent, continuous - as median and interquartile ranges (IQR).

Results: D-dimer was higher in women (n=727) than in men (n=327): [330 (223-519) vs. 255 (167-431) ng/ml] and in carriers of FV G1691A (n=34, 3.1%) compared to non-carriers (n=1068): [553 (336-1015) vs. 304 (206-493) ng/ml]. All values of PiCi% in carriers of FV G1691A were within Q1 of distribution and were significantly lower than in non-carriers [71.9 (35.4-76.4) vs. 88.6 (84.9-90.9)] (Mann-Whitney U test p< 0.0001 for all). In contrast, there was no difference between carriers (n=23, 2.1%) of FII G20210A and non-carriers in D-d (307 vs 321 ng/ml) or PiCi% (89.4 vs. 88.5%). In the total cohort D-d correlated (Spearman) with age (r=0.408), hs-CRP (r=0.320), body mass index (r=0,179), fibrinogen (r=0,170), systolic BP (r=0.136) and inversely with PiCi% (r=-0,151), all p< 0.0001.

Conclusions: In a population-based cohort the strongest determinant of D-d levels were age, hs-CRP and FV G1691A.

PB 1533 | Antithrombin p.Thr147Ala: Not Just Another SNP?

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Background: Hereditary antithrombin (AT) deficiency is a rare autosomal dominant disorder characterised by decreased AT activity in plasma and predisposition to recurrent venous thromboembolism (VTE). Hereditary AT deficiency is caused by mutations in the *SERPINC1* gene.

Aims: We investigated a variant in the *SERPINC1* gene, p.Thr147Ala, in six women of African origin. This variant is known as single nucleotide polymorphism (SNP) rs2227606 with minor allele frequency of 0.67% in Africans and absent in all other studied populations. *In silico* prediction tools for pathogenicity render conflicting results.

Methods: We collected plasma and DNA samples from six affected women. Mutations were identified by sequencing of all exons and intron-exon junctions of *SERPINC1*. Plasma AT activity was measured by chromogenic anti-Xa and anti-IIa methods and antigen by rocket immunoelectrophoresis. Heparin affinity was assessed with crossed immunoelectrophoresis. Recombinant AT molecules were constructed by site-directed mutagenesis and expressed in HEK-293T cells. Secreted AT was purified and studied with functional assays.

Results: Six unrelated women of Black African origin were found to carry the p.Thr147Ala variant. All but one experienced VTE, stroke or obstetric complications. AT anti-Xa activity was reduced in all patients: range 54-76% (reference value >80%) while anti-IIa activity and antigen levels were normal. On crossed immunoelectrophoresis, no AT fraction with aberrant heparin affinity was detected. The purified recombinant p.Thr147Ala protein displayed anti-Xa activity of 61.6±1.58% of the wild type recombinant AT (p< 0.05).

Conclusions: Antithrombin p.Thr147Ala, a variant reported as SNP in Black Africans, is responsible for type II AT deficiency. Type II AT mutations are known to cluster in specific regions, like p.Phe131Leu (Budapest III) in the Balkan and p.Ala416Ser (Cambridge II) in Spain and Scotland. Our data suggest a pathogenic phenotype. Further studies to unravel the exact molecular mechanism are ongoing.

PB 1534 | EPCR 6936A/G Gene Polymorphism and Plasma Levels of Endothelial Protein C Receptor as Risk Factors for Early-onset Venous Thromboembolism in North-Western Russia

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Background: Polymorphism 6936A/G in the endothelial protein C (PC) receptor gene is supposed to be a risk factor for venous thromboembolism (VTE) due to elevated plasma levels of the soluble form of endothelial protein C receptor (sEPCR). The mechanisms linking sEPCR levels to VTE remain to be determined. Thrombin generation test (TGT) in the presence of thrombomodulin (TM) is sensitive to defects of the PC system.

Aims: Aim of our study was to assess possible implication of 6936A/G polymorphism and sEPCR levels for TGT parameters in patients with early manifestation of VTE.

Methods: PCR-RFLP was used for detection of EPCR 6936 A/G polymorphism in 207 patients with VTE before 45 years old and 200 controls. TGT according to Hemker et al. and sEPCR level by ELISA were measured in subgroup of 36 patients and 28 controls. STATISTICA 6.1 was used.

Results: G allele was more prevalent in patients without than with FV Leiden mutation (28,5% vs 18,7%; OR=1,4; 95%CI: 0,8-2,4; p=0,05). Mean sEPCR level in patients was lower than in controls (72,6±30,9 $ngmL^{-1}$ vs.102,0±26,7$ngmL^{-1}$, p=0,08). We didn't find significant difference between subjects with AA or AG genotypes in the levels of sEPCR, probably due to low frequency of heterozygotes in our sample. In patients with FV Leiden mutation sEPCR levels were higher than in those without this mutation (86,4±48,5 vs. 67,9±21,8 p=0,07). Moreover, sEPCR level showed correlation with endogenous thrombin potential (ETP) and peak thrombin (PT) in the presence of TM (R=0,41 and R=0,46 respectively, p<0,05). When PC pathway was challenged by adding TM to plasma, both ETP and PT were markedly reduced. Significant correlation with sEPCR was found for reduction of PT (R=0,49, p<0,05).

Conclusions: Polymorphism 6936 A/G demonstrated no significant correlation with TGT parametres, though sEPCR levels were associated with PC system activity and prothrombotic potential. Prevalence of polymorphism 6936G and sEPCR levels in patients with FV Leiden mutation need further investigation.

PB 1535 | Risk Factor Stratification for Venous Thromboembolim in Patients with Below Knee Injuries Treated with Immobilisation and the Future Role of Thromboprophylaxis

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Background: Below knee injuries are a common presentation in orthopaedic departments, but the incidence of venous thromboembolism (VTE) and need for thromboprophylaxis in these patients is controversial, with the incidence of VTE ranging from 4.3% to 40%. However, it remains unclear if risk assessment can identify a group which could benefit from thromboprophylaxis.

Aims: The primary aims of this study were to determine the incidence and types of risk factors associated with VTE in the selected patient cohort.

Methods: This study was designed as a prospective single cohort study. Patients with below knee injuries, including both fractures and soft tissue injuries, treated with any form of lower limb immobilisation were included in the study. Patients who were receiving oral anticoagulation were excluded from the study. Patients who satisfied the inclusion and exclusion criteria were administered a VTE risk factor questionnaire. The questionnaire assessed age, sex, BMI, performance status, presence of co-morbidities, history of VTE, oestrogen exposure, pregnancy, active cancer, thrombophilia, injury type, smoking, type of immobilisation, and family history. Patients were contacted and radiological imaging reviewed twelve weeks from the date of their initial injury to determine if VTE occurred.

Results: A total of 120 patients were surveyed, 70 males and 40 females. 5 patients developed clinically significant VTE, 3 developed isolated DVT and 2 developing DVT and PE. All but 1 of these patients had 3 or more risk factors. The overall incidence of VTE among this patient cohort was 4.1%, with 4 out of 5 (80%) of episodes of VTE occurring in patients with at least 3 risk factors.

Conclusions: The overall incidence of VTE in this population is 4.1%. This study confirms the propensity of patients with multiple risk factors, with below knee injury to develop VTE. This study suggests that it may be possible to identify a high risk group of patients at the time of injury who may benefit from chemical thromboprophylaxis.

PB 1536 | Paroxysmal Nocturnal Hemoglobinuria (PNH) in Patients Presenting with Cerebral Vein and Sinus Thrombosis (CVST) - Results of Flow Cytometry Based Screening in Indian Patients

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Background: PNH is known to present with thrombosis at unusual sites especially the intracranial sinovenous vasculature and may be missed unless looked for. Thrombotic PNH is associated with large clone sizes that can be detected by flowcytometry. Guidelines suggest routine screening for PNH in these patients, however there is a paucity of data on the prevalence of PNH in patients presenting with CVST.

Aims: To determine the prevalence of PNH in Indian patients presenting with CVST.

Methods: Adults presenting with CVST, confirmed by imaging studies, referred to our laboratory for workup for thrombophilic risk factors were screened for Protein C, S and antithrombin deficiency, positivity for the antiphospholipid antibodies and the Factor V Leiden (FVL) mutation. Flow cytometry was performed on the leucocytes with the "lyse stain wash" technique. PNH was diagnosed by the presence of deficiency of FLAER and CD24 in the neutrophils; and FLAER and CD14 and monocytes respectively. Control samples were included in each run.

Results: Over a period of 5 years, 180 adults, aged 18 to 65 (mean - 31.8) years with CVST and 180 healthy volunteers were screened for PNH. Pregnancy was a risk factor in 18% of the females in the reproductive age group. None of the cases or controls showed presence of PNH clones, with median deficient clone sizes being nil for FLAER in (both neutrophils and monocytes) and CD24 in the neutrophils. CD14 deficiency was not statistically significantly different in cases and controls (0.3 vs 0.2%, *p*=0.08).Protein C, S and antithrombin deficiency were seen in 2.1, 2.8 and 1.4%; positivity for the antiphospholipid antibodies in 12.6% and heterozygosity for FVL in 3% of the cases tested.
Conclusions: Young age, pregnancy and antiphospholipid antibodies are common risk factors for CVST, however PNH clones were not seen in the granulocytes or monocytes. PNH does not appear to be a common risk factor in Indian patients presenting with CVST.

PB 1537 | High Levels of VAMP 8 and Serotonin Transporter Suggest a Tendency of Thrombosis in Spanish Population. Preliminary Results from the RETROVE Project (Risk of Enfermedad TROmboembólica VEnosa)

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Background: Platelet (PTL) hyperreactivity has been associated with thrombosis and with high levels of human vesicle-associated membrane protein 8 (VAMP-8) and serotonin transporter (SERT).
Aims: To determine if Serotonin, SERT and/or VAMP8 are associated with a risk of venous thrombosis.
Methods: A total of 195 healthy controls (87 men and 108 women) and 162 patients with venous thrombosis (80 men and 82 women) were included in our study.
VAMP 8: An ELISA kit was used to determine the levels of VAMP8. PLTs were isolated previously: Plasma Rich Protein was washed with the equal volume using physiological saline (0.9%) and centrifugation at 4500 x g for 10 minutes. PLT pellet was frozen at -40°C. Before determination, PLT pellet was resuspended with Triton X-100 and cooled with ice for 1 hour.
SERT: An ELISA kit was used to determine the levels of SERT. PLTs were isolated as previously described.
Serotonin: An ELISA kit was used to determine the levels of Serotonin. PLTs were isolated as previously described, but the PLT pellet was resuspended with 200µL of distilled water.
Statistics Analyses: the controls and patients were compared by the Mann-Whitney test. p values < 0.05 were considered statistically significant. Interquartiles (25th and 75th percentiles) were calculated for the three parameters; median was calculated also.
Results: VAMP8 and SERT showed statistically significant higher levels in patients than in the controls. In contrast, Serotonin showed lower levels in patients than in controls:
Conclusions: High levels of VAMP 8 and SERT were associated with thrombosis in Spanish population.
SERT is a PLT membrane protein that regulates serotonin levels allowing its recycling. High levels of SERT might explain the decrease in the levels of Serotonin observed in our study.
Further studies are necessary to confirm our results.
RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269

PB 1538 | Genetic Heterogeneity of Protein C Deficiency in Hungary; Genotype-phenotype Correlations

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Background: In the background of hereditary Protein C (PC) deficiency more than 300 different mutations have been identified, so far. Type I (quantitative) and type II (qualitative) PC deficiencies can be distinguished, the latter can be subdivided according to the result of the chromogenic functional assay (CHR), that gives a normal PC activity in the case of the rare type IIb variants.
Aims: Our aims were to map the mutation spectrum of PC deficiency in the Hungarian population and to predict the potential effect of novel mutations detected in this cohort and to investigate the effect of the different mutations on laboratory results.
Methods: Non-related individuals having 70% or lower PC activity were selected (n=134). PC activity was determined by clotting and CHR tests and PC antigen was measured by ELISA. Direct sequencing of PROC gene and MLPA analysis were performed to detect potentially causative mutations. The prediction of pathogenicity of the

TABLE 1 Results

	Median	Median	25th Percentile	25th Percentile	75th Percentile	75 th Percentile	
	Patient	Control	Patient	Control	Patient	Control	p
VAMP8 (pg/10E9PLT)	1502	1138	923	536	2373	1930	0.001
SERT (pg/10E9PLT)	650	541	459	364	989	776	0.001
SEROTONIN (ng/10E9PLT)	144	171	69	110	211	257	0.002

novel missense mutations was evaluated by MutPred, PhD-SNP and Poly-Phen-2 in silico tools.

Results: A total of 44 different mutations were identified in 82 index patients. Among them 18 (41%) novel and 26 (59%) known mutations were found. Of the novel alterations 12 missense, 1 splice site, 4 nonsense and 1 large gene deletion were detected. Most of the mutations (n=28, 64%) resulted in type I, six (14%) caused type II deficiency from which 4 were type IIb and 10 mutations resulted in uncertain laboratory phenotype. Among type IIb, p.Arg57Gln, p.Gly392Arg were suggested as pathogenic by all in silico tools. The consequence of p.Ala333Asp was uncertain and p.Ala408Thr was predicted as rather non-pathogenic variant.

Conclusions: No founder mutation was identified in the Hungarian PC deficient population and a high number of novel variants were described. Since the in silico tools did not clarify the consequences of type IIb mutations biochemical studies are needed to establish their clinical relevance.

PB 1539 | Elevated Plasma Factor IXa Activity in Women Using Hormonal Contraception

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Background: Oral contraceptives (OCP) are associated with a 2-6 fold increase in venous thromboembolism, but the mechanism(s) remain unclear.

Aims: To determine the relationship between plasma FIXa activity and OCP use.

Methods: Plasma from 36 premenopausal female blood donors on hormonal contraception (PreHC), 36 premenopausal females not on hormonal contraception (PreNO), 36 post-menopausal females (POST) and 10 males (MEN) were analyzed for FIXa activity with the enhanced thrombin generation assay. Total protein S (PS), total tissue factor pathway inhibitor (TFPI) and TFPI-α were determined by ELISA. Tissue factor (TF) triggered FIX activation was examined in vitro using FV-deficient plasma to avoid quantitative thrombin generation.

Results: The PreHC group demonstrated significantly higher FIXa activity and a non-normal distribution (Fig 1). Median FIXa activity, TFPI-α and total PS antigen levels with interquartile distribution (IQR) were compared in female subgroups (Table 1). Similar to FIXa activity, TFPI-α was significantly lower in the PreHC group. In contrast, total PS and TFPI (not shown) had significantly lower values in the premenopausal (PreHC and PreNO) compared to post-menopausal (POST) females without a discernible effect of hormonal contraception. MEN were similar to the POST group for all parameters (not shown). Using the aggregate data (n=116), FIXa activity demonstrated modest but statistically significant negative correlations with both TFPI-α ($r=-0.36$, $p < 0.0001$) and total PS antigen ($r=-0.32$, $p < 0.0004$). Total protein S had a moderate strength positive correlation with TFPI-α antigen ($r=0.52$, $p < 0.0001$). Evaluation of TF-triggered FIX activation in FV-deficient plasma demonstrated that Inhibitory antibodies vs. the TFPI K1 and/or K2 domains enhanced TF-dependent FIXa generation by 2-3 fold, while addition of 2.4 nM TFPI or 75 nM PS reduced FIXa levels by ~2-fold.

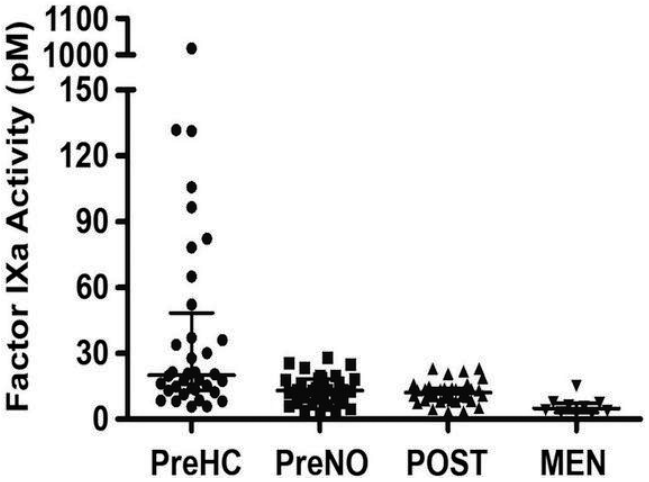

FIGURE 1 Plasma FIXa Activity in Blood Donor Subgroups

TABLE 1 FIXa Actvity, TFPI and Total PS antigen levels in Blood Donor Subgroups (Mann-Whitney)

	Blood Donor Subgroup	Median	IQR	p-value
FIXa Activity (pM)	PreHC	20.1	13.2-48.4	
	PreNO	13.0	7.9-18.2	0.0006
	POST	12.1	8.6-14.0	<0.0001
TFPI-α (ng/ml)	PreHC	12.9	9.2-18.7	
	PreNO	20.1	15.9-24.8	0.0001
	POST	27.9	23.0-35.0	<0.0001
Total PS (μg/ml)	PreHC	20.1	16.0-26.8	
	PreNO	22.9	17.8-27.7	0.74
	POST	31.6	22.6-36.4	0.0037

Conclusions: Plasma FIXa activity is a potential biomarker for hypercoagulability in premenopausal females taking OCPs.

PB 1540 | Analysis of Factor V Leiden Paradox from a Large Monocentric Cohort Study: A Role for Distal Thrombosis?

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Background: Factor V Leiden (FVL) is a stronger risk factor for deep vein thrombosis (DVT) than for pulmonary embolism (PE), hence the so-called FVL paradox.

Aims: To overview the venous thromboembolism (VTE) clinical presentation in our cohort of FVL and prothrombin mutation (PTM) carriers.

Methods: Consecutive patients with a first VTE homozygous or heterozygous FVL and/or PTM were considered. VTE patients in the same study period without thrombophilia acted as controls.

Results: Among 1970 VTE patients, 659 were carriers of FVL and/or PTM. 336 heterozygous FVL, 32 homozygous FVL, 10 homozygous PTM, 238 heterozygous PTM, 35 double heterozygous (FVL and PTM), and 8 FVL pseudohomozygous. A higher percentage of heterozygous FVL (61%) presented with isolated DVT than controls (43%, p< 0.0001). A similar figure was observed in homozygous FVL. There was no difference in heterozygous PTM and in double heterozygous compared to controls. 6 of pseudohomozygous (75%) had isolated DVT. A higher proportion of isolated distal DVT was observed in heterozygous FVL (30.2%) compared both to controls (18.6%, p< 0.001) and heterozygous PTM (18%, p=0.003). Multivariate risk of isolated DVT: 2.15 (95% CI 1.6-2.7) in heterozygous and 2.21 (95% CI 1.07-4.5) in homozygous FVL compared to controls. Heterozygous PTM and double heterozygous had a non-significantly higher risk of isolated DVT.

TABLE 1 Odds ratios (OR) for isolated DVT associated with different thrombophilic defects

	Univariate OR (95%CI)	Multivariate OR (95%CI)
No thrombophilia	1 (ref)	1 (ref)
Hetero FVL	2.22 (1.54-2.3)	2.15 (1.6-2.7)
Homo FVL	2.31 (1.18-3.7)	2.21 (1.07-4.5)
Hetero PTM	1.14 (0.7-1.6)	1.17 (0.8-1.56)
Homo PTM	2.24 (0.88-5.0)	1.98 (0.55-7.09)
Double heterozygosity	1.8 (0.91-2.6)	1.40 (0.71-2.75)
Pseudohomozygosity	3.31 (0.8-19.8)	3.19 (0.87-19.9)

Heterozygous FVL showed a similar risk of isolated DVT as homozygous FVL (0.98, 95%CI 0.93-1.04), a higher risk of isolated DVT (1.83, 95%CI 1.31-2.56) and of distal DVT (3.31 95%CI 1.73-6.33) than heterozygous PTM. There was no difference in the activated protein C resistance ratio in FVL with isolated DVT (1.66 ± 0.20) and FVL with isolated PE and combined DVT/PE (1.59 ± 0.19).

Conclusions: The paradox was limited to heterozygous and homozygous FVL, with a trend in pseudohomozygous. Double heterozygosity presented an intermediate clinical pattern. The higher prevalence of isolated distal DVT might in part explain the presence of paradox only in FVL.

PB 1541 | Paroxysmal Nocturnal Hemoglobinuria (PNH) and Thrombosis: Experience from an Asian Tertiary Centre

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Background: PNH was touted to be the most vicious acquired thrombophilia. We retrospectively reviewed the thrombotic events in our patient cohort that was followed up since January 2005 to date.

Aims: To review the characteristics of thrombotic events, treatment and the outcomes in the patients with PNH.

Methods: Our data was collected from the patients' laboratory and clinical records. Diagnosis of PNH was confirmed on flow cytometry assay (ISCH guidelines).

Results: There were 24 patients with PNH who were followed up since 1/1/2005 to date in our institution. 10 had classic PNH with significant hemolysis, 12 had associated bone marrow failure without significant hemolysis and 2 had asymptomatic PNH clone. Gender ratio was equal and racial distribution was as follows: Chinese 67%, Malay 12.5%, Indian 4% and other races 16.5%. Median age at diagnosis was 38.5 (17-81) and median follow up was 5.75 years with a total person-years of follow up 143.08 years. Venous thromboembolism (VTE) occurred in 4 patients (17% of total and 40% of classic PNH) and arterial thrombosis, coronary artery disease (CAD) in 2(8% of total). The incidence rates were 2.8% for VTE and 1.4% for arterial thrombosis per 100 patient-years. All VTEs responded well to the treatment with no recurrence during the follow up period. Table 1 summarized the characteristics of thrombotic events and their outcomes.

Conclusions: Our patients with PNH did not demonstrate the high risks of vicious thrombotic complications as described in literature. The patients with CAD had multiple cardiovascular risk factors. VTEs responded well to standard anticoagulation therapy with no recurrences while on long term anticoagulation. All patients with unprovoked and visceral VTE are of non-Chinese descent. Considering majority of Singapore population is ethnic Chinese, it seems probable that Chinese patients with classic PNH are at lower risk of developing VTE. Though overall mortality rate was higher in the patients with thrombosis, the mortality was not attributed to VTE.

TABLE 1 Characteristics of thrombotic events, treatment and outcomes in the study cohort

Patients with thrombotic events	Race	Types of PNH	Types of thrombosis	Sites of thrombosis	Risk factors other than PNH	Treatment of thrombosis	Outcome
1	Myanmar	Classic	Venous	Intra-abdominal	None	Warfarin	Alive
2	Indian	Classic	Venous	Intra-abdominal and right lower limb (proximal)	None	Warfarin	Alive
3	Malay	Classic	Venous	Right lower limb (proximal)	None	Warfarin	Death (Cause : sepsis)
4	Chinese	Classic	Venous	Right lower limb (distal)	Severe Sepsis Immobility	Enoxaparin	Death (Cause : sepsis)
5	Chinese	PNH with aplastic anemia	Arterial	Coronary artery	Pneumonia Renal failure Hypertension Hyperlipidemia Diabetes mellitus	Aspirin and clopidogrel	Death (acute myocardial infarction)
6	Malay	PNH with aplastic anemia	Arterial	Coronary artery	Hypertension Hyperlipidemia Diabetes mellitus	Bypass surgery, Aspirin Warfarin (short term)	Alive

PB 1542 | Results of Thrombophilia Testing in Infertile Women and Women Experienced a Miscarriage

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Background: Although some studies suggested an association between thrombophilic risk factors and infertility and miscarriage, others did not confirmed these findings and disagreement regarding this association still exists.

Aims: The aim was to analyze results of thrombophilia testing in infertile women and women experienced miscarriage referred for testing at our laboratory during the two consecutive years (2015 and 2016).

Methods: The study included lupus anticoagulant (LA) investigation, functional assays for antithrombin (AT), protein C (PC), protein (PS), resistance to activated protein C (APCR) and coagulation FVIIII (FVIII) activitiy using commercially available coagulation methods (Siemens, Germany).

Results: Among all patients referred for testing, infertile women accounted for 150/1688; 8.9% (LA), 112/783; 14.3% (PS), 85/911; 9.4% (PC), 90/1054; 8.5% (AT), 25/356; 7.0% (APCR) and 9/451; 2.0% (FVIII). Positive results among all infertile women were: 1/150; 0.7% (LA), 2/112; 1.8% (PS) and 1/25 (APCR). There were no positive results for PC (0/85), AT (0/90) and FVIII (0/9) among infertile women. Women with miscarriage were represented as follows: 132/1688; 7.8% (LA), 93/783; 11.9% (PS), 50/911; 5.5% (PC), 52/1054; 4.9% (AT), 16/356; 3.5% (APCR) and 8/451; 1.8% (FVIII). Positive results among women with miscarriages were: 2/132; 1.5% (LA), 2/93; 2.2% (PS) and 1/16 (APCR) while there were no positive results for PC (0/50), AT (0/52) and FVIII (0/8).

Conclusions: Our results do not support nondiscriminate testing for all thrombophilic risk factors in all infertile women and all women experienced a miscarriage. The clear association between individual risk factors and infertility or miscarriage still remains controversial and inconclusive. A further large prospecitve and retrospective studies are necessary for clarifying and understanding this association. In order to contribute to these findings, we intend to follow and analyze our results during the next 5 years.

PB 1543 | Importance of Thrombophilia Screening in Patients with Ocular Thrombosis

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Background: Ocular thrombosis (OT) is the second most common retinal vascular disease following diabetic retinopathy. It is still questionable if screening of thrombophilia in these cases has an important diagnostic and therapeutic impact as in other non-ocular thrombosis. We here report upon thrombophilia screening in 140 patients with OT.

Aims: We aimed to assess the role of thrombophilia as an etiology of ocular thrombosis, as seen in extremities thrombosis or pulmonary embolism.

Methods: 116 patients with ocular venous thrombosis and 21 with ocular arterial thrombosis and 3 patients with both arterial and venous thrombosis were examined. The patients presented in our department few weeks after incidence of OT. We carried out usual screening for most common inherited and acquired thrombophilia in all patients.

Results: Thromboembolic history was positive in 49 patients (35 %), 40 % of them were younger than 50 years old. Thrombophilia

screening showed: detected antiphospholipid antibodies in 28, lipoprotein (a) elevation in 23, factor VIII:c elevation in 23, antithrombin deficiency in 4, prothrombin (G20210A) polymorphism in 7, factor V Leiden mutation in 14 patients.

Conclusions: These results showed statistically no correlation between common risk factors of venous thromboembolism like factor V Leiden mutation, prothrombin polymorphism, antithrombin deficiency and factor VIII:c elevation and incidence of OT, while antiphospholipid antibodies and lipoprotein (a) elevation seem to play a major role. Positive history of deep venous thrombosis and pulmonary embolism in 35 % of these patients has an important impact on the prognosis and on the choice of therapeutic approach.

PB 1544 | Screening of Thrombophilia in Latin America

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Background: Latin America is an heterogeneous region. A population in excess of 6 hundred million people belong to different ethnicities and cover different socio economic conditions.

Aims: In order to know how thrombophilia defects are investigated in the area the Latin American Group on Thrombosis and Haemostasis (Grupo Cooperativo Latinoamericano de Hemostasis y Trombosis-CLAHT) conducted a survey (March to April 2016).

Methods: The questionnaire was sent to members of different societies dedicated to haematology, thrombosis and haemostasis or internal medicine in various countries. It enquired about tests requested in case of patients with a first thromboembolic event and neither personal nor family history of thrombosis in four different situations: unprovoked event under either < 50 year old (A) or >50 year old (B); provoked event either < 50 year old (C) or >50 year old (D).

Results: Answers: 169 were obtained, mostly from haematologist (71.9%). The overall responses were made up as follows: Uruguay (43.2%), Argentina (17.4%), Brazil (8.3%), Perú (6.8%), Venezuela (6.8%) and other countries (17.5%). A: 74-81% requested tests of inherited thrombophilias (IT) (AT, PC, PS, FVL, P20210A) and antiphospholipid antibodies (APA); there is a surprisingly high number of requests for thermolabile MTHFR and PAI 4G5G (29%-15%) polymorphisms. B: 78% requested cancer screening and 64% APA; again, there is a high number of requests for homocysteinemia (44%) and IT (28-36%). C: 45% requested APA and 41% required no test; however, 27-36% also request some IT. D: 77.5% requested cancer screening despite being a provoked event.

Conclusions: The survey shows the need for more educational actions in order to abolish incorrect requests (MTHFR and PAI in A; APA in C and D; IT in B) by applying International Guidelines in the region, which will result in considerable economic and social benefits.

PB 1545 | First Do Not Harm: Questioning Ethics Aspects of Thrombophilia Law in Argentina

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On behalf of the Women Health Issues in Thrombosis and Hemostasis Group (WHITH-CAHT) and the Argentine Thrombosis and Hemostasis Cooperative Group (CAHT), Buenos Aires, Argentina

Background: Universal study of hereditable thrombophilia (HT)in pregnancy complications(pgC)is not indicated. Risks of treatment with low molecular weight heparin(LMWH)are low. Decision making upon starting treatment in pgC should come from well designed randomized controlled trials(RCT), there is no benefit in preventing early recurrent pregnancy lost(RPL)in women without thrombophilia(TP). RCT in RPL and HT are ongoing. The strongest evidence for the use of heparin and aspirin is in the antiphospholipid syndrome. In spite of this information a law petition was presented by organizations of women with pgC and TP and was finally passed by the Congress.

Aims: To evaluate the law's requirements.To describe its ethic questioned aspects.

Methods: Law's requirements: 1.Psychophysics protection of women with thrombophilia; 2.Early detection of thrombophilia in women in fertile and prefertile age; 3.Inclusion of tests and treatments as obligatory in public/private health programs.

Results: Ethic questioned aspects:1.Patient's autonomy in demanding HT studies vs medical autonomy in the obligation to prescribe them.2.Universal study of HT in healthy women.3.The use of medication in pregnancy that is "off label" or "non-validated" or what some authors call "innovation".4.The allocation of economic resources in public health. 5.The impacts of health related information in the media.

Conclusions: What happened in our country exposes important bioethics issues related to thrombophilia tests and its treatment in pregnancy:

1. Highlights open unresolved questions such as investigation and regulation of research in pregnant women.
2. Differences of research regulations between countries (we were unable to participate in previous trials SPIN-ALIFE for this reason).
3. Universal demand of genetic studies in healthy women.
4. Distribution of economic resources: The role of Public Health in defining Health Programs and Policies when the population demands government action in areas where scientific evidence do not support this petition.

PB 1546 | Extensive molecular characterization of a family with thrombophilia: implication of multiple genetic alterations of low thrombotic risk profile

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Background: Thrombophilia is a complex disease where multiple genetic factors interact with each other and with the environment to generate a gradient of susceptibility to disease.

Aims: Characterize a family with several members with venous thrombosis.

Methods: A biological study of thrombosis in the propósitus was made extending to his relatives. Factor V Leiden mutations, prothrombin gene F2G20210A, factor XII F12C46T, AT Cambridge type II mutation (SER-PINC1G13268T; p.Ala384Ser) and ABO group genotype were included in the study.

Results: The propositus is a female patient with idiopathic popliteal DVT at 79 years old. The thrombosis study resulted in a G / T heterozygous AT Cambridge type II and BO2 of ABO . In her family history of thrombosis: a daughter with popliteal DVT at 30 years old secondary to polytraumatism, at that time in treatment with oral contraceptives and smoker. The genetic study resulted gene F2G20210A heterozygous and A1B of the ABO. Father deceased with a history of paraneoplasic DVT. No other history of thrombosis in rest of the family. A second daughter of the propósitus (currently 49 years): double heterozygote for the mutation F2G20210A and AT Cambridge type II, and A1O2 of the ABO. Grandson of first daughter (currently 30 years): homozygous for F2G20210A and homozygous A1A1 for ABO. His father was also found to have homozygosity for the F2G20210A mutation with no personal or family history of thrombosis in this part of the family. Grandsons by the second daughter (10 and 15 years old at present): both heterozygotes for the AT Cambridge type II mutation, one of them being heterozygous for the F2G20210A mutation and T / T homozygous for F12C46T

Conclusions: In this work we describe a family with Cambridge type II antithrombin in combination with multiple genetic factors. The family had thrombotic clinic in different family members. This example represents the oligogenic composition of thromboembolic disease, where multiple genetic defects coexist modulating the risk of disease.

PB 1547 | Inherited Thrombophilia and Infertility

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Background: Increased tendency for thrombosis i.e. hypercoagulability as a result of multiple genetic mutations is called inherited thrombophilia. According to the World Health Organization (WHO), infertility can be described as the inability to become pregnant, maintain a pregnancy, or carry a pregnancy to live birth. Because of the recently reported possibility of association between infertility and inherited thrombophilia, further research is needed.

Aims: Aim of this study is to see the presence of the most common and clinically significant inherited thrombophilias: factor II prothrombin G20210A, factor V Leiden (FVL) G1691A and methylentetrahydrofolate reductase (MTHFR) C677T mutations among the patients with infertility.

Methods: retrospective study, using the data from the outpatient clinic in the Institute of Transfusion medicine- Skopje, Republic of Macedonia. We have examined 39 females with infertility referred to our Institute in the period of June-September 2016. 2ml of venous blood in K2EDTA was collected for genetic evaluation. Detection of point mutations for factor II prothrombin G20210A, factor V Leiden (FVL) G1691A and methylentetrahydrofolate reductase (MTHFR) C667T was performed, using the Operon kit.

Results: We found 23 patients(58.9%) with one thrombophilia, 7 patients(17.9%) with two thrombophilias and only 9 patients(23.1%) do not have any of the tested mutations. Among the tested patients: 15.4% are MTHFR C677T homozygous and 43.6% are MTHFR C677T heterozygous, 17.9% are factor V Leiden (FVL) G1691A heterozygous and 12.8% are factor II prothrombin G20210A heterozygous.

Conclusions: There is strong presence of the most common and clinically significant thrombophilic mutations among the females with infertility. Expanding the tested group and inclusion of a control group is necessary.

PB 1549 | Impact of the Molecular Study of the PROC, PROS1 and SERPINC1 Genes in Patients with Venous Thrombosis

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Background: The prevalence of deficiencies (d) of protein C(PCd), protein S(PSd) and antithrombin(ATd) are low and probably underdiagnosed. The molecular study of the genes *PROC*, *PROS1* and *SERPINC1* increase the diagnostic capacity, allow to establish a genotype/phenotype correlation and to identify patients at venous thrombosis risk.

Aims: To study the these genes in patients (P) with
(i) PCd and/or PSd and/or ATd or with plasma levels at the lower limit
(ii) normal plasma levels but severe history of venous thrombosis.

Methods: We studied 172P with PE (8.1%), VT(33.7%), TA(7%) and obstetric complications(18.6%); 32.6% had a family history of thrombosis. Functional studies: PSL, PCact and ATact - plasma levels compared to age-adjusted reference values, confirmed in 2 different samples. Molecular studies: ASPCR, Sanger sequencing and MLPA.

Results: Among 172P, 130 had: 34.3% PCd, 38.3% PSd and 3.5% ATd; 1 P PCd + PSd; 34normal levels of PC, PS and AT; 7were anticoagulated. In 102 P 26different mutations (mut) - (12*PROC*, 10*PROS1* and 6*SERPINC1*).The p.Ser501Pro(PS Herleen) was found in 46% of PSd. The *PROC* mutation p.Arg199*was identified in 18P with PCd type I and associated with a severe phenotype. The *PROS1* p.Val1191Cysfs*6, p.Ala307Cysfs*22 and p.R451* mut, were found in severe PSd. Two compound heterozygous *PROS1* p.[Pro76Leu+Ala307Cysfs22] were detected in 2P with PSd; 1P showed combined heterozygosity for *PROC*-13A>G, *PROS1* p.N166H,p.A307Cfs*22; two *novel* mut: *PROC*(p.Cys105Arg); *SERPINC1* p.YTyr95Serfs*18; 60% of P with mut in *SERPINC1* and 18.9% in *PROS1* had normal levels.

Conclusions: In PSd and ATd, the success of mut identified was lower, especially in mild deficiencies. Mut. were detected in patients with normal plasma levels, confirming the failure to detect qualitative changes by the available functional assays. The molecular study should be considered in patients with a significant personal/family history of thrombosis, and „normal/borderline" levels of these proteins.

PB 1550 | Cutaneous Necrosis Revealing Paroxysmal Nocturnal Haemoglobinuria

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of hematopoietic cells characterized by intravascular hemolytic anemia, hypercoagulable state and bone marrow aplasia. Venous thrombosis generally arise in hepatic, intra-abdominal, cerebral, and extremity veins and represent the most common cause of death in patients with PNH. Despite being recognized as a complication, cutaneous thrombosis is uncommon and a variety of findings have been reported.

Aims: We report an exceptional case of cutaneous necrosis of the earlobe and the scalp revealing PNH.

Methods: We report the case of a 37-year-old male patient with minor-thalassemia diagnosed at the age of 5.

Results: He presented with necrotic lesions mainly located in the neck, the scalp and the left earlobe. Laboratory analysis revealed bicytopenia (hemoglobin 9 g/dl; platelet count 33 g/L), with hemolysis and a negative coombs test negative, biological inflammation (CRP of 195 mg/l) and hematuria. There were no other serological, immunological or coagulation alteration. Skin biopsy revealed superficial and deep dermal vessels with foci of necrosis; without vasculitis. Flow cytometry of peripheral blood leucocytes and mononuclear cells confirmed the presence of an PHN clone with 90-95% of positive cells.

Anticoagulant therapy was started with enoxaparin 1 mg/kg twice a day with corticosteroids (prednisone 1 mg/kg/day). After 1 week there were only residual hyperpigmented macules. After 3 years, the patient remains well with VKA treatment and 5mg of prednisone.

Conclusions: PNH is a clonal stem cell disorder resulting from a mutation in the PIG-A (phosphatidyl inositol glycan class A) that block glycosyl-phosphatidyl inositol (GPI) biosynthesis.

Both corticosteroid therapy and anticoagulants may be useful in the treatment of thrombotic episodes and prednisone is used because the complement activation probably initiates thrombosis in patients with PNH. The role of eculizumab in the treatment of acute thrombosis is under consideration.

PB 1551 | Venous Thromboembolism (VTE) in Homozygous Carriers of Factor V Leiden Mutation Patients (FVL)

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Background: Risk of venous thromboembolism is increased 50-100-fold in homozygous carriers factor V Leiden mutation patients particularly in the presence of additional congenital and acquired thrombotic risk factors.

Aims: The 21 years retrospective clinical analysis of homozygous FVL patients diagnosed and treated in the IHTM.

Methods: During last 21 years 132 homozygous carriers of FVL mutations were diagnosed in IHTM. We analyzed 103 homozygous FVL mutation patients (74F ; 58M, age 18-64).

DNA was isolated using GeneMatrix kit (Eurx, Poland). The FVL mutation was identified by PCR/RFLP method according to Bertina et al (1994) using restriction enzyme Mnl1 (Eurx, Poland). Prothrombin G20210A mutation was determined according to Poort et al (1966) using Hind III restriction enzyme (Eurx, Poland).

Results:

1. Homozygous factor V Leiden was detected in 74 female (56.1%) and 58 male (43.9%) patients. Deep venous thrombosis (DVT) was observed in 125/132 patients. Heterozygous G20210A mutation of prothrombin gene occurred with FVL in 5/132 homozygous patients.
2. Mean age of female and male patients was similar (30.5 vs.30.7 yrs) at the moment of the first DVT episode (K-16/74 (21.6%) ; M-6/58 (10.3%).
3. Proximal and distal DVT was more often observed in female than male patients.
4. 2-4 episodes of DVT occured in 17/125 pts (13.6%) more often in female patients
(F-11 ; M-6).

5. All the patients had several thrombotic risk factors, particularly age >30 yrs and familiar DVT (K-8/74 ; M-1/58).
6. Deep venous thrombosis untypical localization occurred in 3.03% FVL patients and thrombotic stroke in 8.3% FVL patients. Dominant thrombotic risk factors were familiar DVT.
7. Treatment of all patients was similiar : low molecular weight heparin (LMWH) followed by anticoagulant vitamin K (VKA).

Conclusions: Venous Thromboembolism can occur in carriers of homozygous FVL mutation particularly above 30yrs old if several pathophysiological, environmental and genetic thrombotic risk factors are present.

PB 1552 | Characteristics and Outcomes of Patients with Cerebral Venous Sinus Thrombosis in Oman

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Background:

1. Cerebral venous sinus thrombosis (CVST) is a rare cause of stroke, usually affecting young to middle aged patients 1. It accounts for 0.5% to 1% of all causes of strokes 1.

Aims:

1. Primary objective: To describe the clinical profile of the patients with CVST, to assess the risk factors involved and the presenting symptoms and signs
2. Secondary objective: The association with inherited thrombophilia and the out come.

Methods:

1. This was a retrospective cohort study which was conducted in 2 tertiary care hospitals in the Sultanate of Oman.
2. The study was reviewed and approved by the ethics committees of the two centers.
3. All Patients admitted with a diagnosis of CSVT were identified using the (ICD-10) coding system of the hospital medical records between Jan 2006 to June 2016.
4. Out of 81 patients were identified, 27 patients were excluded as didn´t meet the inclusion criteria (Omani patients, 14 years or older and radiologically confirmed by CT or MRI).

Results:

1. A total of 54 patients fulfilled the inclusion criteria with radiologically confirmed CVST.
2. The commonest presenting feature was headache (85%); followed by focal motor deficits (48%), and seizures (27%).
3. causes In this cohort were 24% were pregnant or postpartum, 11% had an inherited thrombophilia, 11% were on hormonal therapy, 11% were found to have systemic lupus erythematous, 7% had Antiphospholipid syndrome,13% had infections, and 2% had cancers. 33% there was no cause identified.
4. Thirty-seven percent of patients had multiple sinus thrombosis, 35% had sigmoid and transverse sinus thrombosis.
5. Out come: 30 % had residual neurological deficit on discharge and 2% of patients had died.
6. Age was a predictor for residual neurological deficit (*p=0.003*).

Conclusions:

1. Pregnancy and postpartum were the most common predisposing factors for cerebral venous thrombosis.
2. 30% of patient had residual neurological deficit at discharge.
3. mortality was 2%.
4. further studies needed.

PB 1553 | Anticoagulant Therapy in Disorders in the Fibrinolysis System among Patients with Primary Infertility

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Background: Currently an important role in the failure of in vitro fertilization, pregnancy complications and infertility of unknown origins belongs to thrombophilia. Early pregnancy losses, that may be connected with disorders in fibrinolysis, are of some interest.

Aims: Identification of disorders of the hemostasis system among patients with primary infertility and also the possibility of using low molecular weight heparins for pregnancy without in vitro fertilization (IVF).

Methods: The study involved 34 women diagnosed primary infertility aged 24 to 38 years, with an increased level of D-dimer in their blood. The timing of primary infertility ranged from 3 to 14 years. 26 women had failures with IVF in the anamnesis. Laboratory tests included: activated partial thromboplastin time, international normalized ratio, fibrinogen, XIIa-dependent fibrinolysis, protein C, protein S, antithrombin III, plasminogen, factor VIII, platelet count, level of plasminogen activator inhibitor type 1, D-dimer. The method of polymerase chain reactions identified: polymorphisms of methylenetetrahydrofolate reductase C677T, prothrombin G20210A, plasminogen activator inhibitor PAI-1, fibrinogen 455G/A, FV Leiden Arg506Gln. The analysis was carried out using nonparametric statistics with the use of U-criterion of Mann—Whitney.

Results: Polymorphism of PAI-1 was revealed among 29 patients (85,3%), including 11 patients with an increased level of PAI-1 (P=0,000005). At the stage of pregravid preparation, all the patients used enoxaparin in the complex therapy, the injections starting before ovulation and continuing until the end of the cycle, for 6 cycles or until pregnancy. Among 34 women included in the study, 7 (20,6%) with an excess of PAI-1 became pregnant.

Conclusions: The fibrinolysis system may play a role in primary infertility. The use of enoxaparin at the stage of pregravid preparation promotes pregnancy in some cases. Further observation is required to develop specific recommendations.

PB 1554 | Pattern of Thrombophilia Testing in Patients with Venous Thromboembolism at a Tertiary Care Hospital

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Background: Role of thrombophilia testing in patients with venous thromboembolism (VTE) is controversial. With no existing consensus on indications or panel of tests, there may be significant variability in the pattern of testing within and between institutions.

Aims: Determine the pattern of thrombophilia testing in patients with VTE at Emory University Hospital (EUH).

Methods: Patients admitted at EUH in year 2015, for whom a Hematology consult for VTE was requested were identified (N=85) and charts were reviewed retrospectively. Complete thrombophilia workup was defined as testing for all the following: Protein S (PS), Protein C (PC) and Antithrombin (AT) activity, Antiphospholipid antibodies (APLA), Factor V Leiden and PT G20210A gene mutations. Abnormal results for PC, PS and AT activity under conditions known to falsely affect levels were called ‚wrong time'. Statistics were descriptive.

Results: Of the 85 patients, 43 (51%) patients were males; 47% White, 42% African-American. Mean age at first VTE was 52.6 years. Forty-four (52%) underwent thrombophilia testing; 20 (45%) within 2 weeks of VTE event. Thirteen (29%) had complete workup. Reasons for incomplete workup were missed tests in 41, ‚wrong time' in 9 and no repeat testing for APLA in 1. Other tests included JAK 2 mutation in 11, PNH flow cytometry in 7, and fibrinogen, PAI-1, factor 8, factor 9 and factor 11 activity in 18, 3, 2, 1 and 1 patient respectively. Patients who underwent workup had lower mean age at first VTE (51.4 vs 57.7,p=0.045) and higher proportion of unprovoked episodes (52%vs19%,p=0.002) as compared to those who did not get tested. No significant difference in sex, race, clot location or number of episodes were found between the groups. Workup was positive in 10 (23%) and APLA syndrome (n=3) was the most common thrombophilia.

Conclusions: Thrombophilia testing in patients with VTE is common, with significant variability in the pattern of testing. There is need for establishing clear indications for a clinically relevant panel of tests.

PB 1555 | Meaning of Hereditary Thrombophilia in the Diagnosis of Thrombotic Complications during Combat Gunshot Injury

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Background: Prevention of complications is needed.

Aims: The aim of the study was to investigate the effect of haemostasis system gene polymorphism on venous thrombosis in the wounded from combat gunshot injury.

Methods: The results of the study of genetic mutations of the haemostatic system components at 46 wounded from combat gunshot injuries from 2013 to 2015 were performed. All patients - men, mean age - 29,5 ± 5,1 years. Mine-blast injured 33 (71.7%), bullet - 13 (28.3%) patients.

All the wounded were classified as high risk for venous thromboembolic complications and had 3 - 4 risk factors for their development.

The study group included 13 (28.3%) patients who during treatment at the hospital were diagnosed with venous thrombosis & the control group - 33 (28,3%) - with no signs of thrombosis.

Studied allelic polymorphism of genes: the factors I, II, V, XII, PAI-1, HPA-1, HPA-2, P2Y12, GpIa C677T (MTHFR).

To assess the reliability between the values used χ^2 test, Fisher´s exact test (two-tailed) to assess the strength of the relationship factors studied - criteria φ (phi) and Cramer´s V.

Results: There were no thromboembolic complica t ions & vascular disease before injury; soldiers were not screened previously for the presence of thrombophilia.

TABLE 1 Distribution of the main polymorphisms of genes in the injured

The name of the mutation	Allelic polymorphism	With thrombosis	No thrombosis	Total	Fisher´s exact test	p	φ & V Cramer
FV Leiden	MN	1	0	1	0.28261	p>0.05	0,2-<0,4
Prt G20210-A	MN	3	0	3	0.01884	p<0.05	0,4-<0,6
HPA-2	AB	8	8	16	0.01400	p<0.05	0,2-<0,4
HPA-2	BB	1	0	1	0.16667	p>0.05	0,4-<0,6
Fibrinogen G/A-455	MUT	5	1	6	0.01318	p<0.05	0,4-<0,6
Fibrinogen G/A-455	MN	2	13	15	0.68566	p>0.05	0,1-<0,2
GpIa C677T	MUT	3	1	4	0.06222	p>0.05	0,2-<0,4
PAI-1	4G/4G	5	8	13	0.46890	p>0.05	0,1-<0,2
P2Y12 ins801A (AP)	MUT	1	1	2	0.44286	p>0.05	0,1-<0,2

The presence of genetic mutations were identified in 42 (91.3%) injured. The data obtained are presented in the table.

Conclusions: We have found that, despite the high prevalence of thrombophilia not all lead to the development of thrombosis. The greatest probability of developing clinical manifestations of thrombosis must be expected for the wounded with combinations of several genetic mutations. It has been found that hereditary thrombophilia Prt G20210-A, HPA-2 and Fibrinogen G/A-455 are risk factors for thrombosis ($p< 0.05$).

The presence of the genotype of the wounded predisposition to thrombosis markers may serve as a basis for the definition of „risk groups" & to make recommendations for the prevention & treatment thrombosis in wounded with prothrombotic genotypes.

PB 1556 | Acquired Thrombotic Thrombocytopenic Purpura (aTTP) in Patient with Decompensated/Uncontrolled Hypertension, Hyperhomocysteinemia, Hypercholesterolemia and Hyperlipidemia

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Background: Acquired thrombotic thrombocytopenic purpura (aTTP) is characterized by thrombocytopenia and microangiopathic hemolytic anemia (MAHA) of no obvious origin. Microvascular thrombosis is also reported and causes variable degrees of tissue ischemia and infarction .

Aims: A female patient aged 50 admitted to IHTM in poor general condition, conscious without contact, no hemorrhagic disorders, 1brain ischemia attack was observed in the amplitude of vascularization of right and left internal carotid arteries with transient right and left-sided hemihypaesthesia with such symptoms as: confusion, no logical contact, psychomotor agitation.

Methods: Lab tests showed : RBC $1.7x10^6$/L, Hb 5.7 g/dl , HCT 17.0 % , MCV 97.1 fl , platelet 7.0 $x10^3$/L, RBC -poikilocytosis, mikrocytosis, makrocytosis, schistocytes, reticulocytes: 9.03 % , AST: 67.9 U/l , GGTP: 95.4 U/l , LDH: 1 380.3 U/l , bilirubin.5.30 mg/dl, bilirubin direct 1.32 mg/dl , hypercholesterolemia and hyperlipidemia, antinuclear antibodies (ANA3), lupus anticoagulant (LA) were not detected, heterogous MTHFR 677T and hyperhomocysteinemia (20.90 umol/l), ADAMTS 13 activity < 2% and ADAMTS13 inhibitor 41U/ml. Decompensated hypertension and chronic nicotinism.

Results: A 4-week management consisted in administration of : 27 FFP plasmaphereses (1 epileptic attack Grand-Mal was recorded/maintained after 2-nd procedure), steroids (Prednisone, Methyl-Prednisolone), red blood cell infusions-2 units. ADAMTS13 activity increased from 2 to 62% and the inhibitor decreased from 41 to 12U/ml. Remission was observed for the last 58 months. Nowadays: ADAMS 13 activity (44.0%), no hyperhomocysteinemia, hypercholesterolemia, hyperlipidemia and arterial hypertension is under control.

Conclusions: In patients with TTP/aTTPa, heterozygous MTHFR C677T mutation, hyperhomocysteinemia, hypercholesterolemia, hyperlipidemia and uncontrolled hypertension. the plasmapheresis procedure and steroids prove to be an effective therapy.

PB 2293 | External Validation of Generic and Cancer-Specific Risk Stratification Tools for Predicting 30-day Mortality in Patients Presenting with Pulmonary Embolism and Active Cancer

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Background: Numerous risk stratification tools exist to predict early post-pulmonary embolism (PE) mortality; however, few were specifically designed for use in cancer patients.

Aims: To evaluate 3 cancer-specific (Registro Informatizado de la Enfermedad TromboEmbólica [RIETE], POMPE-C and criteria by Font et al.) and 3 generic (Hestia, Pulmonary Embolism Severity Index [PESI] and Geneva Prognostic Score [GPS]) risk stratification tools for predicting 30-day post-PE mortality in patients with active cancer.

Methods: We identified consecutive, adult, objectively confirmed PE patients with active cancer presenting to our institution from 11/2010-1/2014. We calculated the proportion of patients categorized as low- or high-risk by each of the 6 risk stratification tools and determined each tools' accuracy for predicting 30-day all-cause mortality.

Results: A total of 124 patients with PE and active cancer (mean age 66.2 years, 46.0% with concurrent DVT, 49.2% with metastatic disease and 46.8%, 16.9% and 11.3% receiving chemotherapy, radiation or both, respectively) were included. Mortality at 30-days occurred in 25 (20.2%) patients. The cancer-specific tools (POMPE-C, RIETE and the criteria by Font et al.) categorized between ~32-43% of patients as low-risk and displayed sensitivities and specificities of 88.0-96.0% and 38.4-52.5%, respectively. The generic PESI and Hestia tools also had sensitivities >96.0% but classified < 19% of patients as low-risk. Specificity of these tools were low (PESI=6.1%; Hestia=23.2%). Although GPS classified 43.5% of patients as low-risk, it did so with a sensitivity of 52.0% and specificity of 42.4%.

Conclusions: When risk stratifying newly diagnosed PE in patients with active cancer, cancer-specific tools appeared to exhibit better

TABLE 1 Prognostic Test Characteristics for 30-Day Mortality

Characteristic	RIETE	POMPE-C	Font	Hestia	PESI	GPS
Low-Risk n (%)	53 (42.7%)	40 (32.3%)	51 (41.1%)	23 (18.5%)	7 (5.6%)	54 (43.5%)
Low-Risk Mortality n/N (%)	1/53 (1.9%)	2/40 (5.0%)	3/51 (5.9%)	0/23 (0%)	1/7 (14.3%)	12/54 (22.2%)
High-Risk Mortality n/N (%)	24/71 (33.8%)	23/84 (27.4%)	22/73 (30.1%)	25/101 (24.8%)	24/117 (20.5%)	13/70 (18.6%)
Sensitivity (95%CI)	96.0% (77.7-99.8%)	92.0% (72.5-98.6%)	88.0% (67.7-96.8%)	100% (83.4-100%)	96.0% (77.7-99.8%)	52.0% (31.8-71.7%)
Specificity (95%CI)	52.5% (42.3-62.6%)	38.4% (28.9-48.7%)	48.5% (38.4-58.7%)	23.2% (15.6-33.0%)	6.1% (2.5-13.2%)	42.4% (32.7-52.8%)
NPV (95%CI)	98.1% (88.6-99.9%)	95.0% (81.8-99.1%)	94.1% (82.8-98.5%)	100% (82.2-100%)	85.7% (42.0-99.2%)	77.8% (64.1-87.5%)
PPV (95%CI)	33.8% (23.3-46.1%)	27.4% (18.5-38.4%)	30.1% (20.2-42.1%)	24.8% (16.9-34.5%)	20.5% (13.8-29.2%)	18.6% (10.6-30.0%)
C-statistic (95%CI)	0.774 (0.679-0.870)	0.806 (0.713-0.899)	0.723 (0.616-0.813)	0.725 (0.623-0.827)	0.754 (0.630-0.878)	0.483 (0.353-0.614)

prognostic accuracy than their generic counterparts. POMPE-C, RIETE and the criteria by Font et al. identified a substantially greater proportion of these PE patients likely to survive to 30-days with comparable sensitivity to the generic tools.

PB 2294 | Predictors of 30-day Mortality among Patients with Cancer-associated Thrombosis in the RIETE Database

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Background: Venous Thromboembolism (VTE) is a predictor of fatality among patients with cancer. Identifying who are the pts at the highest risk of 30 d mortality after a cancer-associated thrombosis (CAT), may help us stratify treatment options.

Aims: To derive and validate the variables present at CAT diagnosis which predict 30-day mortality.

Methods: We selected patients with CAT in the RIETE database. The main outcome was mortality within 30d. We analyzed as potential predictors prospectively collected variables including: demographic, comorbidities, cancer specific (type, metastatic, treatment), VTE (location, recurrent), and baseline lab values. We randomly split the database into a derivation (2/3) and validation (1/3) cohort. Data are expressed as mean and percentages, continuous variables categorized in quartiles. To simplify the analysis of high level interactions, we used recursive partitioning and amalgamation (RECPAM) to select groups at risk. We used SPSS 23 for the analysis.

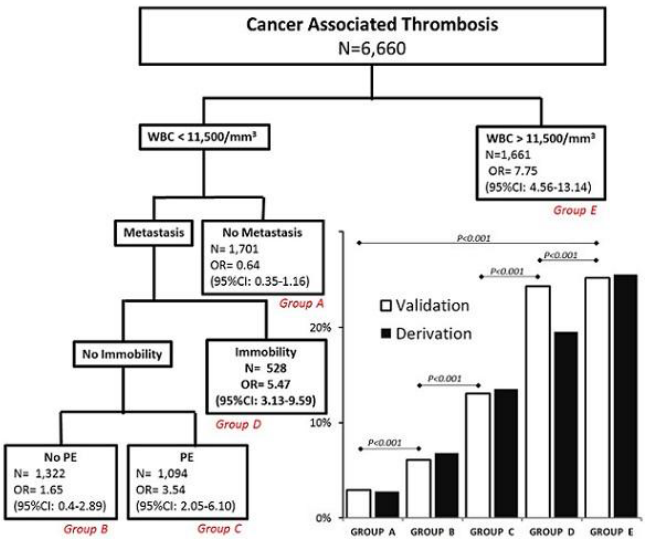

FIGURE 1 Derivation RECPAM and validation mortality rates

Results: In September 2016, there were 10,025 CAT pts (mean age 67, female 47%, metastatic 63%). There were 1,911 (19%) immobile pts for non-surgical reasons at least 4 d prior to diagnosis. The derivation cohort included 6,660 pts of which 827 (12.4%) died within 1 mo. The following variables remained as predictors: WBC > 75th percentile, immobility, metastasis, and pulmonary embolism. RECPAM helped us define 5 risk groups (Fig1). In the validation cohort (n=2,936), 429 (12.7%) of the pts died < 30d. In both the derivation and validation cohorts, there was a significant, incremental risk of death predictable by findings at CAT presentation. The highest risk of death at 30d was among pts with high white count (25%, OR 4.6-13.4) and the lowest probability of death was among pts with no metastases and normal white count (3%, OR 0.4-1.16).

Conclusions: Elevated WBC is more important in predicting CAT early mortality than the combination of PE, immobility, and metastasis.

PB 2295 | Thrombosis after Orthotopic Liver Transplantation for Hepatocellular Carcinoma: Incidence, Implications and Risk Factors

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Background: Whether thrombosis influences the prognosis of patients with hepatocellular carcinoma (HCC) undergoing orthotopic liver transplantation (OLT) is unknown, as well as the role of inherited thrombophilia in its development.

Aims: To estimate the incidence rate (IR) of thrombosis after OLT for HCC, its influence on mortality and re-transplantation and the role of factor V Leiden (FVL) or prothrombin G20210A mutation, both in donors and recipients.

Methods: Historical cohort of HCC patients enlisted for OLT between 2001 and 2013 in two Northern Italian Liver Units and referred to the North Italy Transplant Program allocation agency. IR of thrombosis, re-transplantation and mortality after OLT were calculated. Hazard ratios (HR) were used to compare patients with and without thrombosis. In a nested case-control study (case:control ratio of 1:3) the association between thrombosis and inherited thrombophilia in donors and recipients was estimated as odds ratio (OR) with 95% confidence intervals (CI).

Results: Of the 460 patients (88% men, mean age 52 yrs) included in the cohort, 430 underwent OLT. During a median follow-up time of 7.2 yrs, 26 recipients (6%) developed thrombosis (19 venous and 7 arterial) for an IR of 1.06 (95%CI 0.71-1.53) per 100 pt-yrs. Mortality rate after OLT did not differ between recipients with and without thrombosis (Table 1). Re-transplantation, conversely, was more common in patients with thrombosis (HR 2.50, 95%CI 0.87-7.17).

The risk of thrombosis was higher in recipients with thrombophilia than in those without (OR 4.23, 95%CI 0.99-18.04), especially when the analysis was restricted to venous thrombosis (Table 2).

The presence of inherited thrombophilia in the donor did not increase the risk of thrombosis.

Conclusions: Thrombosis after OLT for HCC increases the risk of re-transplantation but not mortality. The risk of thrombosis is increased when FVL or prothrombin G20210A mutation is present in the graft recipient but not in the donor.

TABLE 1 IRs of mortality and need of re-transplantation. Adj.HR, hazard ratio adjusted for age at OLT, sex and year of OLT.

	N.	pts-yr	IR per 100 pts-yr (95% CI)	IR ratio	Adj.HR
Mortality after OLT					
All recipients	101	2557.41	3.95 (3.22-4.79)		
without thrombosis	95	2402.34	3.95 (3.19-4.83)	Ref.	Ref.
with thrombosis	6	155.07	3.87 (1.42-8.42)	0.98 (0.43-2.23)	0.92 (0.41-2.11)
Planned re-transplantation					
All recipients	33	2440.96	1.35 (0.93-1.89)		
without thrombosis	28	2312.92	1.21 (0.80-1.75)	Ref.	Ref.
with thrombosis	5	128.04	3.91(1.27-9.11)	3.23 (1.25-8.35)	2.50 (0.87-7.17)

TABLE 2 Association between thrombophilia in the recipients and the risk of thrombosis. Adj.OR, odds ratio adjusted for age at OLT, sex and BMI.

	Cases n=26	Controls n= 78	Crude OR (95% CI)	Adj.OR (95% CI)
All thrombosis				
Thrombophilia negative, n (%)	21 (80.8)	74 (94.9)	Ref.	Ref.
Thrombophilia positive, n (%)	5 (19.2)	4 (5.1)	4.23 (0.99-18.04)	4.25 (0.95-18.96)
Venous thrombosis				
Thrombophilia negative, n (%)	14 (74)	74 (95)	Ref.	Ref.
Thrombophilia positive, n (%)	5 (26)	4 (5)	6.26 (1.19-32.85)	7.46 (1.26-43.99)

PB 2296 | Predicting the Risk of Recurrent Venous Thromboembolism in Patients with Cancer: The REMARK Study

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Background: Patients with cancer-associated venous thromboembolism (VTE) often fail (recur) on low-molecular-weight heparin (LMWH). Procoagulant markers may identify high-risk patients and then be used to guide decisions about intensifying anticoagulation.

Aims: To evaluate whether serial measurements of procoagulant markers can identify patients at high risk of recurrent VTE.

Methods: A multicenter, prospective cohort study in which cancer patients with acute symptomatic VTE were enrolled. Patients received standard LMWH therapy. Treatment failure was defined as an extension of VTE, a new VTE, or fatal PE. D-dimer and soluble P-selectin levels were measured at baseline, 1, 4, 5, 12 and 24 weeks post treatment initiation. The relationships between treatment failure and the Louzada risk score, baseline values of the biomarkers, and individual relative changes from baseline were assessed.

Results: We enrolled 117 patients with 19 different cancers (22% lung, 21% colorectal, 9% breast), a mean age of 63 years, equal men and women; 62% had distant metastases. Eleven (9.4%) had recurrent VTE and 23 (20%) died, including 3 fatal PEs and 1 fatal bleeding. VTE recurrence rates were 7.3% with a Louzada score of 0, 8.7% with a score of 1, and 20% with a score of 2 (Figure 1). Median baseline P-selectin levels were higher in patients who recurred (P=0.01), but not D-dimer levels (P=0.12). The area under the ROC-curve was 0.64 (95% CI 0.57-0.81) for baseline D-dimer levels and 0.75 (95% CI 0.60-0.90) for baseline P-selectin levels (Figure 2).

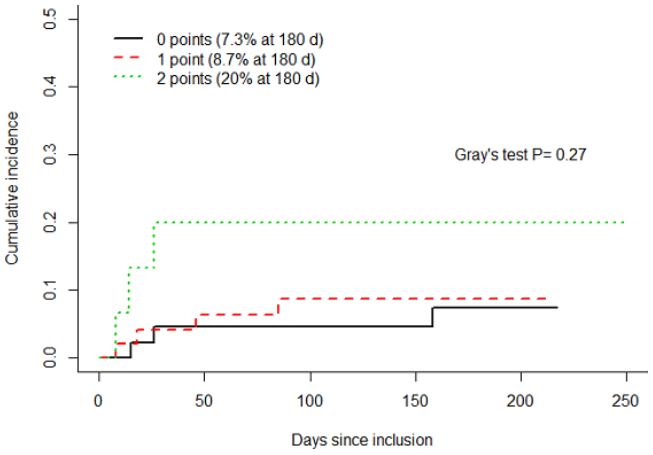

FIGURE 1 Risk of recurrent venous thromboembolism stratified by Louzada score

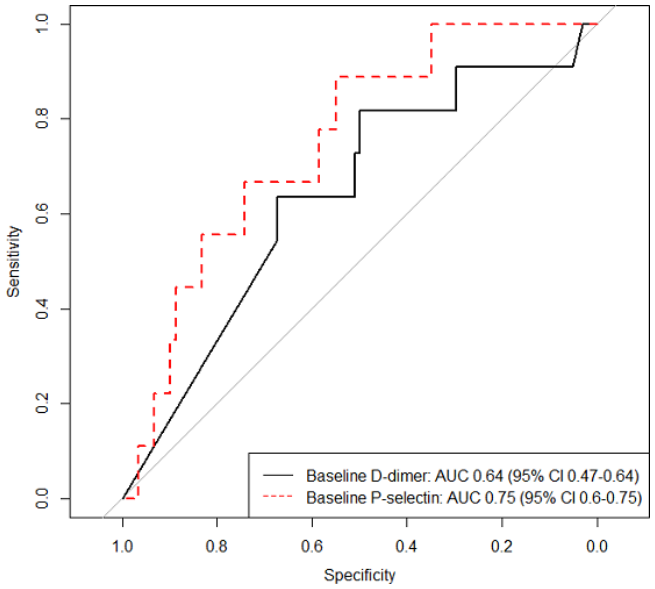

FIGURE 2 ROC-curves for baseline biomarker levels

P-selectin levels at baseline were independently associated with recurrent VTE in competing risk regression analysis, conditional on the risk score (P< 0.01). The decrease in levels of D-dimer and P-selectin from baseline during treatment was not associated with recurrent VTE.

Conclusions: Baseline P-selectin but not D-dimer predict recurrent VTE and may be a valuable addition to clinical prediction rules to select patients for more intensive therapy or closer observation.

PB 2297 | Comparative Analysis of Predictive Models for Thromboembolic Events in Lymphoma Patients

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Background: The models that evaluate potential thromboembolic risk in lymphoma patients in particular are quite limited.

Aims: In this study we compared diagnostic performance of suggested predictive models for general cancer population /Khorana and Padua score/ and Thrombosis lymphoma /Throly/ score that is specific for lymphoma patients.

Methods: The study population included 1820 eligible lymphoma patients who were treated in the clinics of hematology Clinical Center Serbia, Belgrade and Clinical Center Kragujevac during 9 years period.

Initially, the study population was divided based on a split-sample random method into the model derivation and validation cohorts.

Patient's characterics were evaluated by univariate logistic regression analysis, while the final ThroLy model was developed using a stepwise multivariate logistic regression analysis.

Results: In analyzed population ninety-nine patients (5.4%) developed thromboembolic events. Cohorts were balanced regarding the incidence of events (5.3% and 5.5% in derivation and validation cohort, respectively). The variables included in ThroLy score were: previous venous and/or arterial events, mediastinal involvement, BMI>30 kg/m^2, reduced mobility, extranodal localization, development of neutropenia and hemoglobin level < 100g/L. Based on the risk model score, the population was divided into: low (score 0-1), intermediate (score 2-3), and high (score >3). For patients classified *at risk* according to ThroLy score (intermediate and high-risk scores) in derivation cohort, the model produced negative predictive value of 98.5%, positive predictive value (PPV) of 25.1%, sensitivity of 75.4%, and specificity of 87.5%. In validation cohort PPV for Throly score was 28.9%. Padua and Khorana score had PPV of 15.5% and 14.8% in derivation, and 11.5% and 14.8% in validation cohort, respectively.

Conclusions: ThroLy score is more specific for lymphoma patients than Padua and Khorana score but external validation is required.

PB 2298 | Outcomes of Subsegmental Pulmonary Embolism (SSPE) in Cancer Population

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Background: Acute pulmonary embolism (PE) is a significant cause of morbidity and mortality among cancer patients. Modern computed tomographic (CT) scans and CT-pulmonary angiography (CT-PA) has allowed for better visualization of the peripheral pulmonary arteries, resulting in greater detection and incidence of subsegmental pulmonary embolism (SSPE). The clinical significance of SSPE remains unclear, especially among the cancer population.

Aims: To evaluate the incidence of recurrent PE within 6 months of SSPE and overall survival of cancer patients with SSPE.

Methods: We performed a retrospective analysis (2014-2015) of adult cancer patients followed at MD Anderson Cancer Center who were diagnosed with SSPE. The primary outcome was PE recurrence rate within 6 months of SSPE diagnosis. The secondary outcome was overall survival (OS) stratified by SSPE treatment, adjusted for performance status (PS) and tumor stage (TS), and PE recurrence. The study was approved by IRB with consent waiver.

Results: Among 128 patients, 64.1% were male, 75.5% with stage IV disease, and 57.8% were on chemotherapy at time of SSPE diagnosis. The majority of SSPE (73.4%) were diagnosed incidentally by staging CT vs. 26.6% by CT-PA (Table 1).

TABLE 1 Radiographic characteristics of 128 SSPE cancer patients

Characteristic	N	%
Number of SSPE		
- Single	90	70.3
Number of lobes involved		
- Unilobar	98	76.6
Location		
- Unilateral, right	82	64.1
- Unilateral, left	24	18.7
- Bilateral	22	17.2
RV/LV ratio >1	7	5.5

A total of 99 patients (77.3%) were treated with anticoagulation alone, 7 patients (5.5%) had IVC filter placement, 9 patients (7.0%) received both anticoagulation and IVC filter, and 13 patients (10.2%) had no therapy. At 6-month follow-up, 7 of 128 patients (5.5%) had PE recurrence. There was no significant effect of anticoagulation in OS, after adjustment for PS and TS (P=0.11), or by PE recurrence status (P=0.53).

Conclusions: The study showed a similar PE recurrence rate among cancer patients with SSPE treated with anticoagulation when compared to PE outcomes in cancer thrombosis randomized trials. The effect of therapies other than anticoagulation on PE recurrence could not be assessed due to a small number of cases in those subgroups. Anticoagulation did not seem to impact OS in this population.

PB 2299 | Screening for Cancer in Patients with Unprovoked Venous Thromboembolism: A Systematic Review and Individual Patient Data Meta-Analysis

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Background: Unprovoked venous thromboembolism (VTE) may be the first sign of occult cancer. Screening is therefore often considered in these patients. However, precise risk estimates are needed as well as better information on the effect of screening strategies and age on occult cancer diagnosis.

Aims: To evaluate the risk of occult cancer overall and in subgroups by age and type of screening received.

Methods: A systematic review searching MEDLINE, CENTRAL, and Embase until January 2016 was performed. Individual patient data was obtained from the prospective studies that have evaluated occult cancer screening strategies in patients with unprovoked VTE and have followed patients for a minimum of 12 months. Risk of bias was assessed using the QUADAS-2 tool. Risk estimates were obtained in one-stage random effect meta-analyses.

Results: Ten eligible studies were identified and individual data were obtained for 2,316 patients. The mean age was 60 years and 1,334 patients (58%) underwent an extensive screening strategy (Table). The risk of a cancer diagnosis was 3.5% (95% CI 2.8-4.5%) at initial screening, 1.6% (95% CI 1.0-2.6%) between screening and 12 months, and 1.0% (95% CI 0.56 to 1.9%) between 12 and 24 months. At screening, cancers were most often diagnosed by medical history and physical examination (37%). The most frequently diagnosed cancers were colorectal (17%), lung (14%), and pancreatic cancer (9%). Of the solid cancers, 41% were early-stage cancers (i.e. stage 0, 1, or 2). An extensive screening strategy at entry was associated with a two-fold increased rate of occult cancer detection (odds ratio 2.0, 95% CI 1.1-3.4). The 12-month risk of cancer diagnosis increased linearly with age (Figure).

Conclusions: Occult cancer is detected in 5.2% of patients within 12 months of an unprovoked VTE diagnosis. Extensive screening detected significantly more cancers than a more limited screening. Whether this translates into improved outcome remains to be elucidated.

TABLE 1 Patient characteristics

	N=2,316
Age, mean (SD), y	60 (15)
Male, n (%)	1,416 (61)
Index VTE	
PE with or without DVT, n (%)	1,082 (47)
Lower extremity DVT, n (%)	1,216 (52)
Upper extremity DVT, n (%)	16 (0.7)
Current of former smoker, n (%)	901 (39)
Previous VTE, n (%)	192 (8.3)
Estrogen use, n (%)	156 (6.7)

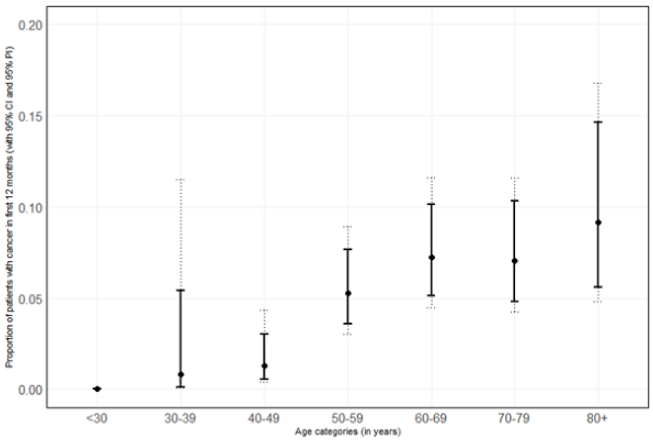

FIGURE 1 12-month risk of occult cancer detection in different age cohorts

PB 2300 | Cancer Cell Derived Extracellular Vesicles Increase Extracellular Vesicle Dependent Tissue Factor Activity of Peripheral Blood Mononuclear Cells in the Presence of Platelets

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Background: Tissue factor bearing extracellular vesicles (TF+EV) are thought to be involved in the pathogenesis of cancer-related coagulopathy and thrombosis in certain cancer types. However, the complete pathomechanism and the contributing cells remain to be clarified. In addition to cancer cells, previous studies showed that circulating monocytes express TF under certain circumstances and release EV into the blood.

Aims: The aim of this study was to investigate whether cancer cell derived EV can induce the release of TF+EV by peripheral blood mononuclear cells (PBMC) *in vitro*. Further, the effect of platelets on the release of TF+EV was investigated.

Methods: PBMCs and platelets of healthy donors (n=5) were isolated and co-incubated with supernatant containing EV of a TF+ prostate cancer cell line (DU145). The EV-TF activity in the collected supernatant of each experiment was determined with a factor Xa generation assay.

Results: The EV-TF activity (pg/ml) of PBMCs alone (mean±SD: 0.61±0.77), PBMCs plus platelets (0.25±0.48) and of platelets stimulated with cancer cell derived EVs (0.84±0.11) was low. Increased EV-TF activity was found in the supernatant of PBMCs stimulated with cancer cell derived EVs (1.54±0.63, p=0.039). The co-incubation of PBMCs with platelets and cancer cell derived EVs resulted in a further increase in EV-TF activity (4.84±1.13) compared to PBMCs incubated with cancer cell derived EVs (p=0.003) and to platelets incubated with cancer cell derived EVs (p=0.010).

Conclusions: EVs derived from a cancer cell line increase the extracellular vesicle dependent TF activity of PBMCs and this effect is

enhanced in the presence of platelets. This observation supports an interrelation of cancer cells with platelets and monocytes in activating coagulation in cancer.

PB 2301 | Impact of Regular Physical Activity on the Risk of Cancer-related Venous Thromboembolism - The Scandinavian Thrombosis and Cancer (STAC) Cohort

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Background: Venous thromboembolism (VTE) is a common complication and a predictor of poor prognosis in cancer. Growing evidence suggests that regular physical activity (PA) may reduce the risk of VTE in the general population, but the effect on cancer-related VTE is unknown.

Aims: To investigate the impact of PA on the risk of cancer-related VTE in a population-based cohort.

Methods: A total of 137 780 subjects from the STAC cohort (comprising data from the Tromsø Study, the Nord-Trøndelag Health Study, and the Danish Diet, Cancer and Health Study), were included. Incident cancer and VTE were registered from the date of inclusion (1993-97) until study end (2007-12). Levels of moderate and high intensity PA were dichotomized (≥1 vs < 1 h/week), and hazard ratios (HRs) adjusted for age, sex and BMI were calculated using Cox regression. Cancer-free subjects with PA < 1h/week were used as reference. VTEs occurring from 6 months before to 2 years after a cancer diagnosis were defined as cancer-related. VTEs were classified as unprovoked or provoked based on the presence of provoking factors other than cancer.

Results: There were 2218 VTEs and 13374 cancers during a median follow-up of 11.8 years. Overall, the risk of VTE was 8.5-fold higher in cancer patients (HR 8.5; 95% CI 7.6-9.5) compared to those without cancer, and it was not influenced by the level of PA at baseline. However, in subjects aged < 60 years, the HR of VTE was lower in those engaging in PA ≥1h/week (HR 10.9; 95% CI 8.1-14.8) compared to those engaging in PA < 1h/week (HR 13.5; 95% CI 11.2-16.2). The impact of PA on the risk of VTE among cancer patients was driven by an effect on unprovoked events (HR for PA ≥1h/week 8.9; 95% CI 6.0-13.4 vs HR for PA < 1h/week 12.6; 95% CI 10.0-15.9), with essentially similar effects for deep vein thrombosis (DVT) and pulmonary embolism (PE).

Conclusions: Our findings suggest that moderate and high intensity PA ≥1h/week may affect the risk of VTE among cancer patients, particularly unprovoked VTE, in middle-aged subjects.

PB 2302 | Immunomodulatory Changes at the Tumor Microenvironment Induced by a Kunitz-type Inhibitor Improve Antitumoral Activity CD8$^+$T-dependent Manner on Orthotopic Kidney Tumor Model

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Background: A Kunitz-type inhibitor domain-containing protein, isolated from the salivary glands of the *Amblyomma cajennense* tick, termed Amblyomin-X, which inhibit coagulation factors, angiogenesis and triggers apoptosis in murine renal adenocarcinoma cells (Renca) by inhibiting the proteasome and endoplasmic reticulum stress.

Aims: Investigation of the immunologic role associated with inhibition of angiogenic factors in the clearance of renal metastasis in lungs.

Methods: Tumor affinity by Amblyomin-X was assessed using orthotopic kidney tumors model, developed in BALB/c by *in vivo* imaging. Its functional and phenotypic immune infiltrate in the tumor microenvironment, apoptosis and cell cycle arrest were evaluated using CBA techniques, flow cytometry and immunohistochemistry. In addition, this model was applied to NSG mice without Amblyomin-X treatment and splenic CD8 T cells from BALB/c mice were adoptively transferred.

Results: herein, we have demonstrated that Amblyomin-X drastically reduced the incidence of lung metastases by inducing cell cycle arrest and apoptosis. In addition, Amblyomin-X treatment modulated cells-immune response and inflammatory cytokines. It increased and activated of CD8T and decreased MDSC´s cells in tumor microenvironment and improves IFNγ and TNFα, as well as decreased IL17 levels. No changes were observed in the IL6 levels. In the *in vivo* imaging analyses, we observed the co-localization of Amblyomin-X with Renca tumor mass for longer time compared to tumor free animal. In NSG mice, Amblyomin-X have shown minor antitumor effect, which was recovered with adoptive CD8$^+$T cells transfer.

Conclusions: In summary, Amblyomin-X has shown antitumor activity with high affinity for tumor cells and dependent on the recruitment of immunologic system to amplify its antitumor property, indicating a strong potential toward a safe drug against cancer and associated thrombosis.

PB 2303 | Elevated Risk of Venous Thrombosis Post Allogeneic Transplantation for Leukemia: Patient Characteristics of a Single Centre Cohort

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Background: Leukemia patients undergoing allogeneic hematopoietic stem cell transplant are at increased risk of venous thromboembolism (VTE) due to need for central venous catheter for supportive care, chemotherapy and graft versus host disease(GVHD). This specific post-transplant population is poorly described.

Aims: We sought to describe the rate of events and patient characteristics of individuals who develop VTE in the post allogeneic hematopoietic stem cell transplant period in a cohort of leukemia patients.

Methods: The study was approved by the Ottawa Health Science Network Research Ethics Board. We retrospectively reviewed the charts of all patients diagnosed with acute leukemia at the Ottawa Hospital from January 2007 to September 2015. Patients' demographics, details of transplant and thrombosis characteristics were added to a database of all leukemia patients diagnosed by our centre.

Results: 166 leukemia patients underwent allogeneic bone marrow transplantation. There were 46 venous thrombosis events (27.7%; 95% confidence intervals (CI): 21.1 to 35.2%). Of these events, 15/166 (9.0%; 95%CI: 5.1 to 14.5%) of the transplant population developed a VTE in the post-transplant period. Descriptive statistics are depicted in Table 1.

TABLE 1 Characteristics of patients developing post allogeneic hematopoietic stem cell transplant thrombosis. N=15

	N	%		N	%
Gender			Leukemia Type		
Male	11	73.0	AML	9	60.0
Female	4	27.0	ALL	5	33.3
			Ambiguous Lineage	1	6.7
Mean Age (sd)		47.6 (16.2)			
BMI (sd)		26.07 (3.17)	Stem Cell Source		
			HLA Matched Unrelated Donor	10	66.7
			HLA Matched Related Donor	4	26.7
			HLA Mismatch 7/8 Unrelated Donor	1	6.7

11/15 (73%) of the patients with post allogeneic hematopoietic stem cell transplant VTE had either acute or chronic GVHD. The median time from transplant to diagnosis of VTE was 206 days(range 0-2498 days). There were 6 leukemia relapses post allogeneic hematopoietic stem cell transplant in this population of 15 patients who developed VTE. Thrombosis predated the diagnosis of relapse in 5/6 cases by a mean of 3.35 months (standard deviation 2.20). The site of VTE and first vs recurrent event is depicted in Table 2.

TABLE 2 Site of VTE and First vs. Recurrente VTE event N=15

Site of Thrombosis	N	%	Post Transplant VTE	N	%
PE	1	6.7	First VTE event	6	40.0
DVT	6	40.0	Recurrent VTE	9	60.0
PE+DVT	2	13.3			
Upper Extremity DVT	2	13.3	On DVT prophylaxis at the time of VTE	1	6.7
Upper Extremity Superficial thrombosis	3	20.0			
Portal Vein Thrombosis	1	6.7			

Conclusions: There is a high rate of VTE in the post allogeneic hematopoietic stem cell transplant leukemia population. GVHD is present in a high number of those that develop VTE. Long term prophylaxis strategies in this high risk population should be investigated.

PB 2304 | Combination of Podoplanin Expression and IDH-1 Mutation Status in Primary Malignant Brain Tumors Identifies Patients at Risk of Venous Thromboembolism

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Background: Venous thromboembolism (VTE) is common in patients with primary malignant brain tumors. Recently, independent studies have reported intratumoral podoplanin expression and IDH-1 mutation status as novel risk predictors for VTE in these patients. However, the interrelation between podoplanin and IDH-1 mutation and their combined effect on risk of VTE has so far not been investigated.

Aims: We explored the association of podoplanin with IDH-1 mutation status and their combined effect on VTE risk assessment in patients with primary malignant brain tumors.

Methods: Immunohistochemical staining against podoplanin and IDH-1-R132H was performed in brain tumor specimens of 213 adult patients (mostly high-grade gliomas [89%]) included in the Vienna Cancer and Thrombosis Study (CATS), a prospective observational

cohort study of patients with newly diagnosed cancer or progressive disease. Primary endpoint was symptomatic VTE within two years.

Results: Eighty (37.6%) tumor specimens stained medium or highly positive for podoplanin. IDH-1 mutation was found in 42 (19.7%) tumors. IDH-1-R132H mutation was only found in podoplanin negative tumors, while all podoplanin positive tumors were IDH-1-R132H wildtype (wt) (Figure 1A).

During follow-up, 29 (13.6%) patients developed VTE. Expression of podoplanin was associated with a high risk of VTE, while mutant IDH-1-R12H was associated with a very low risk for VTE. In competing risk analysis, a combined variable of podoplanin and IDH-1 mutation predicted risk of VTE (Figure 1B). This association was independent of tumor type: subhazard ratio (SHR) for podoplanin positive plus IDH-1-R132H wt tumors, compared to podoplanin negative plus IDH-1-R132H mutant tumors: 4.69, 95%CI: 1.28-17.17; p=0.020).

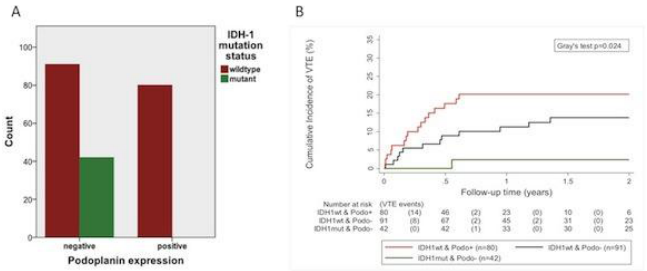

FIGURE 1 (A) IDH-1 R132H mutation according to podoplanin expression. (B) Incidence of VTE according to podoplanin expression and IDH-1 R132H mutation

Conclusions: IDH-1-R132H mutation is only detected in tumors, which are podoplanin negative. Determination of podoplanin and IDH-1-R132H mutation in primary malignant brain tumors identifies patients with both very high and very low risk of developing VTE.

PB 2305 | Podoplanin Promotes Oral Cancer Malignancy by Inducing Intravascular Coagulation in Orthotopic Xenograft Mice Model

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Background: Tumor-associated thrombosis is a lethal cause in patients with cancer. Podoplanin (PDPN), a small transmembrane glycoprotein, mediated tumor cell-induced platelets aggregation (TCIPA) has been studied in different cancer types. Emerging data also indicated that PDPN is a malignant indicator of human oral squamous cell carcinoma (OSCC).

Aims: To define whether and how PDPN promotes OSCC malignant progression.

Methods: A *luciferase*-expressing PDPN positive (P^+Luc^+) OECM-1 cells was used as our study model. P^+Luc^+shLacZ and P^+Luc^+shPDPN sublines were established by introducing short-hairpin RNA targeting on *b-galactosidase* or *pdpn into* P^+Luc^+ cells, respectively. The cell growth rate and the ability to induce platelet aggregation of these cell lines were analyzed *in vitro*. An orthotopic xenograft animal model was established for functional analysis of PDPN in OSCC. The tumor growth rate was analyzed by *in vivo* image system (IVIS). Indicators of tumor-associated thrombosis were analyzed by immunofluorescence staining for tumor sections and enzyme-linked immunosorbent assay (ELISA) for plasma samples.

Results: *In vitro* analysis revealed no difference for the cell growth or the ability to induce TCIPA of shLacZ and shPDPN cells. In IVIS analysis, the tumor growth rate also had no difference between shLacZ and shPDPN tumor ($p > 0.05$). However, the survival time of the mice bearing shPDPN tumors prolonged when compared to shLacZ tumors ($p < 0.001$). Notably, 29.4% (5/17) of mice bearing shLacZ tumor died suddenly, without any signs of deterioration. Comparing to mice bearing shPDPN tumor, mice with shLacZ tumor was associated with a 2.4-fold increase of platelet infiltration to tumor tissue ($p < 0.05$) and a 1.4-fold increase in the plasma levels of thrombin-anti-thrombin complex ($p < 0.05$).

Conclusions: PDPN promotes disease progression by inducing tumor cells-platelet interactions and intravascular coagulation, which reduce lifespan of the mice with OSCC tumors.

PB 2306 | Thrombin Generation in Patients with Active Multiple Myeloma and Impact of Immunomodulators. The ROADMAP Study

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Background: Multiple myeloma (MM) and the associated immunomodulatory (IMiD) treatments are associated with an incresed risk of vascular complications. Thrombin generation (TG) assessment reflects the equilibrium between procoagulant and anticoagulant potenties in plasma.

Aims: We conducted a multicenter study, to explore the relationship between stages of MM and alterations of TG profile.

Methods: MM patients (n=129) were recruited from July 2014 to December 2016 and stratified to the following groups: 44 newly

diagnosed treatment naïve patients (ND), 33 patients receiving IMiDs (IM), 45 patients in complete remission (CR) and 7 patients in partial remission on IMiDs (PR/IM). Patients receiving anticoagulant treatment were excluded The control group (CG) consisted of 30 healthy age and sex-matched individuals. Samples of platelet-poor plasma (PPP) were assessed for thrombin generation (TG) with the TF 5pMPPP-Reagent® on Calibrated Automated Thrombogram (Stago,Asnières,France).TG parameters such as Peak, Endogenous Thrombin Potential (ETP) and velocity (MRI) were studied.The upper and lower normal limits (LNL and UNL) were calculated by the mean±2SD.

Results: Patients with ongoing MM (ND, IM, PR/IM) had significantly lower Peak, ETP and MRI values as compared to the CG. In contrast Peak, ETP, MRI values were similar in CG and CR. PR patients had lower ETP and MRI values as compared to the CR group (Table 1). In ND 8% had TG >UNL and 20% had TG< LNL. In IM 9% had TG>UNL and 57% had TG< LNL. None in PR/IM had TG>UNL and 33% had TG< LNL. In CR, 28% had TG>UNL and 14% had TG< LNL.

TABLE 1 Thrombogram parameters in patients with MM and controls (*p<0.05 versus Control; +p<0.05 versus IM; $p<0.05 versus ND; £p<0.05 versus CR)

	Peak (nM)	ETP (nMxmin)	MRI (nM/min)
Control (LNL - UNL)	288±36 (211-359)	1496±191 (1114-1880)	110±24 (61-159)
ND	247±55*$	1229±179*$	103±40
IM	233±54*	1292±532*	86±30*
PR/IM	220±85*	1163±415*£	79±31*£
CR	285±79$+	1591±402$+	114±39+

Conclusions: Patients with active MM showed attenuated TG which was enhanced in the presence of IMiD treatment. Complete remission was associated with TG normalization . This evolutive profile might indicate that TG assay reflects vascular aggression in MM patients which is followed by the release of natural coagulation inhibitors such as thrombomodulin, heparin cofactor II, sEPCR and TFPI.

PB 2307 | Impact of the Extensive Cancer Screening in Patients with a First Episode, Unprovoked Venous Thromboembolism: A Systematic Review and Meta-analysis

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Background: Diagnosis of malignancy is estimated to be 10% in patients who presents with unprovoked venous thromboembolism (VTE). The impact of extensive cancer screening on mortality in VTE patients remains controversial.

Aims: To explore the benefit of extensive cancer screening among patients with first episode, unprovoked VTE.

Methods: We performed an updated systematic search in MEDLINE, EMBASE and CENTRAL databases, from January 2015 to October 2016. Search strategies were adapted from the recent Cochrane review (CD010837). We included randomised controlled trials evaluating extensive cancer screening (including computed tomography [CT] or a positron emission tomography [PET] scan) versus standard screening in patients with first episode, unprovoked VTE.

Results: We included four randomized trials with 1,644 participants. Median age ranged from 53.4 to 69.3 years. Cancer-associated death occurred in 10 of 817 (1.2%) in patients undergoing extensive screening and 19/827 (2.3%) in control group (4 studies, RR 0.53, 95%CI, 0.25 to 1.14, I^2=0%, P=0.10), Figure 1. All-cause mortality occurred in 20 of 718 (2.8%) patients in extensive group and 25 of 725 (3.4%) in control (3 studies, RR 0.80, 95%CI, 0.45 to 1.42, I^2=0%, P=0.44), Figure 2. Incidence of early malignancy was 2.7% and 1.3% in extensive and control group (4 studies, RR 1.88, 95%CI, 0.90 to 3.95, I^2=0%, P=0.09). Incidence of advanced malignancy was 4.3% and 4.4% in the extensive and the control group (4 studies, RR 1.18, 95%CI, 0.80 to 1.74, I^2=0%, P=0.95).

Conclusions: In patients with a first episode of unprovoked VTE, extensive cancer screening did not significantly reduce cancer-associated mortality and all-cause morality. The meta-analysis did not show differences in the incidence of cancer among patients underwent the extensive screening as compared to the standard of care. The moderate certainty surrounding the effects of the extensive cancer screening warrants further clinical trials.

PB 2308 | Paradoxal Roles of Platelets in Colorectal Cancer Behavior

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Background: The relationship between thrombosis and cancer is well established. Platelets are able to interact with cancer cells and participate in the development of the tumor and metastasis. To date, Tumor Cell Induced Platelets Aggregation (TCIPA) constitutes the main cellular consequence described following the interactions of platelets and cancer cells.

Aims: We investigated the capacity of colorectal cancer cells to interact independently of TCIPA with platelets and determined the consequences of these interactions *in vivo* during colorectal cancer development.

Methods: The interplay between human and mice platelets and human and mice colorectal cancer cells was analyzed *in vitro* in static and dynamic conditions and *in vivo* by real time intravital microscopy using syngeneic, ectopic or orthotopic, colorectal cancer mice models.

Results: We show, by intratumoral microscopy, that single platelets are present in the microenvironment of a primary tumor in different colorectal cancer models. At the site of the primary tumor, platelets interact with cancer cells mainly through K-cadherin interactions and independent of the TCIPA. This interaction induces the activation, the granule release and the spreading of isolated platelets at the surface of the tumor. This k-cadherin dependent interaction of platelets with cancer cells reduces the growth of the primary tumor. In contrast, when occurring at the lumen of a vessel, this interaction facilitates the binding of cancer cells to the endothelium, mainly through an integrin-dependent pathway.

Conclusions: Our results indicate that platelets play a paradoxal role in tumor behavior: in the tumor microenvironment, platelets reduce tumor growth whereas in the bloodstream platelets facilitate the dissemination of the tumor and the formation of metastasis.

PB 2309 | The Modified Ottawa Score and Clinical Events in Hospitalized Patients with Cancer-associated Thrombosis from the Swiss VTE Registry (SWIVTER)

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Background: The modified Ottawa score (MOS) predicted venous thromboembolism (VTE)
recurrence in a cohort of patients with cancer-associated thrombosis mainly managed on an outpatient basis.

Aims: We assessed the prognostic value of the MOS in hospitalized patients with cancer-associated thrombosis.

Methods: In 383 hospitalized patients with cancer-associated VTE from the SWIss Venous
ThromboEmbolism Registry (SWIVTER), 98 (25%) were classified as low-risk, 175 (46%) as intermediate-risk, and 110 (29%) as high-risk for VTE recurrence based on the MOS. Clinical endpoints were recurrent VTE, fatal VTE, major bleeding, and overall mortality at 90 days, estimated with the Kaplan-Meier method, and compared by use of a log-rank test.

Results: Overall, 179 (47%) patients were female, 172 (45%) had metastatic disease and 72 (19%) prior VTE. The primary site of cancer was lung in 48 (13%) patients and breast in 43 (11%). According to the MOS, the rate of VTE recurrence was 4.1% for low, 6.3% intermediate, and 5.5% high risk (p=0.75), the rate of fatal VTE was 0.8%, 1.9%, and 2.0% (p=0.69), the rate of major bleeding was 3.1%, 4.1%, and 3.6% (p=0.92), and the rate of death was 6.1%, 12.0%, and 28.2% (p< 0.001), respectively. None of the MOS items was associated with VTE recurrence: female gender HR 1.26 (95%CI 0.53-2.96), lung cancer HR 1.17 (95%CI 0.35-3.98), prior VTE HR 0.44 (95%CI 0.10-1.91), breast cancer HR 0.83 (95%CI 0.19-3.58), and absence of metastases HR 0.74 (95%CI 0.31-1.74).

Conclusions: In hospitalized patients with cancer-associated VTE, the modified Ottawa score failed to predict VTE recurrence at 3 months but was associated with early mortality.

PB 2310 | Predictive Value of D-dimers and Microparticle-associated Tissue Factor Activity for the Risk of Thrombosis in Pancreatic Cancer

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Background: Venous Thrombo-embolic Events (VTE) frequently occur in patients with pancreatic cancer and contribute to elevated morbidity and mortality. D-dimers and tissue factor-dependent procoagulant activity of MP (MP-TF) have been proposed to help identifying cancer patients at high risk of thrombosis.

Aims: To compare D-dimer levels and MP-TF in pancreatic cancer and in chronic pancreatitis to determine whether these biomarkers are related to cancer or inflammation and to validate their association with thrombotic risk in a pancreatic cancer population.

Methods: 42 patients with pancreatic cancer, 48 with intraductal papillary mucinous tumor of the pancreas (IPMN), a precancerous lesion, and 50 with chronic pancreatitis were recruited. Plasma D-dimer levels were quantified by immunoturbidimetry (*STA®-Liatest® D-Di*, Diagnostica Stago) and MP-TF was determined by a chromogenic endpoint assay measuring factor Xa generation.

Results: D-dimer levels and MP-TF were significantly higher in cancer patients compared to IPMN or chronic pancreatitis.

TABLE 1 D-dimer levels and MP-TF according to pancreatic disease type

	Pancreatic cancer (n=42)	IPMN (n=48)	Chronic pancreatitis (n=50)	p-value (Chi-square or Kruskal-Wallis)
Age, years, median (IQR)	66 (54-72)	65 (56-71)	47 (39-53)	<0.0001
Gender, male, n (%)	26 (62)	17 (35)	41 (82)	<0.0001
D-dimers, µg/ml, median (IQR)	0.91 (0.57-2.16)	0.27 (0.22-0.44)	0.38 (0.22-0.92)	<0.0001
MP-TF, fM, median (IQR)	24 (11-56)	12 (9-19)	16 (10-23)	0.01

Patients with metastatic cancer (n=26) presented higher D-dimer levels (median [IQR] 1.27 [0.69-2.77] vs 0.56 [0.31-082] µg/ml, p=0.005) and MP-TF (median [IQR] 40 [12-96] vs 12 [9-25] fM, p=0.008) compared to patients with localized lesions (n=18). Cancer patients were followed for a median duration of 159 days [IQR 61-180]. VTE occurred in 9 (20.4%) patients. All had metastatic cancer at the time of thrombosis. In univariate analysis, only elevated D-dimers (cut-off level = 75th percentile 2.16 µg/ml) were significantly associated to the risk of VTE in the next 6 months (HR = 6.2, 95% CI 1.4-27.7, p= 0.017). This was not significant after adjustment for age, sex and metastatic status.

Conclusions: Elevation of D-dimers and MP-TF seems to be related to cancer process and to disseminated cancer stage. D-dimers are associated with the occurrence of future VTE but dependently from metastatic status.

PB 2311 | Venous Thromboembolism Leads to Poor Survival in Patients with High Grade B Cell Lymphoma - A 7-year Single Centre Analysis

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Background: Venous thromboembolism (VTE) is a recognised complication of cancer. High Grade B cell Lymphoma (HGBCL) carries a significant risk of VTE. However, little is known about the prognostic significance and epidemiology of VTE in these patients

Aims: To assess the risk and prognostic significance of VTE in HGBCL.

Methods: 203 unselected patients with HGBCL diagnosed between 1st January 2010 to 31st December 2016. These included DLBCL and Grade 3 Follicular NHL at all sites. VTE was diagnosed either on CT scan or Doppler US as per standard.

Results: 14 developed a VTE ie 6.9%. 8 patients were female and 6 were male. 4 were pulmonary embolism, one of which directly contributed to patient's death; 1 splanchnic vein clot; 3 upper limb DVT; and 6 lower limb DVT. None of the VTE events were related to indwelling lines. VTE occurred at an average 21.6 days after diagnosis (range -23 to 95 days). The mean Stage and LDH were high at 3.4 and 694.9 respectively and were statistically higher than the non VTE group (P= 0.029 and 0.0014 respectively). The mean age of patients with VTE was 67.1 years and those without was 63.9 years with no statistical significance between the two groups. 10 (71.4%) patients with VTE have died. The median survival of VTE patients was 12.5 months whereas those without VTE have not yet reached median survival (Fig1). When only the Non VTE Stages 3 and 4 (mean 3.6) were compared to the VTE cohort, there was still a significantly worse outcome in the VTE group with a median survival of 64.6 months vs 4.4 months respectively; P = < 0.001). LDH was higher in the VTE group (P=0.028).

Conclusions: 6.9% developed VTE. This occurred very early in their disease with a mean of 21.6 days from diagnosis. VTE patients had a very poor overall survival which was not associated with age or Stage. It remains unclear why VTE is so predictive for overall survival in HGBCL.

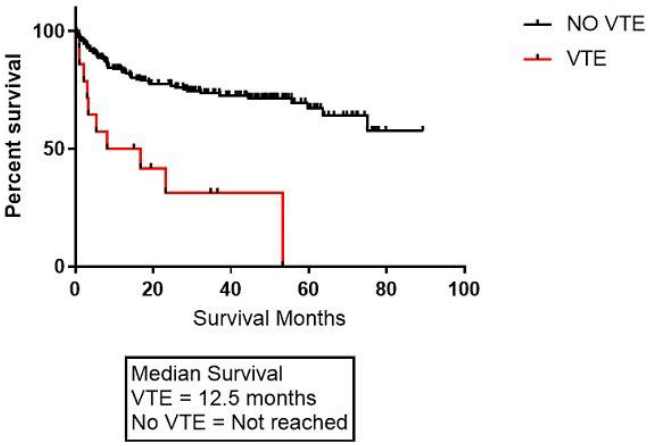

FIGURE 1 Overall Survival in HGBCL patients with VTE and those without VTE

PB 2312 | Performance of Khorana Score Models in Predicting Occurrence of Incidental Venous Thromboembolism (VTE) in Cancer

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Background: VTE is a common complication of cancer and it contributes importantly to morbidity and mortality in this population. The Khorana

score (KS) assesses the risk of developing VTE. In the era of better resolution radiology modalities, clinicians must face the challenge of predicting and managing incidentally found VTE during cancer staging.

Aims: The aim of this study was to evaluate the utility of KS and two modified-Khorana score models in predicting the occurrence of unsuspected VTE in oncology patients.

Methods: The study population included single-institution, prospectively identified ambulatory adult cancer patients scheduled for routine computed tomography (CT) scan of the chest, abdomen and/or pelvis. Exclusion criteria included: prior history of VTE, clinically suspected VTE and/or scheduled for CT scans for suspected VTE, Zubrod performance status score of 4 and current therapeutic anticoagulation therapy. All eligible patient charts were reviewed to assess the KS and two additional previously published KS model modifications. Using non-parametric methods we compared the median of KS, those of the modified models and stratified by incidentally found VTE versus non-VTE patients. Approval by the institutional review board and informed consent from each participant were obtained.

Results: We identified 568 patients with all available information for the calculation of KS and modified models. Of these, 14 had incidental VTE. In the VTE cases, the median score for KS and the modified models were 1.0, 4.0 and 5.0, respectively. KS was unable to predict VTE (p-value of 0.801) whereas the modified models (PROTECHT, Australian) predicted most cases of incidental VTE (Table 1) to be in the "high risk" category, p-value of 0.001 and 0.036, respectively.

TABLE 1 Comparison of Khorana Score (KS) and KS modified models between incidental VTE cases versus controls

Model score	Cases	Controls	Total
Original Khorana score	N (%)	N (%)	N (%)
< 3	13 (2.3%)	536 (94.4%)	549 (96.7)
≥ 3	1 (0.2%)	18 (3.1%)	19 (3.3%)
PROTECHT Model Score			
< 3	2 (0.4%)	267 (47.0%)	269 (47.4%)
≥ 3	12 (2.1%)	287 50.5%)	299 (52.6%)
Australian Model score			
< 3	3 (0.6%)	275 (48.4%)	278 (49.0%)
≥ 3	11 (1.9%)	279 (49.1%)	290 (51.0%)

Conclusions: This study suggests that KS did not predict incidental VTE but adding other characteristics (presence of distant metastases and type of chemotherapy) may improve the performance of KS and potentially identify candidates for VTE prophylaxis.

PB 2315 | Audit: Use of Khorana Score in Newly Diagnosed Patients with Pancreatic Cancer

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Background: Venous Thromboembolism (VTE) is a well-documented cause of morbidity and mortality in cancer patients. Patients with pancreatic cancer are documented as more likely to experience thromboembolic events than other types of cancer and have been proven to benefit from prophylactic low molecular weight heparin (LMWH). The Khorana score has been validated as one means of risk assessing those patients who would benefit from primary prophylaxis; with guidance of low molecular weight heparin (LMWH) if the Khorana score is ≥ 3.

Aims: To audit if pancreatic cancer patients in the Belfast trust were prescribed primary prophylaxis if they had a Khorana score of ≥3.

Methods: This audit retrospectively looked at all cases of pancreatic cancer discussed at the regional hepatobiliary multidisciplinary meeting (MDM) between January 2014 - December 2017. Belfast trust patients were identified and their Khorana score, evidence of VTE prophylaxis and outcome in terms of VTE was gathered from their notes.

Results: 191 patients were identified from the pancreatic MDM; 89 of whom where in the Belfast trust. Of these 12 developed a pulmonary embolism (PE) none of whom were on primary prophylaxis. 7 developed other forms of VTE, only 1 who was on prophylaxis in the form of an anticoagulant for recurrent deep vein thrombosis. 24 patients were identified to have a Khorana score of ≥3 and of these 5 on chemotherapy and therefore to whom the score could apply. Of these 5 none were prescribed primary prophylaxis and 1 developed a PE.

Conclusions: The Khorana score was only applicable to a small number of pancreatic cancer patients, however in these the standard of primary prophylaxis was not met. The Khorana score was not predictive of PE's however of note; of those to whom the standard was applicable 20% developed a PE in this case without prophylaxis.

PB 2316 | Direct Oral Anticoagulants for the Treatment of Acute Venous Thromboembolism in Patients with Cancer: A Meta-analysis of Randomized Controlled Trials

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Background: Previous meta-analyses have assessed the safety and efficacy of the direct oral anticoagulants (DOACs) in patients with cancer associated venous thromboembolism (VTE). However, in the last year, new evidence from subgroup analyses of pivotal clinical trials has become available, providing separate data on patients with a history of cancer and those with active cancer.

Aims: To estimate the efficacy and safety of the DOACs compared with vitamin K antagonists (VKA) for the treatment of VTE in patients with active cancer and in those with a history of cancer.

Methods: After a systematic review of the literaturel, all RCTs comparing DOACs with VKA in the treatment of patients with acute DVT or PE were included. Patients were classified as patients with active cancer, patients with a history of cancer and patients without active cancer or a history of cancer. Pooled risk ratios (RR) and 95% confidence intervals (CI) were calculated using a random effects model.

Results: Of 27,178 patients (13,593 receiving DOACs and 13,585 VKA) included in 6 randomized controlled trials, 1,496 (5.5%) had active cancer, and 1,605 (5.9%) had a history of cancer. DOACs were significantly more effective than VKA in patients with active cancer in preventing recurrent VTE (RR 0.62, 95% CI 0.43, 0.90) and as effective as VKA in patients with a history of cancer (RR 0.50, 95% CI 0.23, 1.07) and in patients without cancer (RR 1.03, 95% CI 0.87, 1.21). The incidence of major bleeding was significantly lower in the DOAC group than in the VKA group in patients without cancer, but not in patients with active cancer or with history of cancer. However, the absence of heterogeneity among different subgroups suggests a similar, better safety profile of DOACs in comparison to VKA in cancer patients.

Conclusions: The results of this meta-analysis suggest a favorable risk-benefit ratio with the use of the DOACs as compared to VKA in patients with VTE associated with active cancer.

PB 2317 | Podoplanin-dependent Platelet Aggregation Mediated by Human Glioblastoma Cell Lines

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Background: Primary brain tumor patients are at high risk to develop venous thromboembolism (VTE). Recently, an association between podoplanin (PDPN)-expressing brain tumors, intratumoral platelet aggregates and increased VTE risk was demonstrated. PDPN is a sialomucin-like glycoprotein with the ability to activate platelets via the C-type lectin-like receptor (CLEC)-2.

Aims: To assess the ability of glioblastoma cells to activate human platelets via PDPN *in vitro*.

Methods: Two human glioblastoma cell lines were investigated: Gli16 (PDPN-positive), isolated from a glioblastoma patient (WHO grade IV), who developed pulmonary embolism six months after diagnosis. AMCH (PDPN-negative), isolated from a patient with gliosarcoma (WHO grade IV). Brain tumor cell-induced platelet activation was measured via 1) platelet-leukocyte aggregates in whole blood by flow cytometry, 2) platelet aggregation in platelet-rich plasma by light transmission aggregometry (LTA), and 3) release of the activation marker platelet factor 4 (PF4) by ELISA.

Results: PDPN-positive Gli16 cells induced higher amounts of platelet-leukocyte aggregates compared to PDPN-negative AMCH cells in a dose-dependent manner. Gli16 cell-mediated platelet-leukocyte aggregation was reduced upon addition of the PDPN-specific antibody NZ-1. PDPN-positive Gli16 cells induced significantly higher platelet aggregation than PDPN-negative AMCH cells (median 34.25% vs. 18.9%; p< 0.001), and Gli16 cell-induced platelet aggregation was significantly reduced upon PDPN inhibition via NZ-1 (median 34.25% vs. 2.55%; p< 0.001) (Fig 1). Furthermore, upon co-incubation with PDPN-positive Gli16 cells high release of PF4 from platelets was induced, which was reduced upon NZ-1 addition. In contrast, PDPN-negative AMCH cells caused only minor release of PF4.

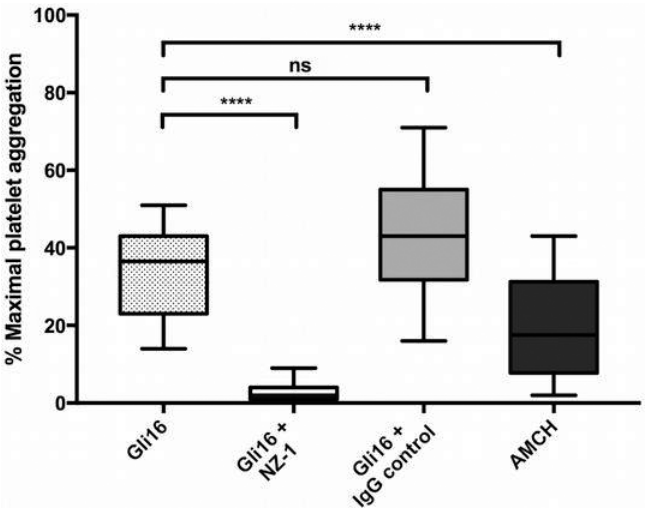

FIGURE 1 Platelet aggregation upon stimulation with human glioblastoma cells in vitro (light transmission aggregometry), ns = not significant

Conclusions: Our results indicate that PDPN plays a crucial role in tumor cell-induced platelet aggregation and thereby might contribute to increased VTE risk in glioblastoma patients.

PB 2318 | Tumor cell-induced Platelet Secretion: Role of Protease-activated Receptors

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Background: Paracrine interactions between cancer cells and platelets have been shown to regulate overall tumor growth. We have recently demonstrated that the release of growth factors by activated platelets promotes cancer proliferation through the upregulation of cancer cell oncoproteins. However, the mechanisms through which cancer cells induce platelet activation and secretion of growth factors remain ill-defined. We hypothesize that the generation of thrombin by

procoagulant cancer cells promotes platelet activation and secretion of growth factors through activation of protease-activated receptors (PARs).

Aims: To investigate the role of PAR signaling in tumor cell-induced platelet secretion.

Methods: The procoagulant phenotype of colon cancer cells was explored by monitoring thrombin generation and fibrin formation in a 96 well plate-based absorbance assay. Platelet granule release in response to procoagulant cancer cells was assessed in a 96 well plate-based luminescence assay. The involvement of PAR-4 and PAR-1 in platelet granule release was determined by testing novel PAR-1 and PAR-4 specific antagonists.

Results: Procoagulant colon cancer cells were able to generate thrombin in platelet poor plasma (PPP). Washed platelets enhanced the ability of colon cancer cells to generate thrombin in PPP. Thrombin-induced platelet granule release was selectively inhibited by PAR-1 and PAR-4 inhibitors.

Conclusions: These preliminary findings suggest a potential role for PARs in mediating platelet secretion in response to thrombin which can be produced by procoagulant cancer cells. Future efforts will determine how targeted inhibition of platelet PARs can affect different steps of the metastatic disease without disturbing hemostasis.

PB 2319 | Can NETs or miRNAs Predict Early Surgery-related Pulmonary Embolism in Patients with Intracranial Tumors?

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Background: Intracranial tumors increase the risk of thrombosis, and often may cause incidental pulmonary embolism (PE) after surgery. New biomarkers are needed to early identify brain cancer patients with high thrombotic risk. Neutrophil extracellular traps (NETs) are highly prothrombotic networks released by neutrophils upon activation. microRNAs (miRs) regulate protein expression.

Aims: To assess the ability of NETs and miRs measured at baseline to predict, the risk of early post-surgical PE in glioma and meningioma patients.

Methods: We recruited and prospectively followed after surgery 10 glioma and 10 meningioma patients. All had negative baseline lung perfusion scan. PE was objectively diagnosed within 7 days after surgery in 5 patients of each group. In plasma samples collected before surgery, we measured NETs markers (DNA, nucleosomes, myeloperoxidase [MPO] and calprotectin), and the expression level of 179 miRs with the Serum/plasma Focus microRNA PCR Panel V4 (Exiqon). Using a multivariable logistic regression model, the predictive ability for post-surgical PE was estimated as the area under the ROC curve (AUC) with R (v3.2.3).

Results: In glioma patients we obtained a good predictive model of post-surgical PE with DNA and MPO as predictors (AUC=0.88 [95%CI: 0.63-1.00], validated AUC=0.77), and also with the expression levels of 8 miRs with Elastic Net. In meningioma patients, NETs markers were not predictors of PE, whilst good prediction was obtained with the expression levels of 6 miRs.

Conclusions: Before surgery, NETs markers (DNA and MPO) might be good predictors of early incidental post-surgical PE in glioma patients. A good prediction could also be obtained with the expression level of 8 and 6 miRs in glioma and meningioma patients, respectively. This information could be useful to tailor post-surgical thromboprophylaxis in these patients. ISCIII-FEDER (PI12/00027, RIC RD12/0042/0029, PIE13/00046, PI14/00079, PI14/00512, FI14/00269, CPII15/00002), GVA (PrometeoII/2015/017), SETH, GR-2011-02347854.

PB 2320 | Thromboprophylaxis Strategy in Cancer Outpatients: CAT AXIS, a Case-vignette Study on Clinical Practice In France

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Background: Data on venous thromboembolism (VTE) long-term prophylaxis in cancer outpatients remain scarce. In the absence of clear and consistent treatment guidelines, the assessment of clinical practice and recommendations implementation is challenging.

Aims: our objective was to describe and better understand the clinical practice regarding thromboprophylaxis use in this context.

Methods: CAT AXIS was a multicenter cross-sectional study based on the completion of physician-profile questionnaires and the assessment of 10 e-mailed credible clinical scenarios of lung, colon and breast cancers by each of participants using the case-vignettes validated method.

Results: A total of 224 physicians participated allowing the completion and the analysis of 2,085 case vignettes corresponding to 765, 703 and 617 fictive clinical scenarios on lung, colon and breast cancers, respectively. The overall rate of thromboprophylaxis prescription was 680/2085 (32.6%) among participants with a comparable proportion for the three types of cancer. Low-molecular-weight heparin (LMWH) was the most frequently used, by 92.7%, 93.8% and 83.9% of participants for lung, colon and breast cancers, respectively; treatment

duration ≥3 months was prescribed by 74.4% of participants using thromboprophylaxis.

Conclusions: In this case vignette study, physicians of different medical specialties would give prolonged thromboprophylaxis in about 30% of patients with lung, colon or breast cancer. In the absence of clear guidance, thromboprophylaxis use seems not so limited and rather empiric. Furhermore, the choice of LMWH by the majority of participants and treatment duration are appropriate based on available data to date.

PB 2321 | Peripherally Inserted Central Catheter (PICC) Related Thrombosis in 230 Patients with Hematological Malignancies. A 6 Years Single Experience Center

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Background: The use of peripherally inserted central catheters (PICCs) is widely extended in patients with hematological malignancies who need be treated by chemotherapy. However, catheter-related thrombosis is one of its main complications. We reported the experience of the PICC-related thrombosis (PRT) in our center.

Aims: To analyze the incidence of PRT, describe the clinical characteristics and management of these patients and identify the risk factors of PRT.

Methods: We performed a retrospective chart review of 230 adult patients diagnosed with hematological malignancies, in whom, experimented nurses tunneled PICCs with different technique: blinding Seldinger from 2010 to 2014 and guided by ultrasonography (US) from 2015 to 2016. PRT diagnose was confirmed by Doppler US. Statistical analysis was performed using the SPSS program (v.20).

Results: Baseline patients' characteristics are shown in Table 1. The overall incidence of PRT was 7% (n=16). The main diagnoses related to PTR were acute lymphoblastic leukemia (ALL=6), Non-Hodgkin Lymphoma (5), and Hodgkin Lymphoma (HL=3). All except one had active disease when PICC was tunneled (15/16=94%). Fourteen patients (88%) were treated by chemotherapy based in L-asparaginase (L-ASA), immunomodulatory drugs or other treatment combined with corticosteroids. The median onset of PRT was 26 days, (range: 0-230); and 8 of them (50%) in the first 30 days after insertion. In 11 cases (69%) D-Dimer was elevated. All PICC were removed within 72 hours of PRT and treated with LMWH to a median of 4 months (range: 1-11). Finally, in the univariate analysis ALL, HL and L-ASA had significant impact on PRT (Table 2). However, in the multivariate, HL was the only risk factor of PRT
(OR 8,38; CI 1,05-66,5; p=0,044).

TABLE 1 Baseline patients' characteristics:

Median age (range)	58 years (14-86)
Sex: male / female	128 (56%) / 102 (44%)
Hematological malignancies: n (%)	NHL: 105 (45,7%) / Acute myeloid leukemia and myelodysplastic syndrome: 60 (26,1%) / ALL: 22 (9,6%) / Multiple myeloma and Amyloidosis: 19 (8,3%) / HL: 17 (7,4%) / Others: 7 (3%)
Status disease when PICC was tunneled	Active: 188 (81,7%) / Remission: 42 (18,3%)
Drugs related thrombosis	Immunomodulatory drugs (IMIDS): 24 (10,4%) / L-Asparaginase (L-Asa): 21 (9,1%) / Erythropoietin: 16 (7%)
Thromboprophylaxis	Low Molecular Weigh Heparin (LMWH): 27(9,1%) / ASA: 21 (9,1%) / Oral anticoagulants: 3 (1,3%)
PICCs tunneled technique	Guided by US: 127 (55,2%) / Blinding: 103 (44,8%)
Catheter tip location	Right atrium (RA): 78 (33,9%) / Cava-RA: 152 (66,1)

TABLE 2 Univariate (U) and multivariate (M) analysis to identify risk factors of PRT

	U: OR [IC 95%]; p-value	M: OR [IC 95%] p-value
Male sex	1,36 [0,48-3,87]; 0,57	1,09 [0,358-3,361]; 0,87
Age (>50 years)	2,59 [0,93-7,24]; 0,07	1,28 [0,374-4,396]; 0,69
ALL / HL / NHL	15,75 [2,91-85,12]; 0,001 / 9 [1,38-58,78]; 0,022 / 2,10 [0,39-11,1]; 0,283	4,79 [0,21-110,03); 0,33 / 8,38 [1,06;-66,5]; 0,04 / 2,12 [0,39-11,59]; 0,28
IMIDs / L-Asparaginase / Stimulant growth factors	0,85 [0,1-7,02]; 0,88 / 7,82 [2,453-24,95]; 0,001 / 1,67 [0,6-4,67]; 0,33	1,43 [0,157-13,027]; 0,75 / 3,68 [0,23-59,16];0,38/---
Seldinger technique / Right atrium location tip	0,54 [0,18-1,60]; 0,27 / 1,85 [0,58-5,95]; 0,3	NS

PB 2322 | The Management of Non DVT-PE Cancer-associated Thrombosis: A Single Centre Experience

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Background: Thrombosis is a common complication of cancer and its treatment. The management of deep vein thrombosis (DVT) and

pulmonary embolism (PE) is well documented, however the management of other thromboses (non-lower limb DVT or PE thrombosis (NDVTPE)), is less clear.

Aims: To review the natural history and management of patients with cancer-associated NDVTPE thrombosis.

Methods: All patients at a large tertiary hospital for thrombosis and cancer diagnosed with upper limb VTE (ULVTE) or splanchnic veins (SVT) and cancer between June 2014 and December 2015 were identified using an electronic database. Data was collected on tumour type, stage, anti-neoplastic treatment, thrombosis management, complications of anticoagulation and clinical outcome.

Results: 36 (18 male and 18 female) patients were identified, 18 ULVTE and 18 SVT, with results summarised in table 1. The median age and sex distribution were as expected for patients with cancer. Most patients had advanced disease. In addition, 77% for both ULVTE and SVT had cancer near the site of thrombosis suggesting localised effects. Most thromboses were diagnosed at cancer diagnosis or during treatment. The median Khorana score was 2 (range 0-3), with 56% patients having a score of 2 or over. Symptomatic thrombosis was higher in ULVTE (73%) than SVT (50%). No anticoagulation was given in 22% patients with SVT and all others received low molecular weight heparin (LMWH). There were no haemorrhagic complications in this group. 2 patients with ULVTE did not receive anticoagulation, most received LMWH and one received rivaroxaban. Two patients in this group had treatment-related bleeding. Mortality rate was high with a median time to death of 99 days.

TABLE 1 Features of patients in NDVTPE cancer-associated thromboses

	Upper Limb and Neck	Splanchnic
Mean Age (Range)	66.5 (53-90)	64.5 (44-82)
Sex (M/F)	9/9	9/9
Advanced disease (%)	13 (73%)	12 (67%)
Symptomatic (%)	13 (73%)	9 (50%)
Thrombosis at presentation cancer (%)	4 (22%)	5 (27%)
Thrombosis during cancer therapy (%)	9 (50%)	8 (44%)
Thrombosis post-cancer treatment (%)	5 (27%)	5 (27%)
Anticoagulation	16 (88%)	14 (77%)
Bleeding	2	0

Conclusions: These results show that NDVTPE cancer thrombosis are associated with advanced stage cancer and carry a poor prognosis. Many SVT are asymptomatic but are associated with cancer near the site of thrombosis. There are few complications of LMWH treatment but with too little data on other agents.

PB 2323 | Bioinformatic Analysis of Collaborative Interaction between Platelet and Cancer Cell Derived Microparticles in Evolving a Post Code Mechanism for Breast Cancer Metastasis and Identification of CD9 as Potential Drug Resistance Marker

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Background: Metastasis and drug resistance are the main cause of cancer-related death and major challenge in disease management. The process of tumor cell spreading from primary site to distant tissue is a complex process and involvement of platelets and coagulation factors. However, the mechanisms involved and comprehensive analysis to understand the molecular processes is not yet completely understood in order to evolve with new potential targets or biomarkers.

Aims: We aimed to use patient and cell line based data available from literature and develop a map of molecular pathways and identify potential biomarkers/targets and validate in in-vitro 3D cell culture system.

Methods: The gene expression data of patients (placlitaxel responders vs non responders) were downloaded from Gene Expression Ominibus, drugs and their targets from drug bank database. The gene expression data was analyzed using R-script for LIMMA followed by WGCNA and Cytoscape for network construction. The 3D cell culture was performed in gelatin-hydrogels and MDA MB-231 cells were used for drug resistance assays.

Results: The gene expression and network analysis showed that several novel genes were associated with breast cancer mestasis including WFDC2, WNT6, SMPDL3B, PCOLCE, TNFRSF10A, ST8SIA4, MAPRE2, PIK3R3, DLX4 and CD9 which had similar profiles in 3D cell cultures and non-responder patients. Further microparticles proteome analysis of platelets and cancer cells suggested novel interactome suggesting physical associations between the microparticles possibly leading to larger particles with specific surface protein signature suited for internalization at distal tissues leading to metastasis. These protein signatures could be termed as post code also have CD9 for specific tissues which are targets for metastasis.

Conclusions: Our results for the first time demonstrate that there is a possible mechanism of post code for cancer metastasis and CD9 could be a potential marker also for drug resistance.

PB 2324 | Tumour Microvesicles Interact with Endothelial Cells under Venous Flow in vitro

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Background: Tumours shed microvesicles (MV) into the circulation that can then interact at sites remote to the tumour source, particularly those MV that express tissue factor (TF), which are thought to possibly play a role in venous thromboembolism (VTE) in cancer patients. The pathophysiology relating to cancer-induced VTE are not yet fully understood but we have previously shown that tumour MV appear to bind to endothelial cells in static conditions and confer procoagulant activity via tissue factor. This suggests that there may be a role of the endothelium in cancer-related VTE in patients.

Aims: Here, we aimed to investigate the interactions of tumour-MV on human umbilical vein endothelial cells (HUVECs) under shear flow rates.

Methods: MV released from AsPC-1 tumour cells labelled with 5(6)-carboxyfluorescein diacetate N-hydroxysuccinimidyl ester (CFSE) were initially analysed by flow cytometry for degree of CFSE labelling (Figure 1). Having established reproducible, fluorescently-labelled MV they were flowed through a Vena8 Endothelial+™ biochip pre-coated with a confluent monolayer of HUVECs and imaged using a CELLIX imaging system.

Results: CSFE staining of tumour cells was shown to be an effective method of labelling shed MV for a period of up to 48h (Figure 2). The degree of labelling to background was shown to decrease from (29.91%) to (9.38%) over the course of 6 days. When using CFSE-labelled ASPC-1 MV in a shear flow system (under venous shear rates) initial data observed showed a coalescence of tumour MV on HUVECs.

Conclusions: CFSE can be used to label MV released from tumour cells. Based on our initial observations labelled MV appeared to interact with endothelial cells on a biochip, under flow conditions. This may have implications for clot formation in cancer patients.

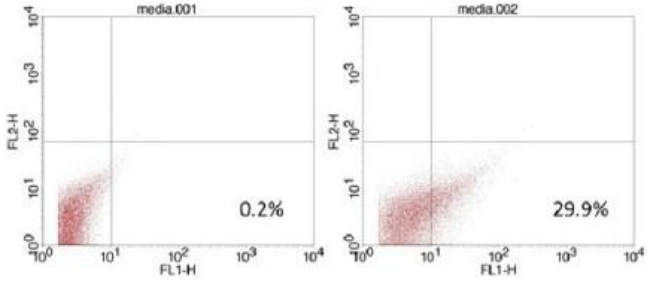

FIGURE 1 Flow cytometry detection of fluorescence intensity of tumour MVs. Right panel shows MVs derived from AsPC-1 cells labelled with 2 µM CFSE wh

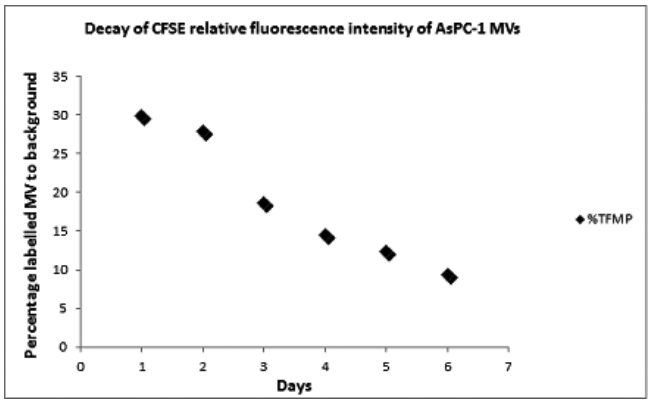

FIGURE 2 Chart shows labelling intensity of MV released by AsPC-1 tumour cells over the course of 6 days.

PB 2325 | The STEP-CAT Cohort Management Study: Step-Down to Prophylactic Doses of Enoxaparin after a Minimum of 3-6 Months of Anticoagulation for the Treatment of Cancer-associated Thrombosis

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Background: Clinical guidelines recommend a minimum of 3-6 months of anticoagulation with weight-adjusted low molecular weight heparin (LMWH) in cancer patients with acute venous thromboembolism (VTE). There are no recommendations beyond 3-6 months. We hypothesize that prophylactic dose LMWH is a safe option for extended duration secondary prevention of cancer-associated thrombosis (CAT). We therefore propose a pilot study to assess the feasibility of extended duration prophylactic dose LMWH in cancer patients with treated acute VTE. The data obtained from this study will help inform the design of a future trial on prophylactic dose LWMH for secondary prevention of CAT.

Aims: Primary aim is to determine study design feasibility. Secondary aims are to determine the cumulative incidence of VTE recurrence and bleeding.

Methods: This is a multicentre, open-label, single arm pilot study of prophylactic dose enoxaparin in cancer patients with acute VTE after 3-6 months of therapeutic dose LMWH. 150 subjects from 7 Canadian hospital centres with an active malignancy and an objectively confirmed VTE treated with weight adjusted LMWH for 3-6 months will be recruited. Eligible subjects will receive 40 mg daily of enoxaparin

for a maximum of 26 consecutive weeks or until any of the following occurs: recurrent VTE; bleeding necessitating permanent discontinuation of study drug; other adverse event necessitating discontinuation of study drug; or withdrawal of informed consent. Follow up will consist of four visits to determine feasibility and adverse outcomes.

Results: To date one study centre is active and has enrolled 7 patients. The 6 remaining sites will be active by end of February 2017. It is anticipated that enrollment will be completed by April 2018.

Conclusions: Little data exists on reduced dose extended-duration LMWH for secondary prevention of CAT. STEP-CAT will be the first study to assess the feasibility of prophylactic dose LMWH for the treatment of CAT following standard 3-6 months of anticoagulation.

PB 2326 | Myeloma Plasma Cells Express Tissue Factor and Trigger Thrombin Generation

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Background: Multiple myeloma is a hematological cancer characterized by clonal expansion of malignant plasma cells within the bone marrow. Myeloma plasma cells (MPCs) occupy and alter the stromal tissue of the bone marrow thereby enhancing their survival and growth. Beyond their key roles in blood coagulation, tissue factor (TF) and thrombin are also involved in cellular cross-talk and modulation of cell proliferation and migration.

Aims: To modelize the procoagulant fingerprint of MPCs by studying their TF expression and effect on thrombin generation (TG) of human plasma.

Methods: TF expression of MPCs was analyzed for TF expression by flow cytometry using anti-TF human murine IgG1 monoclonal antibody (Ref: 4503, American Diagnostics). TG in the presence of MPCS and anti-TF antibody was assessed with the Calibrated Automated Thrombogram assay as described elsewhere (Gerotziafas et al Thromb Res. 2012;129:779-86). In control experiments, TG was assessed in re-calcified normal PPP

(a) in the absence of plasma cells recalcification and

(b) after addition of PPP Reagent® 5pM TF - 4 µM procoagulant phospolipids (PPL) from Diagnostica Stago (France).

Results: MPCs (10^6 cells/ml) express TF (Mean Index of Fluorecence = 6 %) in contrast to normal lymphocytes, neutrophils or HUVECs which expressed no detectable TF. MPCs, accelerated TG in a TF-dependent manner, and increased the Peak and the endogenous thrombin formation (ETP) values compared to the control. However, the procoagulant activity of plasma cells was significantly lower compared to TG triggered by physiologically relevant concentrations of TF (5 pM) and PPL(Table 1).

Conclusions: MPCs express low amounts of TF and induce a mild increase of TG. The identification of the procoagulant fingerprint of plasma cells could lead to new therapeutic strategies aiming to interrupt the cross-talk between MPCs and the surrounding stromal tissue.

TABLE 1 Effect of plasma cells on TG of normal PPP (n = 7). ETP,PEAK ,MRI: mean rate index.*p<0.05 versus control; $ p<005 versus PPP reagent

cells/µL	MRI (nM/min)	ETP (nMxmin)	Peak (nM)
1000	21.03±18.68*$	902.99±310.74*$	85.26±48.02*$
500	18.83±15.77*$	902.83±262.25*$	83.31±48.72*$
250	20.30±17.87*$	812.53±360.95*$	79.55±49.29*$
100	17.60±15.98*$	814.99±327.55*$	75.49±49.29*$
50	17.73±16.05*$	831.52±283.30*$	75.61±40.38*$
25	16.22±14.62*$	728.25±302.54*$	68.97±41.11*$
0 (control)	10.56±6.64	680.02±218.49	56.19±24.50
PPP reagent (5pM TF /4µM PPL	127.48±37.97	1558.55±198.22	300.77±41.26

PB 2327 | Radiotherapy Does Not Affect Coagulation

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Background: Cancer is associated with an increased risk of thromboembolic disease. Thromboembolic disease adds substantially to morbidity and mortality in cancer patients and is the second leading cause of death in cancer patients next to the underlying cancer. Only sparse knowledge exists about the influence of radiotherapy on coagulation and whether radiotherapy itself causes an increased risk of thromboembolic disease.

Aims: The aim was to investigate if adjuvant radiotherapy causes activation of the coagulation system.

Methods: In total 40 women with invasive breast cancer were enrolled after local excision and optionally chemotherapy. The radiotherapy contained up to 50 Gy administrated by 25 radiation fractions. Blood samples were obtained before and immediately after the first, the middle and the last radiation fraction. Platelet function was measured using impedans aggregometry and thrombin generation was determined by calibrated automated thrombogram. Furthermore, von Willebrand factor, factor VIII, fibrinogen, and C-reactive protein (CRP) were measured.

Results: All women had normal platelet aggregation prior to radiotherapy, and means of platelet aggregation stayed within reference interval. Platelet aggregation did not change during a single radiation dose, or throughout the entire radiation period.

Thrombin generation was not affected by radiotherapy, neither by a single radiation dose, nor throughout the entire radiation period. All means of thrombin generation stayed within reference interval. Von Willebrand factor, factor VIII, fibrinogen, and CRP did not change during radiotherapy.

Conclusions: Overall, the coagulation was not affected by radiotherapy. Radiotherapy did not affect the coagulation system nor by a single radiation dose or during the entire radiation period in breast cancer patients receiving adjuvant radiotherapy.

PB 2328 | Usefulness of D-dimer in the Diagnosis of Deep Vein Thrombosis after Neurosurgery for Glioblastoma and Meningioma

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Background: Patients undergoing craniotomy for high grade glioma and meningioma are at risk for deep vein thrombosis (DVT) despite the use of multimodality prophylaxis. D-Dimer (DD) measurement may help early detection of DVT, but its transient increase after surgery may lead to misinterpretation.

Aims: To verify the diagnostic usefulness of DD variation during postoperative monitoring in the early detection of DVT confirmed by ultrasound (US) in patients undergoing craniotomy for brain tumor.

Methods: Patients undergoing craniotomy for high grade glioma or meningioma were tested for DD before (t0), 3 days (t1) and 7 days (t2) after neurosurgery. Each patient received US of lower limbs before and 7±1 days after surgery to test the presence or absence of DVT. All patients received antithrombotic prophylaxis with elastic stockings, intermittent pneumatic compression and LMWH.

Results: Fifty-seven patients were studied. Eleven patients (19.3%) developed DVT within 7 days after surgery. Median DD levels in patients with and in those without DVT were 147 (125-839) and 142 (77-267) ug/L at t0, 1488 (537-2787) and 572 (313-901) ug/L at t1, 2032 (653-4871) and 416 (242.5-625) ug/L at t2 respectively. Temporal variation of postoperative DD levels was significantly different between patients with and those without DVT (p=0.019). In each patient the t2/t1 ratio in DD values was calculated: using a t2/t1 ratio cut-off level of 1.04 was used, D-dimer ratio demonstrated a sensitivity of 63.6% (95% CI: 0.30-0.89), a specificity of 71.7% (95% CI: 0.56-0.84) and a negative predictive value of 91.2 % (95% CI: 0.75-0.97) for DVT. The t2-t1 difference in DD values was also determined: if a cut-off level of 69 ug/L was used, DD difference demonstrated a negative predictive value of 94.7% (95% CI: 0.82-0.99) for DVT.

Conclusions: In patients undergoing craniotomy for brain tumor a close monitoring of DD during the hospital stay may be useful to exclude a suspicion of DVT.

PB 2329 | Predictors of Mortality in Lung Cancer with Pulmonary Embolism: Concurrence of Pulmonary Embolism with Lung Cancer and Central Emboli

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Background: Patients with lung cancer commonly experience pulmonary embolism (PE).

Aims: The aim of the present study was to examine the clinical features of patients with lung cancer and PE and to investigate prognostic factors in these patients.

Methods: This retrospective study divided patients with lung cancer and PE into a group of patients with PE diagnosed concomitantly with lung cancer (concurrent group) and a group with PE detected after lung cancer (sequential group), compared the clinical characteristics of patients in the two groups, and investigated prognostic factors in these patients.

Results: The study population consisted of the concurrent group (27 patients [10.1%]) and the sequential group (240 patients [89.9%]). The concurrent group exhibited higher percentages of stage I cancer at the diagnosis of PE (6 [22.2%] vs. 8 [3.3%], $p < 0.001$) and right ventricular dilation on computed tomography (14 [51.9%] vs. 41 [17.1%], $p < 0.001$), as well as lower rate of small cell carcinoma (1 [3.7%] vs. 49 [20.4%], $p = 0.036$) than the sequential group. PE concurrent with lung cancer (hazard ratio [HR] 2.64, 95% confidence interval [CI] 1.57-4.43, $p < 0.001$) and central PE (HR 1.46, 95% CI 1.02-2.10, p=0.04) were independent predictors of mortality in patients with lung cancer and PE.

Conclusions: PE concurrent with lung cancer is characterized by more severe PE and infrequent small cell carcinoma. PE concurrent with lung cancer and central emboli may be independent prognostic factors in patients with lung cancer and PE.

PB 2330 | Patients with Venous Thromboembolism Events Admitted in a Tertiary Care Hospital: Follow-up for One Year and Correlation with Cancer

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Background: Venous thromboembolism (VTE) is a significant health problem, especially in cancer patients, as cancer is a strong risk factor for VTE. Also VTE is a well-known marker of occult cancer.

Aims: Evaluate the number of patients admitted in our hospital with acute VTE during 2015, associated risk factors, as cancer, mortality and the incidence of newly diagnosed cancer in the following year.

Methods: A retrospective study of 363 consecutive patients admitted with VTE events during 2015. Pulmonary embolism (PE) with or without deep venous thrombosis (DVT) was diagnosed in 103 (28,4%) patients and DVT alone was diagnosed in 260 (71,6%) patients. Presence of known risk factors, co-morbidities and newly diagnosed cancer during the following year after VTE event were obtained from medical records.

Results: In the PE group the mean age was 69,3 years, 63% females. Known risk factors were present in 32 patients (31%): active cancer in 19 patients (4 under anticoagulants), other risk factors in 13 patients. Idiopathic VTE was diagnosed in 71 patients (68,9%). Twenty-four patients died during follow-up, 10 with previous diagnosed cancer and 2 with newly diagnosed cancer. In the DVT group, the mean age was 50,9 years, 44,2% females. Known risk factors were present in 61 patients (23,4%): active cancer in 56 patients (5 with central venous catheter; 4 of this patients were under anticoagulants), other risk factors in 5 patients. Idiopathic DVT was diagnosed in 191 (73,46%) patients. Six patients died during follow-up, 5 with cancer. Four patients with PE and 2 with DVT developed cancer during the first 6 months of follow-up, all with previous idiopathic thrombotic event.

Conclusions: The number of active cancer in VTE patients was 20,7%, similar to other published studies. The rate of newly diagnosed cancer is lower (1,65%) than published. Though thromboprophylaxis in active cancer is not currently recommended in ambulatory setting, we believe there is a need to identify high-risk patients who will benefit from it.

PB 2331 | Cement Pulmonary Embolism as a Complication of Percutaneous Vertebroplasty in Cancer Patients

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Background: Vertebroplasty is a minimally invasive procedure commonly performed for compression vertebral fractures secondary to osteoporosis or malignancy. Leakage of bone cement into the paravertebral venous system and cement pulmonary embolism (cPE) are well described, mostly in patient with osteoporosis.

Aims: Little is known about its clinical sequel and outcomes in cancer patients. Here, we report our experience with cPE following vertebroplasty performed in cancer patients.

Methods: All consecutive cancer patients who had vertebroplasty were retrospectively reviewed. Procedure was performed through a percutaneous approach with injection of barium opacified polymethyl-methacrylate cement.

Results: A total of 102 cancer patients, median age 53 (19-83) years, were included. Seventy eight (76.5%) had malignant vertebral fractures while 24 (23.5%) had osteoporotic fracture. Cement PE was detected in 13 (12.7%); 10 (76.9%) had malignant fractures while the 3 others had osteoporotic fractures. Cement PE was mostly asymptomatic; however, 5 (38.5%) had respiratory symptoms that led to the diagnosis. Only the five symptomatic patients were anticoagulated.

Cement PE was more common with multiple myeloma (MM); seen in 7 (18.9%) of the 37 patients included compared to only 3 (7.3%) of 41 patients with other malignancies.

The incidence of cPE was not different in cancer patient with osteoporotic (12.5%) or malignant (12.8%) fractures (HR= 0.972; 95% CI, 0.244-3.86; P= 0.99). However, patient with osteoporotic fractures were older 66 vs. 51 years, P = 0.0076.

Conclusions: Cement PE, mostly asymptomatic, is a relatively common complication following vertebroplasty. Multiple myeloma is associated with the highest risk. Large scale prospective studies can help identify risk factors and clinical outcomes that can lead to better prevention and therapeutic strategies.

PB 2332 | Renal Cell Carcinoma with Neoplastic Thrombosis: A Treatment Challenge for a Rare Entity

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Background: Renal cell carcinoma (RCC) is well known for angiotropism, with up to 10% of tumors presenting extensive neoplastic tumoral thrombosis (NTT). Surgical debulking treatment with thrombectomy sometimes requires an extracorporeal circuit. In this setting, antithrombotic therapy (AT) and its duration are not entirely well defined.

Aims: To describe the clinical profile and management in patients diagnosed with RCC and NTT in a tertiary hospital.

Methods: A retrospective analysis was performed in a consecutive series of patients over the age of 18 with the diagnosis of RCC and NTT in our center between January 2015 and December 2016. We studied demographic characteristics, clinical presentation, oncologic and antithrombotic treatment strategies and outcome.

Results: A total of 9 patients were included in the analysis (6 cases were female and the median age at presentation was 61 years (47-75)). The pathologic diagnosis was of clear cell renal carcinoma in almost all cases (7/9). All patients had tumoral vena cava infiltration, although thrombotic extension was larger involving renal vessels, vena cava and up to the right atrium in 6 patients (66%). Cytoreductive radical surgery (nephrectomy and cavotomy with thrombectomy) was selected in 8 subjects and required cardiopulmonary bypass in 5.

All cases received postoperative incremental doses of low weight heparin (LMWH) with laboratory anti-Xa determination. At discharge and during the oncological therapy (mostly sunitinib, axitinib or bevacizumab), all of them were treated with LMWH. After one year of stable oncologic disease, 3 (33%) patients were on oral anticoagulation, 1 with direct oral anticoagulants and 2 with vitamin K antagonists.

Conclusions: RCC with NTT is a rare entity with extensive thrombotic manifestations. Cytoreductive radical surgery with thrombectomy is feasible and AT with LWMH is the treatment preferred during the first 12 months. Further studies are needed in order to better characterize this specific population.

PB 2333 | Silent Venous Thromboembolism in Patients Undergoing Thoracic Surgery in the Course of Lung Cancer

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Background: Venous thromboembolism (VTE) often occurs after thoracic surgery but can even occur before surgery in patients with lung cancer.

Aims: The aim of the study was the investigation of the incidence of silent deep vein thrombosis (DVT) and pulmonary embolism (PE), confirmed by objective imaging tests, before and after thoracic surgery in the course of lung cancer.

Methods: Venous ultrasound (US) imaging was performed to detect DVT before and after thoracic surgery in patients with non-small cell lung cancer. Plasma D-dimer (DD) levels as well as Caprini and Khorana risk scores were examined in all patients. All cases with signs or symptoms of PE after surgery were examined by computed tomography pulmonary angiography (CTPA).

Results: The study was perfomed in 200 patients (M:K = 122:88) with lung cancer and hospitalized for thoracic surgery. All patients received primary antithromobotic prophylaxis by low molecular weight heparins. DVT was detected by US in 3 patients before thoracic surgery. There was no symptoms od DVT and DD Level was elevated in one case.

PE was diagnosed by CTPA in 2 patients after surgery; there was no DVT in these cases.

DD levels were elevated in both cases.

Conclusions: Silent or subclinical VTE may occur before surgical treatment in many patients with non-small lung cell cancer. Risk factors for VTE as well as elevated DD level before treatment might not be sufficient for identification silent DVT in thoracic surgery. PE represent potentially lethal postoperative complication despite pharmacological prevention of VTE.

PB 2334 | Demographic Data and Outcomes of Pediatric Patients with Tumor and Thrombosis

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Background: Thromboembolic events (TEs) could be occurred in cancers and lead to serious complications. Risk factors of developing thrombosis in cancers is reported including advanced disease, cancer drugs. Regarding to different race and underlying thrombophilia, the cancers associated with thrombosis is conducted in Thailand.

Aims: To describe demographic datas, risk factors, treatment and outcomes

Methods: Cancer patients with TEs age 0-18 years between 1996-2016 in Srinagarind Hospital, Khon Kaen were reviewed.

Results: 57 cancer patients with TEs were included with median age 12 years (3 months-18 years). Most common site of thrombosis was deep vein thrombosis (42.1%). Majority of patients were hematologic malignancies (36.8%) followed by solid tumor (28%) and soft tissue sarcoma (24.6%). The most common risk factor was L-asparaginase. Tumor invasion/compression had increased risk of TEs in children with solid tumor and sarcoma. All of patient with sarcoma and TEs associated with advance diseases (stage III-IV). Two patients had catheter-related thrombosis. More than half of patients were treated by low-molecular weight heparin (53.6%). Other treatment were warfarin(12.6%), aspirin(3.5%), observation (26.8%) and combined treatment(3.5%). 48.2% of all patients improved thromboembolism. The outcome of TEs was categorized into complete (17.5%) and partial (10.5%) resolution, stable (15.8%) and progressive (3.5%) disease. Some patients (14%) with TEs clinically improve with no imaging follow up. 36.8% of patients were not record thromboembolism outcomes due to cancer advance. 61.4% of patients were alive. Two patients (3.5%) died from pulmonary embolism and brain infarct. Most common cause of death was cancer progression.

Conclusions: There were multifactorial in cancer with thrombosis. Adolescence, advance stage of tumor, chemotherapy had increased risk for TEs. Cancer progression was major cause of death in patients with tumor and TEs.

PB 2335 | Retrospective Cohort Study of Venous Thromboembolism (VTE) Rates in Ambulatory Cancer Patients, and Association with Khorana Score

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Background: Guidelines do not recommend cancer outpatients receive thromboprophylaxis unless high VTE risk, with Khorana score suggested for risk stratification.

Aims: To investigate VTE incidence in outpatients with pancreatic, endometrial, colorectal, ovarian and cervical cancer at major cancer centre and role of Khorana score in risk assessment.

Methods: Data from patient records for 1 year after diagnosis Dec 2012-14 (2009-14 pancreatic).

Results: 26/98 pancreatic patients developed non-hospital-associated thrombosis in first year with 30 individual clots (Table 1).13/26

patients were asymptomatic, 11/26 had VTE at diagnosis. 9/160 endometrial patients developed non-HAT VTE with 10 clots. 3/9 were asymptomatic, 3/9 had VTE at diagnosis. 20/212 colorectal patients developed non-HAT VTE with 21 clots. 9/20 were asymptomatic, 0 had VTE at diagnosis. 21/201 ovarian patients had non-HAT VTE with 23 clots. 8/21 were asymptomatic, 3/21 had VTE at diagnosis. 0/95 cervical patients developed non-HAT VTE.

TABLE 1 Site of VTE events by cancer group

	Pancreatic	Endometrial	Colorectal	Ovarian	Cervical
Lower limb DVT	6	1	4	5	0
Upper limb DVT: PICC-associated	3	2	7	4	0
Upper limb DVT: Non-PICC-associated	1	0	0	0	0
Upper limb DVT: Total	4	2	7	4	0
PE	5	7	8	12	0
Intra-abdominal	14	0	2	0	0
IVC	1	0	0	2	0
Total	30	10	21	23	0
Total number of patients	98	160	212	201	95

Excluding those with VTE at presentation, 46/87 pancreatic, 21/157 endometrial, 3/212 colorectal, 26/198 ovarian, 20/95 cervical patients had high-risk Khorana score (≥3). High Khorana score was associated with higher 1 year VTE rate in endometrial [high-risk 3/21 VTE(14.3%) vs. low-risk 3/136(2.2%), p=0.007]; but not pancreatic [high-risk 8/46 VTE(17.4%) vs. low-risk 7/41(17.1%), p=0.97],colorectal [high-risk 0/3 VTE(0%) vs. low-risk 20/209(9.6%), p=0.57], ovarian [high-risk 2/26 VTE(7.7%) vs. low-risk 16/172(9.3%), p=0.79] or cervical cancer [high-risk 0/20 VTE vs. low-risk 0/75].

Conclusions: VTE is a significant burden in pancreatic (26.5%), endometrial (5.6%), colorectal (9.4%) and ovarian (10.4%) cancers. Khorana score predicted VTE risk in endometrial cancer only. A review of factors that can improve prediction of non HAT in cancer require further investigation.

PB 2337 | Subclinical Portal Vein Thrombosis in Liver Cirrhosis Patients Increase Risk of 30 Days Mortality: Preliminary Study

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Background: Portal vein thrombosis (PVT) is a common complication of liver cirrhosis patients with a prevalence ranging from 1% to 16% patients. Mostly PVT was diagnosed radiographic studies as an incidental finding. There is still limited data about the natural history of PVT and its survival in patients with liver cirrhosis.

Aims: The aim of this study is to know 30 days-mortality of PVT in liver cirrhosis patients.

Methods: This was a cohort prospective study evaluating liver cirrhosis patient with PVT in our general hospital, Karawaci, Tangerang, Banten from 2014-2016. Patients with heart failure, chronic kidney disease and cancer other than hepatocellular carcinoma were excluded. Liver cirrhosis patient with or without PVT was followed until 30 days after admitted in our hospital. Diagnosis of liver cirrhosis and PVT were using multi slice CT scan. PVT is defined as occlusive thrombosis of the main portal vein.

Results: Fifty five patients fulfilled the criteria for liver cirrhosis in the study period. Thirty five patients (63.6%) were male. Thirty seven (67.3%) patients were fulfilled Child Pugh B criteria, 10 patients (18.2%) were fulfilled Child Pugh A criteria and others were Child Pugh C criteria. Twenty nine patients (52.7%) had positive hepatitis B infection and 4 patients (7.3%) had hepatitis C infection. Fourteen patients (25.4%) fulfilled criteria of HCC. Five patients with HCC were positive PVT. Four patients with PVTT died in hospital. The mean of D-dimer level using ELISA technique was 5.6 $\pm$ 0.9 g/L. All patients with PVT died within 30 days after they were diagnosed.

Conclusions: All patients with PVT in Liver cirrhosis patients died within 30 days after they were diagnosed. Further study need to be done to confirm this result.

PB 2338 | Relationship of Platelets Parameters with JAK2 V617F Mutation Status and Thrombotic Risk in Myeloproliferative Disorders

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Background: Blood count is essential in the diagnosis of myeloproliferative disorders which are closely associated with JAK2 V617F mutation and thrombotic events. The diagnostic and prognostic performance of platelets parameters are not clearly demonstrated in these disorders.

Aims: The aim of this study is to assess the relationship between platelets parameters with both JAK2 mutational status and the thrombotic events.

Methods: 53 patients diagnosed with myeloproliferative disorders were enrolled during a period of 5 years. Arterial and venous thrombotic events were recorded.

Platelets parameters including platelets count (PC), thrombocytocrit (PCT), platelet distribution width (PDW) and mean platelet volume (MPV) were analyzed at diagnosis using automated hematology analyzer SYSMEX XT-4000 i.

JAK2 V617F mutation was detected by allele specific real-time quantitative fluorescence PCR.

Results: The mean age was 62 years [24-92], the sex ratio was 22/31. Thrombotic events were recorded in 13 cases.

Of the 53 MPDs, JAK2 V617F mutation was detected in 44 (83%) patients.

Platelets parameters were compared between patients with JAK2 V617F mutation and wild-type. No significant difference was found between the two groups: PC (611045/mm3 vs 785222/mm3; p=0.17), PCT (0.61% vs 0.71%; p=0.4), PDW (12.17 fL vs 11.13 fL; p=0.24) and MPV (9.78 fL vs 9.5 fL; p=0.6).

There was also no association to thrombotic events: PC (675300/mm3 vs 533923/mm3; p=0.29), PCT (0.65% vs 0.54%; p=0.34), PDW (12.02 fL vs 11.9 fL; p=0.871) and MPV (9.79 fL vs 9.56 fL; p=0.64).

Conclusions: According to this study, there was no impact of platelets parameters on JAK2 mutation status and they were not reliable markers in thrombotic risk assessment. Some studies showed that platelets indices could be predictive of JAK2 mutational status.

PB 2339 | Genetic Forms of a Thrombophilia in Cancer Patients with Recurrent VTE

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Background: High rate of the recurrent venous thromboembolism (VTE) as often complication in cancer patients has induced us to carry out association between congenital defects of a hemostasis system and the recurrent VTE in oncogynaecological patients.

Aims: Determination of the rate and structure of genetic forms of a thrombophilia in gynecological cancer patients with recurrent VTE.

Methods: The control group includes 30 women with gynaecological cancer without any VTE episodes and has not family history of VTE.

Laboratory tests: Detection of FV Leiden mutation, prothrombin G20210A mutaton, gene PAI-1 G4/G5 polymorphism, gene MTHFR C677T mutation, genes of platelets glycoproteins polymorphism: GP IIb/IIIa, GP Ia/IIa, GPIba, GP ADP.112 patients with VTE episodes in the past: 62 has ovarian cancer, 30 uterine carcinoma, 20 cervical carcinoma.

Results: We have detected the incidence of FV Leiden mutation is 22 (19,6%); homozygous gene MTHFR mutation is 46 (41,1%); heterozygous gene MTHFR mutation is 58 (51,8%); prothrombin mutaton is 18 (16,1%); gene PAI-1 polymorphism is 32 (28,6%); platelets glycoproteins polymorphism is 50 (44,6%).In control group FV Leiden mutation was in 3 patients (10%); homozygous gene MTHFR in 2 (6,7%), heterozygous gene MTHFR mutation in 5 cases (16,6%); gene PAI-1 polymorphism in 3 (10%) and platelets glycoproteins polymorphism is 3 patients (10%).

Conclusions: Presence of multigenic thrombophilia is the expressed trigger of VTE. So patients with multigenic thrombophilia should be permanently treated by anticoagulats (LMWH) under the control of thrombophilia markers, such as D-dimer, TAT complexes and platelet factor 4.

PB 2340 | Acute Pulmonary Embolism in Cancer Patients: Clinical Findings and Outcome

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Background: As a special group in pulmonary embolism (PE), the baseline characteristics, better therapeutic strategy and prognosis of patients with concurrent malignancy need to be investigated. (*Zhang et al, 2014).*

Aims: To evaluate clinical findings of PE in patients with cancer and to analyse outcome according to prescribed anticoagulation therapy.

Methods: The prospective cohort study included 195 patients (25 patients with cancer) with PE from a single centre in time period from June 2014 till December 2016. The data were analysed by SPSS 21.0.

Results: The overall 6-month mortality in patients with cancer and non-cancer patients was 32% [13.7-50.3] vs 6.6% [2.4-10.8] (p< 0.001). Mean time of death from discharge was 40.25 ± 50.9 [4.9-75.6] days. DVT was localized in lower limbs in 7 cases: 71% [38.0-100.0] - in upper leg, 29% [4.9-62.0]) - in lower leg. Dyspnoea, chest pain and swollen limbs were not significantly associated with malignancy (Relative risk-1.00 and Odds Ratio-1.03), moreover cough was less present in patients with cancer (Risk difference -0.2 [1.0-39.0]).

Mortality rate at 6 months was not significantly associated with clot localization in pulmonary arteries and higher D-dimer value (p=0.093 and p=0.562, respectively).

For long-term treatment after VTE 56% [36.5-75.5] received direct oral anticoagulants (DOAC), 32% [13.7-50.3] - vitamin K antagonist (VKA) and 12% [0-24.7] - low molecular weight heparin (LMWH). 6-month mortality was significantly higher for patients receiving DOACs (n=2; 14% [0-32.6]) compared to VKA (n=5; 63% [29.0-96.0]) (p=0.019). Bleeding during use of anticoagulants occurred in 3 cases (all DOACs; 21% [0-42.9]).

Conclusions: Mortality rate in patients with cancer and PE is significantly higher than in control group - 32% vs 6.6%. Patients who received DOACs had better survival compared to those who used VKA. In real-life practice LMWH is chosen in separate cases instead of being a first choice.

PB 2341 | Subarachnoid Hemorrhage and General Anesthesia Inhibit the Brain (G)Lymphatic System in Mice and Non-human Primates

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Background: Although we thought that the brain was devoid of a lymphatic system, recent studies demonstrated the existence of a brain

wide circulation of cerebrospinal fluid (CSF) which removes brain extracellular wastes: the glymphatic system. The pathophysiological mechanisms influencing the activity of this glymphatic system remain poorly understood.

Aims: Subarachnoid hemorrhage (SAH) is a frequent disease characterized by the presence of blood in the brain subarachnoid space, commonly leading to severe neurological deficits. We hypothesized that SAH may interfere with the brain glymphatic system by obstructing the CSF circulation routes. Since general anesthesia is associated with an increased deposition of brain extracellular proteins, we also investigated whether general anesthesia influences the glymphatic system activity.

Methods: We first evaluated the circulation of the brain CSF in mice and non-human primates (NHP), using contrast enhanced MRI. Then, animals were subjected to a minimally invasive SAH prior to an MRI evaluation of the impact of SAH on the brain parenchymal CSF circulation. To evaluate the impact of general anesthesia, we developed original methods of MRI and near infrared fluorescence in awake and anesthetized mice.

Results: We first demonstrated in non-human primates that the CSF actively penetrates the brain parenchyma, confirming the existence of the active circulation of CSF first described in rodents. SAH dramatically impaired the brain circulation of CSF, because of paravascular space obstruction by fibrin clots. Regarding the impact of general anesthesia, we demonstrated that the glymphatic system is mainly active during wakefulness and significantly impaired under general anesthesia.

Conclusions: Both subarachnoid hemorrhage and general anesthesia negatively affects the glymphatic system, with potential long-term consequences on extracellular protein accumulation such as the beta-amyloid peptide.

PB 2342 | Association between Hepatic Triglyceride Content and Coagulation Factor Levels: The Netherlands Epidemiology of Obesity (NEO) Study

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Background: Hepatic triglyceride content (HTGC) has been linked to levels of coagulation factors (F)VIII, IX and XI in one small study. However, it is unclear whether HTGC contributes to coagulation factor levels beyond overall and visceral fat.

Aims: To assess the association between HTGC and levels of fibrinogen, FVIII, FIX, and FXI while taking into account total body fat (TBF) and visceral adipose tissue (VAT).

Methods: In a cross-sectional analysis (NEO study), we included a random subset of participants who underwent magnetic resonance (MR) imaging and MR spectroscopy to measure VAT and HTGC (n=2,075). We excluded participants with missing data on coagulation factors, with liver disease, venous thrombosis or on anticoagulation (n=129). We examined associations between HTGC and coagulation factors by linear regression, adjusted for age, sex, ethnicity, education, alcohol intake, physical activity, smoking, estrogen use and menopause status (first model), adding TBF and VAT in a second model. Consent and ethical approval were obtained.

Results: 1,946 participants (52% men; median age 56 years, IQR 50-61) were analysed. As shown in Table 1, in the first multivariate model, coagulation factor levels increased dose-dependently across HTGC quartiles compared with the lowest quartile (reference category). With further adjustment for TBF and VAT (model 2), the associations between HTGC and levels of fibrinogen, FVIII and FXI across quartiles disappeared, while the association between HTGC and FIX levels, albeit attenuated, remained, as did the dose-response relationship.

Conclusions: HTGC was associated with levels of various coagulation factors of which FIX levels remained associated with HTGC after adjustment for TBF and VAT. Our results shed more light on the relation between obesity and venous thrombosis risk, including the potential that fatty liver contributes to venous thrombosis risk beyond total body and visceral fat.

TABLE 1 Association between hepatic triglyceride content (HTGC) and coagulation factor levels in 1,946 participants from the NEO study

	Mean differences of coagulation factor levels (95% Confidence Interval) compared with the reference category							
	Fibrinogen (mg/dL)		Factor VIII (IU/dL)		Factor IX (IU/dL)		Factor XI (IU/dL)	
HTGC (%) quartiles	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Q1 (<0.29)	281 (reference)	281 (reference)	119 (reference)	119 (reference)	103 (reference)	103 (reference)	112 (reference)	112 (reference)
Q2 (0.29-0.98)	6.3 (-5.3, 17.8)	-5.5 (-18, 6.7)	1.4 (-5.4, 8.2)	-1.2 (-8.3, 5.9)	9.8 (6.7, 12.9)	6.6 (3.6, 9.6)	4.2 (0.2, 8.1)	2.4 (-1.8, 6.5)
Q3 (0.98-1.84)	13.7 (1.0, 26.3)	-10.3 (-25, 4.3)	2.5 (-4.1, 9.1)	-1.9 (-9.2, 5.3)	18.4 (14.8, 22.1)	11.7 (7.8, 15.6)	7.2 (2.9, 11.6)	2.1 (-2.7, 6.9)
Q4 (≥1.84)	14.4 (1.8, 26.9)	-14.1 (-31, 2.2)	6.6 (0.4, 12.8)	3.2 (-5.1, 11.5)	26.1 (22.4, 29.8)	19.0 (13.8, 24.1)	8.4 (4.4, 12.5)	4.9 (-0.3, 10.2)

PB 2343 | Portal Vein Thrombosis Associated with Abdominal Inflammatory Conditions

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Background: Portal vein thrombosis (PVT) is an unusual-site thrombosis predisposed by conditions like cirrhosis, malignancies, myeloproliferative neoplasia (MPN) and abdominal inflammatory conditions (AIC). AIC represents the most acute of these etiologies and many patients do not have any other underlying chronic medical conditions. Given that management of patients with PVT is unclear, we investigated whether AIC conditions differed from the others in etiology, therapeutic intervention and prognosis.

Aims: To compare clinical and pathological features of patients with PVT associated with AIC versus other etiologies.

Methods: After IRB approval, medical records from 2005 to 2015 were reviewed by institutional data mining software to identify patients diagnosed with PVT by ICD9. The subgroup of PVT associated with AIC was selected for chart review. AIC was defined as due to an acute event occurring within 60 days preceding PVT diagnosis without history of other chronic diseases associated with PVT.

Results: PVT was identified in 698 patients. The most common etiologies were cirrhosis (198; 28.4%) cirrhosis with concomitant malignancy (198; 28.4%); followed by malignancy (151; 21.6%), idiopathic (95;13.6%), AIC (35; 5%) and MPN (21; 3%). Among AIC, the most common predisposing factors were pancreatitis (10; 28.6%), post-surgical (8; 22.9%), diverticulitis (5; 14.3%), and bacteremia (4; 11.4%). 82.9% received anticoagulation. Follow-up imaging was available for 24 cases with evidence for venous thrombosis recurrence in one case. Patients with AIC were younger, more likely to be black, have higher BMI, and were 3x more likely to receive anticoagulation (Figure 1).

TABLE 1 Baseline characteristics and anticoagulation in patients with PVT

	AIC PVT (n=35)	Non-AIC PVT (n=663)	Total PVT (n=698)	P*
Age, median (IQR25-75)	54 (40-66)	61 (54-69)	61(53-69)	0.0004
Gender, male (%)	14 (40%)	383 (57.8%)	397 (57%)	0.04
Hispanic (%)	11 (31.4%)	282 (43.7%)	293 (42%)	0.15
African-American (%)	14 (40%)	156 (23.5%)	170 (24%)	0.02
BMI, median (IQR 25-75)	29 (23.2-37.1)	26 (23.3-31.7)	26.9 (23.3-32.0)	0.001
Anticoagulation rate (%)	29 (82.9%)	88 (13.3%)	117 (16.7%)	0.0001
Heparin	8 (27.6%)	29 (33%)	37 (31.6%)	0.26
Coumadin with heparin bridge	20 (69%)	48 (54.5%)	68 (58.1%)	
DOACs	1 (3.4%)	11 (12.5%)	12 (10.2%)	

Abbreviations: AIC: abdominal inflammatory conditions; IQR: interquartile range; BMI: body mass index; DOACs: direct oral anticoagulants. P value represents the comparison between AIC and non-AIC PVT.

Conclusions: AIC is most commonly triggered by pancreatitis and surgical procedures. Patients with AIC are more likely to receive anticoagulation and have a low venous thrombosis recurrence rate. Whether the low recurrence rate is due to the presence of a provoked risk factor which abates or due to the increased use of anticoagulation is unclear.

PB 2344 | Role of ADAMTS13 Deficiency in the Pathogenesis of Portal Vein Thrombosis Complicating Liver Cirrhosis

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Background: Portal vein thrombosis (PVT) dramatically changes the prognosis of cirrhotic patients, especially those waiting for liver transplantation. However, the possible contribution to PVT pathogenesis of von Willebrand factor (VWF) and ADAMTS13, produced by liver stellate cells (LSC), is scarcely documented.

Aims: The aim of our study was to assess the presence of alterations of VWF and ADAMTS13 plasma levels in cirrhotic patients with PVT.

Methods: Thirty patients with PVT (group A) and 60 without PVT (group B) were enrolled and signed an informed consent. Biochemical and haemostatic parameters were measured. ADAMTS13 activity was studied by a synthetic fluorescent 86-amino acid substrate (FRET method). VWF level and activity were analyzed by chemiluminescence assays (Accustar, Werfen) and western blotting agarose gel SDS-electrophoresis.

Results: ADAMTS13 activity was significantly lower in group A (median=16.5% vs. 69.1%, p=0.0047). Group A showed a significantly higher VWF:act, (median:308% vs 203%, p=0.032), whereas no difference was observed for VWF:Ag, FVIII level and presence of thrombophilic factors. In multivariable logistic regression analysis performed on data concerning both group A and B, only the ADAMTS13 activity (p=0.007) resulted to be independently and inversely associated with PVT. ROC analysis showed that a cut-off level of 18% ADAMTS13 could predict the risk of PVT development (AUC 0.709, [SE=0.077, p=0.005])

Conclusions: ADAMTS13 activity is independently and inversely associated with PVT. The decrease of ADAMTS13 activity likely causes an intra-sinusoidal deposition of ultra-large VWF multimers that would favor thrombi formation in sinusoids and PV circulation. Prospective investigations are under way to clarify whether ADAMTS13 level could be considered a predictive biomarker of PVT development in cirrhosis.

PB 2345 | Haemostatic Changes in Cirrhotic Patients Treated with Direct-acting Antiviral Therapy

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Background: Advanced liver disease is characterized by severe alterations of haemostatic balance that can lead to both bleeding and thrombotic complications. Standard coagulation tests failed to detect this disequilibrium. Thrombingeneration test (TG) with and without thrombomodulin (+/- TM) has been reported as the best coagulation test able to quantify the coagulation impairment in patients with liver cirrhosis.

Aims: To evaluate the impact of HCV eradication following direct-acting antiviral (DAA) therapy in coagulation profile of patients with HCV-related liver cirrhosis as well as to correlate these changes with both the improvement of liver function test and the risk of portal vein thrombosis (PVT) development.

Methods: Patients with HCV-related cirrhosis treated with DAA were prospectively enrolled. TG test (+/- TM) was performed at baseline, at the end of therapy and 12 weeks (12W) after the end of therapy. During follow-up, PVT onset was recorded.

Results: Sixty patients were enrolled (Child A/B 50/10). All of them reached 12W after the end of therapy follow-up. Albumin ($p< 0.004$) and platelet count ($p< 0.001$) significantly increased after the end of therapy. Compared to baseline value (0.4), the endogenous thrombin potential ratio significantly improved after the end of treatment (0.8; $p< 0.001$) and 12W after (0.9; $p=0.002$). The 12W after end of treatment-lag time (time to coagulation start in TG test) was significantly longer in comparison to pre-antiviral values ($p< 0.001$). No statistically significant correlation was found between TG parameters and liver function tests at multivariate analysis. Median follow up was 9 months (4-14). During follow-up, one PVT episode was recorded (incidence 2%).

Conclusions: Eradication of HCV is associated with significant changes in TG profile, possibly related to the improvement of liver function. This amelioration may partially correct the disequilibrium of the haemostatic imbalance of liver cirrhosis, leading to a reduction in the risk of PVT development.

PB 2346 | The Risk of Venous Thromboembolism in Women with Polycistic Ovarian Syndrome: An Italian Study

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Background: The risk of venous thromboembolism (VTE) in reproductive-aged women varies from 0.5 to 1.2 cases/1000 patient/year. The polycystic ovarian syndrome (PCOS) affects up to 12-21% of reproductive-aged women. A reduction of global fibrinolytic capacity, increased plasmatic levels of PAI-1 and Ag t-PA has been repeatedly observed in PCOS. Women affected by PCOS show several comorbidities linked to increased cardiovascular and thromboembolic risk as visceral obesity, hyperinsulinism, diabetes mellitus, hypertension, metabolic syndrome. Moreover, they are often treated with long-term hormonal therapy, leading to a further modulation of the thromboembolic risk. Notwithstanding, the clinical relevance of VTE risk in women with PCOS is virtually unknown.

Aims: The objective of the study is to assess both the incidence rate of VTE in women with an objective diagnosis of PCOS and the role of the associated VTE risk factors.

Methods: We have designed a multicenter retrospective cohort study The main inclusion criteria was an objective diagnosis of PCOS, classified in four phenotypes, according to the consensus of AE-PCOS Society. All enrolled patients were scheduled for a follow-up visit to investigate the occurrence of thromboembolic events.

Results: From 1994 to 2016 the data of 1017 consecutive patients with PCOS were collected from 7 centers in Italy. The overall amount of follow-up time was 20034 years. During that time 14 subjects received an instrumental diagnosis of VTE (9 deep venous thrombosis and/or pulmonary embolism and 5 superficial venous thrombosis). Among these, 3 were unprovoked, 4 were associated with non-hormonal risk factors and 7 were associated to hormonal therapy. The overall VTE incidence rate was 0.7/1000 patients/year (95% CI: 0.4-1.2).

Conclusions: These preliminary data show that in our cohort of women affected by PCOS, the disease does not seem to increase the risk of VTE, even in patients treated with hormonal therapy.

TABLE 1 Baseline characteristics

Baseline characteristics	n (SD)
Mean age at menarch	12.2 years (1.54)
Mean BMI	24.6 (5.73)
Mean time of hormonal therapy	62 months (56)
	n (%)
Phenotype A	535 (52.6)
Phenotype B	302 (29.7)
Phenotype C	105 (10.3)
Phenotype D	75 (7.3)
Hormonal therapy users	834 (82)

PB 2347 | Fibrinogen Estimation in Patients with Liver Disease

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Background: Fibrinogen is mainly produced in the liver and hence, conditions like cirrhosis, may affect the levels of fibrinogen, leading to bleeding diatheses. There are different tests that can be used to measure quantitative and qualitative fibrinogen levels however, there is no agreement yet on which test is preferred.

Aims: Comparing 4 different methodologies ie the Clauss Fibrinogen, PT-derived Fibrinogen (PT-Fg), Fibrinogen Antigen (Fib-Ag) and Functional Fibrinogen Thromboelastography (FF-TEG) for estimating fibrinogen levels in patients with liver cirrhosis and healthy controls.

Methods: A total of 55 cirrhosis patients were recruited. 26 were Child Pugh (CP)-A, 14 CP-B and 15 CP-C. 20 healthy control individuals were also enrolled. The FF-TEG was performed using TEG® so as to obtain the estimated functional fibrinogen level (FLEV), whilst the Clauss Fibrinogen, PT-Fg and Fib-Ag were estimated on Sysmex® CS-2100*i*.

Results: All 4 fibrinogen assays gave significantly different fibrinogen levels. However, these assays were strongly and positively correlated with each other in both cohorts. The PT-Fg and the FLEV gave higher results than the Clauss and the Fib-Ag. When grouped according to CP severity score, there was a non significant trend in all assays showing fibrinogen to be raised in CP-A, normal in CP-B and decreased in CP-C when compared to normal. There was only a significant difference between CP-A and CP-C groups using the Fib-Ag, Clauss and PT-Fg but not the FLEV. Of note is that the PT-Fg overestimated fibrinogen levels by a mean of 1.03g/L when compared with Clauss.

Conclusions: Whilst the 4 assays correlate well with each other, there were no apparent differences according to CP severity groups and healthy controls in the FLEV. This is in contrast to the results obtained by the other 3 assays and points to normal fibrin clot strength in liver cirrhosis despite differences in fibrinogen levels.

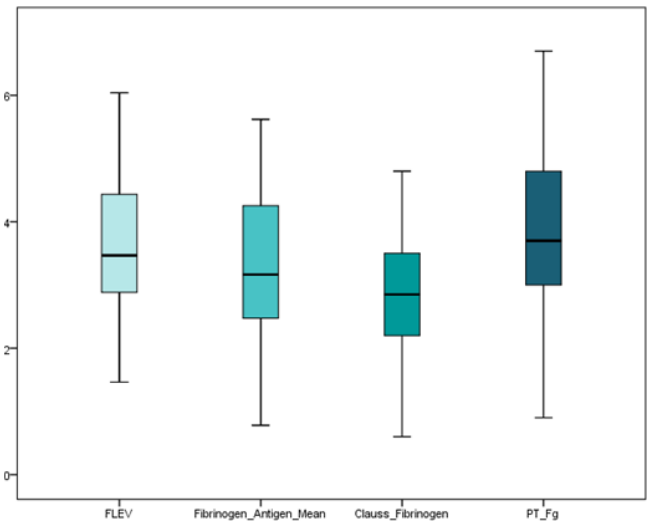

FIGURE 1 Box Plots of FLEV, Fib-Ag, Clauss Fibrinogen and PT-Fg in the liver cirrhosis population

PB 2348 | A Rat Model to Evaluate Formation of Venous Thromboembolism after Prolonged Tourniquet Application

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Background: The incidence of symptomatic venous thromboembolism (VTE) in patients undergoing total knee arthroplasty (TKA) is ~10% when no prophylaxis was administered. Despite modern anticoagulant prophylaxis post-operatively, some patients still develop VTE. We previously reported that prolonged tourniquet use during TKA was associated with VTE in the early postoperative period. Hereby, we used a rat model to investigate the pathophysiology of venous clot formation after tourniquet use.

Aims: To determine if deep vein thrombosis is associated with tourniquet application on the ipsilateral thigh of rats.

Methods: Male Sprague Dawley rats were anesthetized with isoflurane. A pneumatic pressure cuff was applied to the leg on the experimental side above the knee for a predefined time at a constant pressure three times above the arterial pressure. The leg without tourniquet served as control. Heparin (500 units) was injected intravenously immediately before block dissection of vessels from the ipsilateral and contralateral sides to prevent perimortem clot formation. Rats were then euthanized. The histology of the harvested vessels was examined after H&E, platelet- and fibrin-specific staining.

Results: The weight of the rats was ~325g. In the experimental leg where the tourniquet was applied for 3 hours at 260 mmHg extensive venous clots were noted whereas no clot was detected in the contralateral leg without tourniquet. Fibrin- and platelet-specific stains confirmed composition of clot in the experimental side but only little fibrin and platelet deposition was found in the control side. Pool of leukocytes was also found at the tourniquet application site.

Conclusions: This animal model will shed light on the pathophysiology of thrombosis caused by the use of hemostatic tourniquet during surgery. Its application during TKA may initiate venous clot formation; therefore, prophylactic dose of anticoagulant administered post-operatively is insufficient to abort clot propagation which then results in thrombotic complications.

PB 2349 | Increased Baseline TF Activity in Women with Unexplained Pregnancy Loss: An Additional Argument for a Basal Endothelial Dysfunction?

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Background: Tissue factor was identified as a crucial mediator of fetal and placental damage in mouse models of recurrent miscarriage. During pregnancy, an increased level of tissue factor activity (TFa) was reported in women with previous pregnancy loss, compared to healthy women.

Aims: To explore, at distance of pregnancy, the tissue factor pathway in unexplained pregnancy loss.

Methods:

Design: Incident case-control study.Patients: 199 women referred for unexplained pregnancy losses (≥ 2 losses before 21 weeks of gestational age, or at least one later loss), and 201 parous women without pregnancy loss.Intervention: Blood samples collection, at ≥2 months after any obstetric event.

Main outcome measure(s): Plasma levels of thrombomodulin activity (TMa), TFa, free tissue factor pathway inhibitor (f-TFPI).

Results: Among the patients (mean age: 32.5), 182 women experienced early losses and 28 suffered at least one later loss. Controls were significantly older (mean age: 34). When compared to controls, plasma levels of TFa were significantly higher in patients (all patients: OR, 2.2; 95% CI, [1.6-3.1], early loss: OR, 2.2; 95% CI, [1.6-3.1], later loss OR, 2.6; 95% CI, [1.5-4.5]). TMa and f-TFPI were not significantly different between groups. The TFa/ f-TFPI ratio was significantly higher in cases than in controls ($p < 0.001$). Adjusting for age and elapsed time since the last obstetric event did not influence our results.

Conclusions: This study suggests a baseline procoagulant activity in women with history of pregnancy loss. This provides an additional argument for basal vascular dysfunction in women with pregnancy loss.

PB 2350 | HABP2 (PHBP/FSAP) is the Major Plasma Protease that Promote Extracellular Histone Degradation

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Background: HABP2 (hyaluronan binding protein 2), alternatively designated plasma hyaluronan-binding protein (PHBP) or factor VII activating protease (FSAP), is a circulating single-chain zymogen consisting of an acidic amino acid-rich N-terminal region (NTR), 3 EGF-like domains, a kringle domain, and a serine protease domain. HABP2 converts itself to an active two-chain form, HABP2a, through autoproteolysis, which is triggered by polyanionic or polycationic substances. HABP2 activity is implicated in the regulation of the hemostasis as well as inflammatory processes. Cytotoxic extracellular histones which trigger a systemic activation of both the coagulation cascade and inflammatory pathways, potently promote HABP2 autoactivation.

Aims: To explore the role for HABP2 in histone degradation.

Methods: Histone-binding plasma proteins were identified by PMF analysis. Histone degradation was assayed by measuring fluorescence liberated from immobilized Rhodamine-histones during incubation with human plasma or plasma enzymes.

Results: Human plasma showed significant activity to degrade histones. Histone-binding plasma proteins identified by PMF analysis included prothrombin and HABP2. None of prothrombin, thrombin, protein C, or activated protein C were superior to HABP2 or HABP2a in histone degradation. Immobilized anti-HABP2 or histones removed histone-degrading activity from plasma, and anti-HABP2 inhibited histone degradation in plasma.

Conclusions: HABP2 is a major plasma factor responsible for extracellular histone removal.

PB 2351 | Adjunctive Hemostatic Application of Highly Adhesive Drug Loadable Powder for Partial Hepatectomy Bleeding in a Swine Model

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Background: Topical agents can be effective as adjuncts to aid in hemostasis when bleeding is not controllable with general methods. UI-SAH is a highly adhesive drug-loadable powder that has been developed for adjunctive hemostatic use. The hemostatic effects are achieved by forming a hydrogel and showing high adhesiveness and biodegradation on bleeding site. And besides, amine containing therapeutic molecules can be easily loaded into UI-SAH without chemical modification.

Aims: The aims of this study were to confirm 1) the effectiveness of the application of UI-SAH powder in hepatectomy bleeding swine model, 2) the prevention of organ adhesion after gelation at the bleeding site and 3) the feasibility of the drug loading capacity.

Methods: Adjunctive hemostatic application of UI-SAH was evaluated by hepatectomy bleeding in a swine model. After inducing bleeding, we topically applied UI-SAH on the bleeding site. The hemostatic effect and organ-adhesive were observed using blood loss, hemostatic time and organ-adhesion. The therapeutic effect of drug-loaded UI-SAH was evaluated in tumor bearing mice model.

Results: Severe bleeding was effectively controlled after applying the UI-SAH and re-bleeding was not observed until 72 h after treatment in a swine. In addition, Hydrogel that were converted from powder immediately after contacting water were act as anti-adhesion barrier.

In a tumor bearing mice model, the tumor volume of anti-cancer drug-loaded UI-SAH group was significantly decreased approximately 50% compared with those of positive control group on day 35 after tumor inoculation.

Conclusions: This study confirmed that the adjunctive hemostatic application of UI-SAH is effective for the in partial hepatectomy bleeding model due to high adhesiveness and the suppression of organ adhesion. Furthermore, tumor suppression can be accelerated by loading of the anti-cancer drug in the UI-SAH. The present study suggests that UI-SAH is promising candidate for adjunctive surgical hemostasis and local anti-cancer therapy.

PB 2352 | The ratio "Tissue Factor / Tissue Factor Pathway Inhibitor" as a Major Determinant of Hypercoagulabilty in Cirrhotic Patients

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Background: Patients with cirrhosis present abnormalities along both procoagulant and anticoagulant pathways, predicting the risk of bleeding or thrombosis is difficult. In this context we evaluated the contribution of thrombin generation assay (TG) and the determinations of Tissue factor pathway inhibitor (TFPI), tissue factor activity (TFa) procoagulant phospholipid (PPL), thrombomodulin activity (TMa) and the Pc pathway system to better understand the haemostasis in cirrhotic patients.

Aims: Analyze the thrombin generation, the expression of TFa , TMa or TFPI, and determine whether their concentrations decrease as liver disease progresses in cirrhotic.

Methods: Blood sample of 42 patients (12 Child A, 19 Child B and 11Child C) with confirmed cirrhosis and 30 healthy subjects were analysed for thrombin generation performed in the presence/absence of thrombomodulin, (Cat Assay, Stago,France), TFa and Thrombomodulin activities, and protein C and S by the Staclot protein C and S, Free TFPI by the Asserachrom Free TFPI (Diagnostica Stago, France).

Results: Comparatively to controls cirrhotic patients have a significant decreased of TG ($p < 0.001$), the ratios of TG in presence and absence of TM were increased ($p < 0.01$) and related to the severity of disease. Proteine C and S were decreased in comparison with controls ($p < 0.01$) and in function of the Child. TFa ,TMa and PPL were significantly increased ($p < 0.01$) whereas TFa and TMa were associated with the severity of disease, PPL did not change between the chlid groups. TFPI levels were comparable between patients and controls, however the ratios TF/TFPI increased with the Child and were significantly different in comparison with controls ($p < 0.01$).

Conclusions: It has been shown that TFPI plasma levels are reduced in congenital and acquired PS deficiency, probably due to the existence of complex between PS and TFPI.The findings may have clinical implication for the treatment or prophylaxis of thrombosis in cirrhotic patients.

PB 2353 | Upper Regulation of Plasma Endocan and D-Dimers in Individuals with Compensated Liver Disease

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Background: Endocan is a unique proteoglycan of endothelial cell origin that increases in response to endothelial dysfunction or injury. Similarly, D dimers in the plasma of individuals with compensated liver disease (CLD) are increased as a consequence of the mixed hyper- and hypo-coagulable state that exists in this population.

Aims: The goals of this investigation were to quantitate endocan and d-dimer levels in the plasma of individuals with CLD and to determine if a relationship exists between d-dimer and endocan in the plasma of individuals with compensated liver disease.

Methods: Sixty four individuals with CLD were identified. Blood samples were collected in both citrate and EDTA, separated by Centrifugation and the plasma was collected and frozen at -70° C until being assayed. Fifty normal healthy volunteers were included as controls. Endocan levels were determined using a commercially available ELISA kit (Lunginov,Nord, France). D-dimer levels were determined utilizing a commercially available kit (Diagnostic Stago, Parsippany, New Jersey, USA). The relationship between these samples of endocan and D-dimers was determined by linear regression analysis.

Results: Endocan levels in CLD were increased (2.3+ or -0.3pg/ml) as compared to the values of the control population (1.4+ or -0.3 pg/ml ($p < 0.05$). D-dimer levels in the plasma of the CLD subjects were markedly increased (2730+ or -610ng/ml) as compared to the values obtained in the healthy controls (230+ or -110 ng/ ml)($p < 0.05$). The relationship between these 2 biomarkers with a positive regression analysis is consistent with a common pathogenic mechanism determining their plasma levels.

Conclusions: Low levels of endotoxin in the portal venous circulation induce an endothelial injury within and limited to the liver that is responsible for the upper regulation of hepatic endothelial cell endocan production and secretion as well as the increased levels of D- dimers.

PB 2355 | Effect of Extract Ethanol of Poguntano Leaves in Hemostasis Disturbances in Alloxan Induced Diabetic Rats

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Background: The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population, and over 1,5 millioon of deaths were caused by the complications of diabetes. The elevated of blood glucose, free fatty acid and insulin resistance will cause the endothelial dysfunction, hemostasis

disturbances that lead to vascular complications that can increased the morbidity and mortality. Poguntano (Picria fel-terrae Merr) from family Scrophulariaceae found in most part of Indonesia, has been used as traditional plant for treatment of diabetes, fever, malaria and cancer.

Aims: The purpose of this study was to investigate the effect of extract ethanol of Poguntano in alloxan induced diabetic rats

Methods: This is an experimental study using alloxan induced diabetic rats, and were divided into three groups (control diabetic group, group given insulin injection and group given Extract ethanol of Poguntano 200 mg/Kg body weight), and control normal group. The duration of study was 4 weeks, blood glucose and Tissue Factor were measured for all groups.

Results: Extract ethanol of Poguntano 200 mg showed significant results ($p< 0.001$) in lowering blood glucose in Alloxan induced diabetic rats at four week after treatment, but did not show superior to insulin group ($p=0.892$). Tissue Factor level were lower significantly in diabetic rats treated with poguntano 200 mg ($p< 0.001$), but did not showed significant results in the insulin group ($p=0.799$).

Conclusions: In our study we have found that extract ethanol of Poguntano 200 mg showed statistical significant in lowering blood glucose and the Tissue Factor level in alloxan induced diabetic rats.

PB 2356 | Recurrent Budd-Chiari Syndrome (BCS) in a Patient with Polycythemia Vera (PV), Protein S Deficiency and Hyperhomocysteinemia (HH) and Abnormal Resistance to Activated Protein (APCR) in the Absence of Factor V Leiden Mutation (FVL) Following Liver Transplant

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Background: Budd-Chiari syndrome (BCS) is a rare condition recognized in imaging studies and characterized by thrombotic obstruction of the hepatic venous outflow.

Aims: Presentation of the BCS clinical course in a patient with PV and additional trombotic risk factors following liver transplant (LT).

Methods: Factor V Leiden mutation was detected using RFLP/PCR method (EURx,Poland); APCR and protein S deficiency using Siemens reagent.

Results: A 35 year old patient with BCS and PV, progressive fulminant hepatic impairment as result of decompensated liver cirrhosis was subjected to orthotopic cadaveric LT using piggy-back technique. Percutaneous endoscopic jejunostomy (PEJ) was used for nutritional support. Crossmatching was negative as no antibodies against lymphocytes were found in the recipient's serum. The transplanted organ took up function immediately after surgery. On the second day an inferior vena cava (VCI) obstruction was reported just below the right atrium. CT angiography revealed thrombotic obstruction of the hepatic venous outflow, hepatosplenomegaly, ascites that required paracentesis. Gastroscopic findings included oesophageal varices grade 1, reduction in daily urine output, increased blood urea, HH (19.8 mmol/L; n: 5.0-12.0 mmol/l), hypoproteinaemia, hypoalbuminemia, protein S deficiency - 53% (n: 67.5-139%), APCR 0.48 (n: 0.7-1.2); absence of FVL (G1691A) mutation. Balloon technique was applied for VCI obstruction which resulted in resolution of ascites. Successful therapy followed with Enoxaparinum natricum twice a day, hydroxycarbamidum and immunosuppressive corticosteroids, Mycophenolas mofetil and Tacrolimusum.

Conclusions: Protein S deficiency with HH and abnormal APCR in the absence of Leiden mutation may be considered risk factors for recurrence of hepatic vein thrombosis in a patient with PV following liver transplant.

PEDIATRICS

PB 511 | Safety, Efficacy and PK/PD of Rivaroxaban Tablets in Children with VTE. An Einstein Junior Phase II Evaluation

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Background: Rivaroxaban is being developed for VTE treatment in children, targeting the exposure of young adults treated with rivaroxaban 20 mg once-daily.

Aims: To investigate the safety and efficacy of rivaroxaban tablets for VTE treatment and to characterize its PK/PD profile.

Methods: Children 6-18yrs with VTE who completed ≥ 2 months of anticoagulation received 30 days of body weight-adjusted once-daily rivaroxaban tablets at a 20 mg equivalent dose, based on physiologically-based PK (PBPK) modeling predictions and Phase I data. Repeat imaging was performed at Day 30 and compared to baseline. PK/PD samples were taken on Day 15 and Day 31. PK results were compared with the pediatric PBPK model predictions and with the adult reference population of VTE patients treated with rivaroxaban 20 mg once-daily. PD results were compared to an adult VTE treatment population.

Results: 24 children (54 sites/10 countries) were enrolled (12-18yrs, n= 11; 6-12yrs, n= 13). No major bleeding or recurrent VTE occurred.

Repeat imaging was improved in 94%. Individual plasma concentrations, as well as AUC, C_{max} and C_{trough} at steady state, were in the prediction range of the PBPK model and the adult reference range of VTE patients. Children < 30 kg tended to have a C_{trough} at the lower end of values observed in adults. Individual values for PT/aPTT were covered by the adult reference range and for both a linear correlation between change from baseline and plasma concentrations was confirmed. A linear relation of anti-factor Xa activity and plasma concentration was observed.

Conclusions: 30 days with once-daily rivaroxaban tablets, at a dose targeting the exposure with rivaroxaban 20 mg once-daily in adults, was safe and efficacious. PK/PD profiles were as predicted. However, C_{trough} levels were at the lower end of the adult distribution in children < 30 kg, suggesting they require a twice-daily rivaroxaban regimen. Our results need confirmation in the Einstein Junior Phase III study.

PB 512 | Safety, Efficacy and PK/PD of a Rivaroxaban Suspension in Children with VTE. An Einstein Junior Phase II Evaluation

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Background: Rivaroxaban is being developed for VTE treatment in children, targeting the exposure of young adults treated with rivaroxaban 20 mg once-daily. A rivaroxaban suspension was developed to enable greater dose flexibility, easier administration, and better acceptance.

Aims: To investigate the safety/efficacy of rivaroxaban suspension for VTE treatment and to characterize its PK/PD profile.

Methods: Children 0.5-12yrs with VTE who completed ≥ 2 months of anticoagulation received 30 days of body weight-adjusted rivaroxaban suspension (1mg/mL) in a 10 mg equivalent dose using a twice-daily regimen, based on physiologically-based PK (PBPK) modeling predictions and Phase I data. Repeat imaging was performed at Day 30 and compared to baseline. PK/PD samples were taken on Day 1, 15 and 31. PK results were compared with pediatric PBPK model predictions and with an adult VTE reference population. PD results were compared to an adult VTE treatment population.

Results: 56 children (81 sites) were enrolled (6-12yrs, n=19; 2-6yrs, n=25; 0.5-2yrs, n=12). No major bleeding or recurrent VTE occurred. Repeat imaging was improved in 91%. Nearly all individual plasma concentrations, as well as AUC, C_{max} and C_{trough} at steady state, were in the prediction range of the PBPK model and the adult reference range. Individual values for PT/aPTT were covered by the adult reference range and for both a linear correlation between change from baseline and plasma concentrations was confirmed. A linear relation of anti-FXa activity and plasma concentration was shown.

Conclusions: 30 days of twice-daily rivaroxaban oral suspension, at a dose targeting the exposure with rivaroxaban 20 mg once-daily in adults, was safe and efficacious. PK/PD profiles were as predicted. The twice-daily rivaroxaban regimen resulted in consistent C_{trough} levels, regardless of body weight. Our results need confirmation in the Einstein Junior Phase III study.

PB 514 | Long-term Outcome in Children on Anticoagulant Therapy after Extracardiac Conduit Fontan Operation (FO)

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Background: FO is performed in children with tricuspid atresia and a variety of congenital heart defects. Several modifications have improved early and late mortality and morbidity, mostly due to thromboembolic events (TE) and arrhythmias. The incidence of thrombotic events and the event-related mortality are high, reaches 25-38%. There is no consensus about the best strategy to prevent TE.

Aims: To evaluate the occurrence of TE and/or bleeding events in children prospective cohort with the same type of FO under anticoagulant prophylaxis therapy.

Methods: Between August 1999 and July 2016, we conducted a prospective study to evaluate the occurrence of TE and bleeding events in 114 children (mean age 7.6 years, range 2-21; 60.5% boys) who underwent a modified FO (Fontan-Kreutzer) palliative procedure using a cavopulmonary connection with extracardiac PTFE conduit or none prosthesis. Acenocoumarol was initiated (INR target: 2.0-3.0) immediately after withdrawal of chest tube drainage (mean 6.4 ± 3.8 days, range 2-25). Quality of anticoagulation monitoring was evaluated with Rosendaal´s program. All parents signed informed consent. The protocol was approved by Institutional Ethical Committee.

Results: The total follow-up time was 982 years (mean 8.6 years). Eleven patients were lost to follow-up (mean 34 months, range 6-72). Patients remained in the desired INR range 67.7% of the time, 14% above (>3) and 18.3% below (< 2). At last follow-up, 23 patients had reached adulthood (mean age of 25.6 years, range 22-35). TE and bleeding events are described in table: 2/3 patients presented TE events (one dying) associated to interruption of acenocoumarol due to pneumonia; the third had had poor treatment compliance.

TABLE 1 Thromboembolic events (TE) and bleeding complications after surgery

Major events	Frequency and incidence rate per 100 patient-years	Fatalities related to TE/bleeding (months after surgery)	Events (months after surgery)
TE (N=3)	2.6%, 0.32	pulmonary embolism+ pneumonia (12)	1 case of stroke+ pneumonia (36); 1 case of stroke related to poor anticoagulant compliance (9)
Bleeding (N=4)	3.5%, 0.44	hemoptysis (156)	1 case of pleural puncture procedure and hemothorax (1); 2 cases of hemoptysis requiring cauterization by bronchoscopy with good outcome (36, 60)

Conclusions: Primary prophylaxis with oral anticoagulation, initiated rapidly after modified FO surgery could be considered a a safe and effective therapy in children. Studies on bridging strategies in these patients are necessary in order to limit the morbi-mortality associated.

PB 516 | e-Health Usefulness in Oral Anticoagulation Therapy in Children: Point of Care Instruments and Mobile APPs

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Background: Use of oral anticoagulation in children has increased during the last decade due to medical and surgical improvements. Point of care (POC) instruments for INR measurements may avoid frequent visits to the hospital and increase time in therapeutic range (TRT). Self-testing is easy to learn. However, self-management generally induces anxiety in caregivers. e-Health instruments as mobile APPs could be useful to overcome it.

Aims: Prospective clinical study in a Pediatric Universitary Hospital to evaluate safety, efficacy and quality of life of a POC instrument (CoaguChek), and a mobile application (TAONet Me).

Methods: After an initial training, patients started self-testing and management of anticoagulation. INR results, drug doses schedule, and questions were introduced in a mobile app. Information was daily reviewed by a pediatric nurse.

Results: A total of 38 patients were included in this program. Of them, 71.1% were boys. The median age was 11 years [range 2-16 years]. Reason for anticoagulation was: 13 mitral mechanic heart valve (MHV), 5 aortic MHV, 1 tricuspid MHV, 1 pulmonar MHV, 14 Fontan surgery, and other reason in 3 cases.

All families successfully completed an initial evaluation test. POC devices and mobile app were considered useful in improving their quality of life, by maintaining a direct communication with professionals, while decreasing medical appointments.

After a follow-up of 6-months, 26 of them had performed >30 INR measurements and were eligible to evaluate TRT. A statistically significant improvement of TRT from 56.1% to 64.1% ($p< 0.05$) was found. No thrombotic or mayor haemorrhagic events were diagnosed during the study.

Conclusions: POC and Mobile app instruments proved to be a useful tool in decreasing caregiver anxiety and improving their quality of life. Moreover, self-control and self-management of anticoagulation resulted in a significant increase in TRT, as compared to previous data.

PB 517 | Feasibility and Safety of Full Dose Anticoagulation Therapy in the Context of Thrombocytopenia in Children with Symptomatic Thromboembolism during Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma Treatment

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Background: There are no evidence-based guidelines for anticoagulation therapy (ACT) with low molecular weight heparin (LMWH) in relation to low platelet count.

Aims: To evaluate feasibility and safety of LMWH in children receiving myelosuppressive therapy.

Methods: Medical records of patients who received ACT with LMWH for symptomatic thromboembolism (TE) during ALL/LL therapy between 2007-2015 were reviewed for demographics, details of ALL/LL diagnoses, TE diagnoses and therapy, platelet counts during ACT, LMWH dose modifications, and bleeding episodes. ALL/LL was treated per the Dana-Farber Cancer Institute (DFCI) ALL Consortium protocols. Institutional ACT policy was full dose LMWH for platelets >30x10^9/L, half-dose between 20-30x10^9/L and hold LMWH for platelets < 20x10^9/L. LMWH was held for 24 hours prior to invasive procedure.

Results: Thirty-nine TEs were diagnosed in 33 patients during the study period. Six patients had more than 1 episode. Two patients (6%) were diagnosed with TE during Induction phase, 30 (91%) in asparaginase-intensive Consolidation II, and 1 (3%) during Maintenance; with mean time to TE 5.75 months from ALL/LL diagnosis. Platelets were measured

weekly with a mean count of 264x10^9/L (range 10-1373x10^9/L). On 29 occasions, platelet nadir was < 50x10^9/L and twice it was < 20x10^9/L. Four patients required platelet transfusions to facilitate ACT and 1 patient required LMWH dose adjustment for thrombocytopenia. One (3%) patient had major bleeding episode while on ACT (platelet count: 222x10^9/L). Ninety-two procedures were completed without bleeding complications. Although asparaginase was held with TE diagnosis, 32 patients completed all scheduled doses as per protocol (1 discontinued treatment due to pancreatitis).

Conclusions: Ability to administer full dose LMWH, expected bleeding rate, and completion of asparaginase doses while on ACT shows full-dose ACT is feasible and safe in children with ALL/LL who develop TE while receiving therapy according to DFCI ALL Consortium protocols.

PB 518 | Anticoagulant Effects of Dabigatran in Paediatric Patients Compared with Adults: Combined Data from Three Paediatric Clinical Trials

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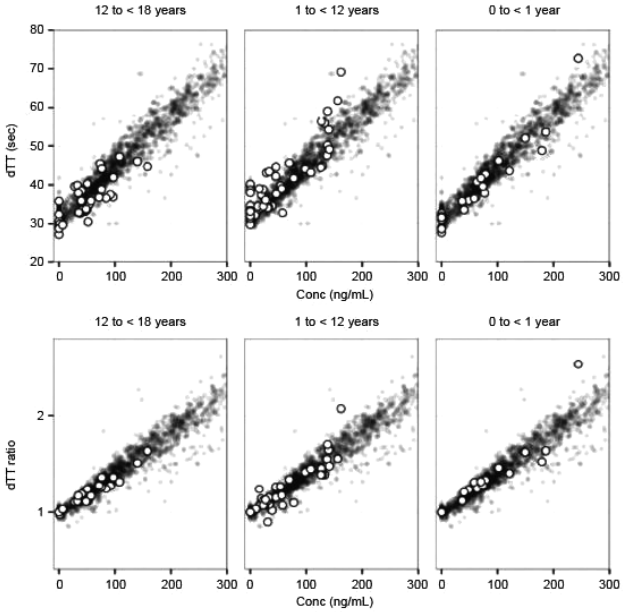
Background: Throughout childhood, physiological age-related changes in the haemostatic system affect responses to anticoagulants and mean coagulation assay responses may differ from those in adults.

Aims: To compare the relationships between plasma dabigatran concentration and coagulation assay results in children treated for venous thrombotic events (VTE) with those in adults.

Methods: Data from three trials in which children (n = 35) of different age groups received age- and weight-adjusted doses of dabigatran etexilate (DE) after standard of care for VTE were compared with those from adult volunteers (healthy or with mild/moderate renal dysfunction in two phase I trials) and adult patients (symptomatic VTE or undergoing hip replacement surgery in two phase III trials). The effects of dabigatran concentration on diluted thrombin time (dTT), ecarin clotting time (ECT) and activated partial thromboplastin time (aPTT) in children and adults were analysed graphically and with modelling.

Results: In the graphical analysis concentration-dTT relationships were consistent in children of all ages and adult volunteers (Figure 1) and patients; for ECT and aPTT, relationships based on ratios over baseline were similar across all ages. However, absolute clotting time data showed the same exposure resulted in longer clotting times in those aged 0 to < 1 year versus adult groups. Modelling showed concentration-clotting time relationships to be largely comparable between adults and children, except in those aged < 2 months (n = 4), in whom there was an upward shift of 10-15% or 10-20% in ECT or aPTT, respectively, relative to adults.

Figure 1. Concentration–dTT relationships for paediatric patients (white circles) and adult volunteers (grey circles) for each of the three paediatric studies



Top row shows untransformed dTT values; bottom row shows dTT expressed as a ratio over baseline.
dTT, diluted thrombin time

Conclusions: The effects of DE on aPTT, ECT and dTT were similar in children and adults with the exception of those aged < 2 months who showed a slight upwards shift in absolute ECT and aPTT relative to adults, although only four subjects were < 2 months. These data suggest that age-related differences in the haemostatic system will have little impact on response to dabigatran.

PB 519 | Factor Levels in Heparinized Critically Ill Pediatric Patients

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Background: Activated partial thromboplastin time (aPTT) and anti-factor Xa levels (Xa) are often discordant in critically ill heparinized pediatric patients, and this discordance is associated with morbidity and mortality. This is particularly manifest in patients requiring mechanical circulatory support (MCS). The underlying abnormalities that lead to discordance are poorly understood.

Aims: We sought to:
1) describe the variations in soluble factors associated with discrepancy of the aPTT/Xa ratio in critically ill pediatric patients;
2) describe the impact of MCS on the differences in factor levels.

Methods: Data was pulled from the electronic medical record of all heparinized patients. Statistical comparisons between patients with and without MCS were made using a t-test with unpaired t-test with

unequal variance. To demonstrate the factor variation related to aPTT/anti-Xa ratios, we conducted regression with fixed ranges of anti-Xa (0.2-1 IU/ml) in all patients.

Results: Regression analyses revealed a strong negative correlation between factors V, VII, IX, X. XII and antithrombin and the aPTT/anti-Xa ratio. Only a moderate negative correlation was observed between factors II and XI with the aPTT/anti-Xa.

TABLE 1 Regression Statistics of aPTT/Xa Ratio to Individual Factors in Pediatric Patients. * r = 0.3 to 0.5 or - 0.3 to -0.5; ** r < -0.5 or > 0.5

	m	r
Factor II**	-12.3	0.41
Factor V**	-4.2	0.81
Factor VII**	-12.6	0.57
Factor VIII	-35	0.06
Factor IX**	-10.6	0.57
Factor X**	-8.3	0.67
Factor XI*	-21	0.36
Factor XII**	-2.6	0.51
Antithrombin**	-13.5	0.74

Circulating levels of factors V, VIII, IX, XII were significantly diminished in patients on MCS relative to those not on MCS.

TABLE 2 Variation in Factor Levels between Patients in whom MCS Is/Is Not Applied. *p<0.05

	non-MCS	MCS	p-value
Xa	0.36	0.34	0.81
Factor II	55%	65%	0.3
Factor V	78%	55%	0.02*
Factor VII	59%	55%	0.69
Factor VIII	197%	140%	0.01
Factor IX	76%	62%	0.03*
Factor X	64%	57%	0.49
Factor XI	46%	44%	0.7
Factor XII	63%	44%	0.01

Conclusions: These results imply that consumption of multiple factors is responsible for discrepancies between the aPTT and anti-Xa in heparinized patients. Consistent with contact activation on an artificial surface, loss of factor XII was more significant in patients requiring MCS. The relative loss of factor VIII in MCS is consistent with previous studies showing loss of high molecular weight von Willebrand Factor multimers in MCS. The relative diminution in factors V and IX associated with MCS was unexpected. Future studies will be needed to determine if the loss of factors V and IX is associated with morbidity in this context.

PB 520 | Dosing and Therapeutic Drug Monitoring of Tinzaparin in the Pediatric Intensive Care Unit (PICU)

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Background: Tinzaparin dosing only leads to target anti-Xa levels (AL) after 8.8 (± 7.3) doses in PICU patients when using ACCP guidelines[1].

Aims: By determining the first AL after the first tinzaparin dose we aimed to achieve target AL quicker. Second aim was to describe tinzaparin dosing and therapeutic drug monitoring.

Methods: A retrospective analysis of tinzaparin dosing and AL in PICU patients. The study was approved by the institutional ethics committee.

Results:

Fifty-six children, median age 8.5 months [0-47.5] with newly started therapeutic tinzaparin. In 84% (47/56), the first AL was below target range, 0.22 (±0.13) IU/mL. A dose adjustment was made in 81% (38/47). Target AL were reached after a mean of 4.9 (± 3.0) doses. The dose needed to achieve target AL was significantly higher than the recommended dose in the age groups 0-2 months (p = 0.018), 2-12 months (p = 0.028) and 1-5 years (p = 0.012). Children with edema required a significantly higher final dose (328 ± 63 U/kg) than children without edema (283 ± 37 U/kg, p = 0.030). Patients with inotropes had significantly lower first AL (0.25 ± 0.19 IU/mL vs 0.41 ± 0.17 IU/mL; p = 0.023) and needed more time to reach target AL than patients without inotropes (5.4 days ± 3.0 vs 2.7 days ± 2.0; p = 0.027). Patients with mechanical ventilation had a lower first AL (0.24 ± 0.18 IU/mL vs. 0.38 ± 0.17 IU/mL; p = 0.007) and needed more time to reach target AL (5.7 days ± 2.9 vs 2.5 days ± 1.7; p = 0.002) than patients without mechanical ventilation.

Conclusions: The final tinzaparin dose needed to achieve target AL is significantly higher than recommended by the ACCP in children under 5 years. Edema further impacts on AL warranting an extra 10-15% dose increase. Concurrent use of vasopressors or mechanical ventilation leads to lower first AL and longer time to reach target AL, but a similar final dose. Decreased renal function does not lower tinzaparin dosing requirements. Based on our results, we recommend new starting doses for children admitted to PICU.

TABLE 1 results of tinzaparin drug monitoring

Age	0-2 months (n=15)	2-12 months (n=14)	1 - 5 years (n=18)	5 - 10 years (n=5)	10 - 18 years (n=4)	Total (n=56)
Recommended dose (IU/kg)	275	250	240	200	175	
Edema, n (%).	8 (53)	7 (50)	11 (61)	3 (60)	2 (50)	31 (55)
Inotropes, n (%)	11 (73)	10 (71)	18 (100)	4 (80)	4 (100)	47 (84)
Mechanical ventilation, n (%)	10 (67)	11 (79)	14 (78)	3 (60)	2 (50)	40 (71)
First anti-Xa level (IU/ml)	0.31 (+/- 0.23)	0.28 (+/- 0.18)	0.29 (+/- 0.21)	0.19 (+/- 0.04)	0.22 (+/- 0.05)	0.28 (+/- 0.19)
Target level reached, n.	4 (27)	2 (14)	3 (17)	0	0	9/56 (16)
Days to target level	4.1 (+/- 2.3) n=11	3.9 (+/- 2.0) n=8	5.7 (+/- 3.1) n=11	10 (+/- 5.7) n=2	4.0 n=1	4.9 (+/- 3.0) n=33
Final dose (IU/kg)	330 (+/- 43)	308 (+/- 50)	314 (+/- 67)	250	194	
Delta dose/kg (IU/kg)	53 (+/- 43), p = 0.018	55 (+/- 50), p = 0.028	74 (+/- 70), p = 0.012	50, p = 0.157	19, p = 0.317	60 (+/- 53) p < 0.001

PB 521 | Safety and PK/PD of a Single Rivaroxaban Administration in Children. An Einstein Junior Phase I Evaluation

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Background: Rivaroxaban is being developed for treatment of venous thrombosis in children.

Aims: To investigate the safety and to characterize the pharmacokinetic (PK)/pharmacodynamic (PD) profile of a single rivaroxaban administration in children.

Methods: Children aged 0.5-18yrs who had completed anticoagulation for any type of thrombosis were eligible. A single body weight-adjusted 10-mg equivalent dose of rivaroxaban was administered as either tablet (6-18yrs) or as oral suspension (0.5-12yrs). Physiologically-based PK (PBPK) modeling was used to predict age-specific weight-adjusted doses of rivaroxaban targeting the adult exposure of 10 mg once-daily. Serial PK and PD samples were taken up to 24 hours after dosing. PD parameters were the prothrombin time (PT), activated partial thromboplastin time (aPTT), and anti-factor Xa (aXa) activity.

Results: 30 children (45 sites/13 countries) were enrolled (12-18yrs, n=4; 6-12yrs, n=13; 2-6yrs, n=7; 0.5-2yrs, n=6). No clinically relevant bleeding occurred. Individual plasma concentrations, AUC, Cmax and C_24h were in line with PBPK predictions and the adult PopPK model. For both PT and aPTT, a linear correlation between change from baseline and rivaroxaban plasma concentrations was found, in line with data obtained in adult healthy volunteers. A linear relationship between rivaroxaban plasma concentration and aXa activity was confirmed.

Conclusions: A single rivaroxaban administration using tablets or oral suspension targeting the adult 10 mg exposure was safe in children. PK data were in line with the PBPK model, validating the model for predicting rivaroxaban doses in children. PD data in children were in line with data from adult healthy volunteers indicating that developmental differences in hemostasis do not influence the PK/PD response of rivaroxaban in children above age 0.5 years. Our results need to be confirmed in the ongoing Einstein Junior phase II study.

PB 522 | Neonatal Portal Vein Thrombosis: Characteristics and Role of Anticoagulation

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Background: Portal vein thrombosis (PVT) is a relatively common complication in neonates. The benefit of anticoagulation has not been clearly defined.

Aims: To describe the short and long-term outcomes of neonates with PVT treated according to an institutional protocol (anticoagulation vs no anticoagulation) with respect to thrombus progression, resolution, lobar atrophy, portal hypertension, and bleeding complications.

Methods: Data from all consecutive patients referred to a single Thrombosis Center between Jan 1st, 2008 till Oct 8th, 2015 were retrospectively collected. Institutional review board approved the study. Groups were compared using the chi-test, Fisher exact test, and Mann-Whitney U test, as appropriate.

Results: 221 neonates were included, with median follow up 3.10 years (0.014-8.33). Treatment group (TG) consisted of 112 and

non-treatment group (NTG) of 109 patients; basic data are provided in Table 1. Thrombus burden (Grade 1, 2 and 3) for each case was captured. The rate of progression in the TG was lower than in NTG (p=0.0426). With respect to resolution of the thrombus and development of lobar atrophy no differences between TG and NTG group were observed. After controlling for treatment, thrombus burden was predictive for both thrombus resolution (p< 0.0001) and lobar atrophy (p< 0.0001).

1 case of portal hypertension (incidence 0.45%) was noted (NTG group; 5.5 months since the diagnosis of PVT). Major bleeding occurred in 10 out of 112 (8.93%) of treated patients.

TABLE 1 Basic demographic data

	Treated group (n=112)	Non-treated group (n=109)	P value
Male:female ratio	1.73 (71:41)	1.87 (71:38)	0.8834
Corrected gestational age at the time of diagnosis of PVT; Weeks - median (range)	38 (25-43)	37.5 (25-43)	0.2040
Number of days since insertion of UVC to diagnosis of PVT; median (range)	6 (0-22)	6 (0-29)	0.7948
Follow up, Years - median (range)	3.17 (0.07-8.33)	2.75 (0.014-7.61)	0.4472
PVT grade initial, Number (%)			
Grade 1 (single branch non occlusive)	n=27 (24.1%)	n=52 (47.7%)	0.0126
Grade 2 (single branch occlusive)	n=68 (60.7%)	n=47 (43.1%)	0.1665
Grade 3 (more branches, parenchymal changes)	n=17 (15.2%)	n=10 (9.2%)	0.3077

TABLE 2 Late sequelae - details

	Treatment group (n=112)	Non-treatment group (n=109)	P value
Resolution of the PVT; number (%)			
Complete	68 (60.7%)	83 (76.1%)	0.2928
Partial	14 (12.5%)	6 (5.5%)	0.1077
None	30 (26.8%)	20 (18.4%)	0.2733
Risk of left hepatic lobe atrophy - based on resolution of PVT; number (%)			
Full resolution	10 (14.7%)	10 (12.0%)	0.8119
Partial resolution	4 (28.6%)	0 (0.0%)	0.5392
No resolution	24 (80%)	15 (75.0%)	1.0
Portal hypertension	n=0	n=1	NA

Conclusions: The only significant late sequelae of neonatal PVT, portal hypertension, is a rare event. Anticoagulation was associated with decreased PVT progression. Increased thrombus burden is a predictor of decreased thrombus resolution and increased lobar atrophy. No difference in rate of lobar atrophy and portal hypertension in TG vs NTG was observed.

PB 523 | The Incidence, Assessment and Management of Mental Health Issues in Young People on Anticoagulation Therapy Secondary to Idiopathic Venous Thromboembolism

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Background: Idiopathic venous thromboembolism (VTE), is a relatively rare condition in young people, which necessitates anticoagulation therapy (AT) and resultant lifestyle changes. The impact of commencing AT on the mental health of young people has not previously been reported.

Aims: This study aimed to identify the prevalence of mental health issues in young people diagnosed with idiopathic VTE.

Methods: The retrospective study selected young people (10-19 years) from the warfarin database at The Royal Children's Hospital, Melbourne with idiopathic VTE diagnosed between 2003 and 2014. Demographic details, assessment data and treatment details were extracted from the medical record. A mental health assessment was defined as any written documentation containing details about the patient's mood, specifically symptoms of depression or anxiety.

Results: Thirty eight eligible young people were identified (20 males). Median age was 15 years (range 12-19). Primary diagnoses were: 23 deep vein thrombosis (DVT), 10 pulmonary emboli (PE), and 5 combined DVT and PE. Six patients had a documented HEEADSS assessment (Home environment, Education and employment, Eating and peer-related Activities, Drugs, Sexuality, Suicide/depression and Safety), 3 of which were referred to the Adolescent Medicine department. A mental health assessment was documented for 23.7% of patients. Three patients had pre-existing depression prior to their VTE. 28.9% (11) of patients were referred from Clinical Haematology to a mental health service. Of these, 11 patients 54.5% had a documented mental health diagnosis, including the 3 patients with pre-existing depression.

Conclusions: 15.8% of young people diagnosed with an idiopathic VTE were diagnosed with a mental health disorder, suggesting a higher prevalence of mental health disorders in this sub-population of young people compared to population-based estimates. Furthermore, this study highlights the significant impact idiopathic VTE and AT has upon the mental health of young people.

PB 524 | Investigating the Experience of Parents who Have Given their Children Enoxaparin at Home

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Background: Enoxaparin is commonly used in paediatrics for outpatient treatment and prevention of thrombosis. Parents are often responsible for the safe preparation and administration of enoxaparin when their child goes home. However, little is known about the parental experience of administering enoxaparin to children post-discharge.

Aims: To explore the experience and educational needs of parents whose children require enoxaparin anticoagulation at home.

Methods: A qualitative, descriptive methodology was employed using two focus groups to generate data. An experienced moderator facilitated session with parents who had enoxaparin to their child post-discharge for at least 6 weeks. These sessions were recorded, transcribed and thematically analysed.

Results: 12 parents participated in two focus groups. The figure presents the emergent key themes and subthemes. Without exception, parents described trauma, and felt overwhelmed and distressed at the reality of giving their child clexane injections at home. Furthermore, many reported that they felt insufficiently trained and had particular difficulty preparing the correct dose from manufactured stock solutions. Parents also reported problems with communication, and felt that they were not adequately educated on the practicalities of enoxaparin administration prior to discharge by clinical staff. These practicalities included the supply of weight-appropriate equipment and dosages and rationale for use of subcutaneous injection catheters versus direct injection. Communication problems contributed to the trauma of the experience, as parents felt isolated and uninformed when they were discharged home.

Conclusions: Parents felt unprepared and traumatised when they had to administer enoxaparin to their child at home. Further research is needed regarding the psychosocial impact of parental administration of enoxaparin post-discharge and the optimal approach to supporting this extension of parent care-giving.

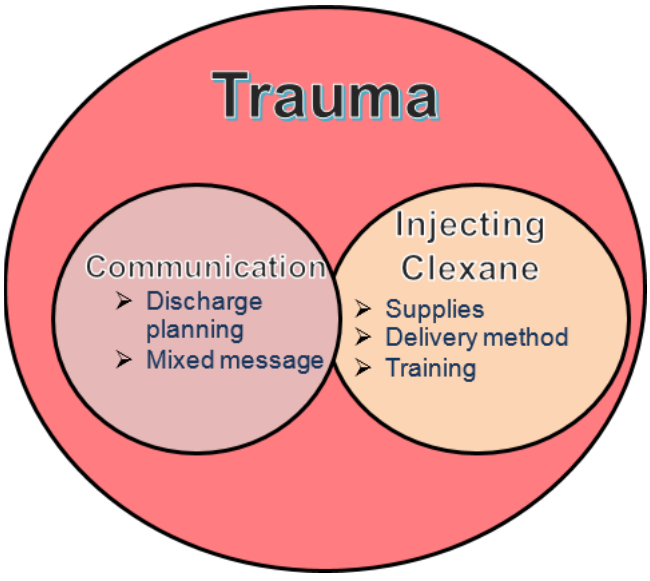

FIGURE 1 Emergent key themes and subthemes

PB 525 | A Pilot Feasibility and Safety Multicenter Trial of Administering Weight Adjusted FIXed Dose of Low Molecular Weight Heparin (Enoxaparin) to Neonates with Thrombosis (FIXET): Study Protocol

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Background: Enoxaparin is a commonly used low molecular weight heparin (LMWH) for the treatment of neonatal thrombosis that is monitored with anti-factor Xa (anti-Xa) levels. The therapeutic range

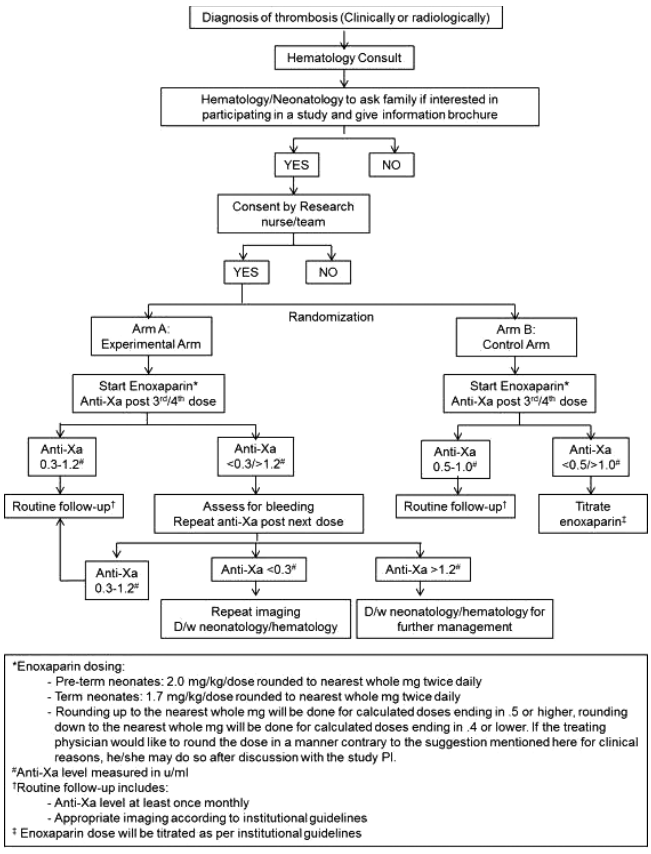

FIGURE 1 Proposed process for screening, recruiting, treating and following a subject

of anti-Xa (0.5 - 1.0 u/ml) was extrapolated from adult studies. The burden of pain to neonates due to venipunctures and of resources to the health care system also warrants an assessment of the utility of monitoring LMWH therapy with anti-Xa levels.

Aims: The aim of this trial is to determine the feasibility and safety of doing a randomized control trial to compare the approach of treating thrombosis in neonates with enoxaparin using weight adjusted fixed dose to variable dose titrated to maintain a pre-determined anti-Xa range (0.5-1.0 u/mL).

Methods: We plan to recruit 20 neonates among four centers over the study period. Figure 1 outlines the study schema.

Premature and term neonates with thrombosis diagnosed by imaging will be included. Key feasibility outcomes include screening/recruitment ratio, recruitment rate, and completeness of data collection. Bleeding and resolution of thrombosis will be measured as secondary outcomes.

Premature and term neonates will receive enoxaparin at a dose of 2.0 and 1.7 mg/kg/dose, respectively, rounded to nearest whole mg twice daily. Patients will have blood sampling for anti-Xa levels drawn after the 3rd or 4th dose from starting or adjustment, followed by at least once monthly. Patients will have appropriate imaging according to institutional guidelines. The duration of enoxaparin therapy will be at the discretion of the treating physician.

The trial has been approved by Hamilton Integrated Research Ethics Board and Health Canada. The trial is registered with clinicaltrials.gov.

Results: The trial has recently started and to date 2 patients have been enrolled and randomized.

Conclusions: We hope to establish feasibility and safety through this pilot study to progress to a full trial and decrease the burden of monitoring in this population.

PB 526 | Secondary Antithrombotic Prophylaxis in Children with Right Atrium Thrombosis

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Background: Current recommendations on treatment of right atrium thrombosis (RAT) in children are based mainly on data obtained from studies in infants with cardiac disease. Here we present a retrospective analysis of low molecular weight heparin (LMWH) use in children with RAT hospitalized in large Hematology-Oncology tertiary hospital.

Aims: To analyze efficacy and safety of LMWH use in children with RAT.

Methods: We analyzed retrospective medical data about secondary antithrombotic prophylaxis (SAP) with LMWH in 23 children (13 boys, 10 girls) aged 1-17 years. All patients had confirmed diagnosis of RAT and had at least 2 echocardiography exams to show thrombus size dynamics (change in size for more than 5 mm). Moreover we analyzed presence of symptomatic PE and all clinically relevant bleeding (CRB) episodes occurred 24 hours after LMWH injection. SAP was accepted as effective when there were no increase in thrombus size or pulmonary embolism (PE) episodes and safe when there were no episodes of CRB.

Results: All patients had central venous line (CVL) at the time of diagnosis and no one had any clinical symptoms of thrombosis or cardiac insufficiency. After RAT evaluation, all patients started SAP with LMWH (dalteparin or enoxaparin) at dose of 100 IU/kg twice daily s/c. LMWH dose was reduced to 50% in cases of platelet drop less than 100 10^9/L, and withheld in cases of platelet drop less than 30 10^9/L. During the observation period (5-426 days, Me=55 days), reduction of thrombus size (TS) appeared in 10 (43,5%), increase of TS - in 2 (8,7%) patients, while absence of any TS dynamics was found in 5 (21,7%) children. No PE or CRB episodes were found during the observation period.

Conclusions: Secondary antithrombotic prophylaxis with LMWH in children with right atrium thrombosis is effective and safe.

PB 527 | Thrombotic Storm in an 11 Year Old Boy

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Background: Thrombotic storm was first described by Kitchens in 1998 as a clinical pattern of developing acute to subacute thrombosis over a period of days to weeks with progressive involvement of multiple sites, including several unusual sites.

Aims: To describe the case of a 11 years old boy with thrombotic storm.

Methods: An 11 year old boy presented with progressive headache for 7 days along with facial asymmetry noted on the day of admission. He had had no significant illness in the past and was developmentally normal. At presentation, he was conscious, oriented and alert and had normal vital signs. He had left sided lower motor neuron facial palsy; rest of the physical examination was unremarkable. His further clinical course and laboratory investigations are summarized in Tables 1 and 2 respectively.

Results: Anticoagulation was initiated with intravenous heparin. In view of a clinical possibility of catastrophic antiphospholipid antibody syndrome (elevated IgM anti-β2 GPI antibody titres), he was also given injection methylprednisolone pulse therapy (30 mg/kg/day for 5 days) followed by oral prednisolone. Although he also had low levels of Protein C and Protein S at this time, it was difficult to interpret this finding during the stage of acute thrombosis. Protein C and Protein S levels were also tested in both parents and were in normal range.

Heparin was replaced by oral warfarin and the INR was targeted between 2 to 3.

He has remained well over 14 months of follow-up. Prednisolone has been tapered and stopped. Warfarin is being tapered now. We plan to stop warfarin after 2 months and repeat the procoagulant work-up thereafter.

TABLE 1 Course during hospital stay

Day of hospital stay	Event	Management
Day 1	Cortical sinus venous thrombosis evident on MRI brain	Subcutaneous low molecular weight heparin (LMWH)
Day 2	Status epilepticus	Multiple anti-epileptics and continued on LMWH
Day 4	Pulmonary thrombosis evident on CT angiography (sudden onset respiratory distress and cardiac arrest, requiring cardiopulmonary resuscitation)	Intravenous infusion of unfractionated heparin maintaining aPTT between 70-90s, ventilated from day 4 - day 8
Day 5	Thrombus in right superficial femoral vein and popliteal vein (picked up on ultrasound doppler)	Continued on unfractionated heparin infusion
Day 22	Thrombus in left superficial femoral, common femoral and popliteal vein (symptomatic in form of pain and swelling and confirmed on ultrasound doppler)	Continued on unfractionated heparin infusion
Day 24	Thrombus in right internal jugular vein and external jugular vein (symptomatic in form of neck pain and confirmed on ultrasound doppler)	Continued on unfractionated heparin infusion
Day 34	No new thrombus and recanalization of vessels	Heparin infusion tapered and initiated on oral warfarin
Day 45	Discharged with no neurological deficits	On oral warfarin with target INR between 2-3

TABLE 2 Investigations carried out during admission

Investigation	Result
Haemoglobin	83 gm/L
White blood cell counts	10.8 × 109 /L (P80;L12;M7;E1)
Platelet counts	165 × 109/L
HIV serology by ELISA	Non-reactive
ANA	negative
ANCA	negative
HLA B5	negative
C3; C4	170mg/dl(normal); 53 mg/dl (normal)
Prothrombotic work-up: i)CD55/CD59 (for paroxysmal nocturnal hemoglobinuria); ii)Protein C; iii) Protein S; iv)Factor V Leiden mutation; v)IgG Anticardiolipin antibody (GPLU/mL); vi)IgM Anticardiolipin antibody (MPLU/mL); vii)IgG Anti β2 GPI (U/mL); viii)IgM Anti β2 GPI (U/mL); ix) Serum homocysteine	i)Normal ii)58% (normal range: 63-123) iii)24% (normal range 67-143) iv)Absent v)2.1 (Normal <10) vi)1.1 (Normal <7) vii)1.3 (Normal <5) viii) initially 8.0 (Normal <5); was normal on follow-up after 3 months ix)9.35 µmol/L (Normal: 5-15)

Conclusions: Thrombotic storm is a rare and potentially life threatening condition. It is extremely uncommon in young children. Early and aggressive anticoagulation remains the cornerstone of management.

PB 528 | Variation in Practice in Relation to Antithrombotic Therapy and Monitoring After Paediatric Cardiac Surgery: Results of a United Kingdom Survey

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Background: Evidence for dosing, monitoring and intensity of antithrombotic therapy after paediatric cardiac surgery is limited.

Aims: To identify current UK practice.

Methods: A survey was sent by electronic mail to a haematologist in each of the 11 UK centres.

Results: 8/11 centres returned data. Initial post-operative anticoagulation is exclusively with unfractionated heparin (UFH). Initial therapeutic UFH dose is 28 U/kg/hr in infants and 20 U/kg/hr in older children in 6/8 centres. Four centres primarily use APTT and 4 centres anti-factor Xa activity (aXa) to guide dose adjustment. Target range for APTT is 60 to 80, 85 or 90 seconds or ratio 2-2.5 and for aXa is 0.35 -0.7 IU/mL, monitored a minimum of 24 hourly. Prothrombin time and fibrinogen monitoring vary from 1-3 times weekly to 4 hourly. Thresholds for platelet and fibrinogen transfusion are 30-50 $x10^9$/L and 1.0 g/L, respectively. No centre monitors antithombin (AT) levels routinely during therapeutic UFH. AT level is measured when difficult to achieve therapeutic anticoagulation and replacement therapy given at a threshold of 30-100 IU/dL using plasma (1 centre), AT concentrate (3) or either (3). UFH dose is reduced during AT replacement in 3/7. UFH infusion is used to maintain line patency in 5 centres, at a dose of 5-25 U/kg/hr, unmonitored in 2. Two centres give a third group 20 or 28 U/kg/hr UFH, without a target APTT/aXa, monitored only for safety. Low molecular weight heparin (LMWH) is dosed as per international guidelines in 2/8 centres, monitored in all children, timing of aXa levels varying from 2-6 hours post dose. Five centres avoid warfarin in infants. Two have prescribed a direct oral anticoagulant outside of a trial. Antiplatelet therapy, predominantly aspirin, is monitored in selected cases in 4, using a variety of techniques. Five centres use warfarin after uncomplicated Fontan surgery, 4 longterm, the remainder using aspirin.

Conclusions: Variation in practice within the UK exists, highlighting a need for research and guideline provision.

PB 529 | Successful Endovascular Thrombectomy of an Extensive Cerebral Venous Thrombosis with Bilateral Thalamic Infarction in A 16 Year Old Girl: Case Report and Therapeutic Algorithm

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Background: Cerebral vein thrombosis is a rare but potentially life threatening disease in children. Overall mortality is 10%; prognosis is worse in the presence of thrombosis of the inner cerebral veins or parenchymal lesions. There is uncertainty with regard to the optimal treatment strategy. Endovascular interventions have not been included in treatment recommendations so far.

Aims: To illustrate that mechanic thrombectomy is a successful treatment for selected patients with cerebral venous thrombosis. To present a treatment algorithm considering endovascular thrombectomy as treatment option for pediatric patients with cerebral vein thrombosis.

Methods: Case Report.

Results: A 16 year old girl presented with a 24 hours history of headache, progressive detoriation and decreasing levels of consciousness. MRI revealed a thrombosis of the great cerebral vein, the straight, left sigmoid and transverse sinus. Despite therapeutic anticoagulation with low molecular weight heparin the clinical condition worsened; MRI now showed growing thrombosis with bilateral hemorrhagic thalamic infarction.

Mechanical thrombectomy was performed by combining aspiration of thrombotic material via a microcatheter system (Sofia 5F) and additionally capturing thrombotic material via a stent retriever system (Trevo XP 4/20). Recanalization of the sigmoid and transverse sinus as well as partial recanalization of the great cerebral vein could be achieved and maintained. The clinical condition improved rapidly; one year after the event the patient continues her training as a saleswoman, while still suffering from headaches and impaired vision.

Conclusions: Given the potential benefit, mechanic thrombectomy needs to be considered in patients with (i) progressive thrombosis despite therapeutic anticoagulation (ii) secondary congestive bleeding or (iii) increased bleeding risk. We propose a therapeutic algorithm for children and adolescents with cerebral vein thrombosis treated at our center.

PB 530 | Management of Henoch-Shönlein Purpura in Children with Heparin. Russian Long-term Experience

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Background: Henoch-Schönlein purpura (HSP) is an acute immunoglobulin A-mediated disorder characterized by a generalized vasculitis involving the small vessels of the skin, the gastrointestinal tract, the kidneys, the joints. The usuall managemen of itt in many countries includes: steroinds and NSAID. Not much articles are devoted to heparin treatment and bed rest.

Aims: To show one of option for treatment of this disease with good outcomes which is used in Russia as a standard for all childen.

Methods: We have analyzed 242 patients case histories. All patients were admitted to hematological department of State Medical Hospital1 from 2006 to 2016y. All of them had rash, joint and abdominal pain and diagnosis of HSP was made, other conditions was excluded. Children were treated due to Russian standards of care.

Results: All children recieved heparin continuous infusion. Dosage was from 400U/kg/ day to 560U/kg/day. It was controlled by APTT. Bed rest was for 10 days. Steroids was given 2mg/kg prior to tird pain free day. Rash was gone on the 4-9th day (average 5,6), no abdominal pain was on the 4-12th day (average 8,2). Steroids were sropped on the 3-19th(average 6,3). Also hypoallergenic diet was recommended. Only 8 patiens had relapses. No creatinine and urea elevation was found. Only 19 patiens develop urinary syndrome and only 6 required surgical treatment.

Conclusions: We strongly recommend to keep in mind our heparin treatment option. We do not have hemorrhagic complications, we use steriods for shorter period of time. Only 3% of patients develop urinary syndrome. Only 2% need surgical testicular or gastrointestinal complications.

PB 531 | Inherited Antithrombin Deficiency in Children: Treatment of Massive Cerebral Sinus Venous Thrombosis in a Neonate with a Mutation in Gene SERPINC1 (Exon 3 C481c>t)

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Background: Inherited atithrombin deficiency (ATD) is a proven major risk factor of thrombosis. In children with venous thromboembolism (VTE), ATD is described to generate 6.6% of events and cause severe thrombosis in children. Its management is difficult in smaller infants

Aims: To expose the management of a neonate with ATD and severe sinus venous thrombosis (SVT) in a tertiary center

Methods: Clinical case review

Results: A 4 days old neonate with normal pregnancy and delivery, only single coil of umbilical cord, was admitted to hospital with seizures. Cerebral magnetic resonance image (MRI) showed massive SVT and disseminated ischemic injuries. Enoxaparin was started at 2mg/

Kg/12h. First anti-Xa 0.1 UI/mL. His mother had ATD (42-46%). No VTE history. Neonate AT was 18%. We started AT concentrate at 40UI/Kg. Successive AT concentrate doses were modified to obtain AT around 100% (median 109%, [47-169]). Median AT concentrate was 85UI/Kg/day, [70-120]. We achieved adequate anticoagulation with a median of anti-Xa of 0.69 UI/mL, [0.42-1.10]. After 5 weeks of treatment, MRI showed improvement with almost complete resolution of thrombosis, small thrombosis rests. After 59 days of AT treatment, indefinite oral anticoagulation was started. No hemorrhage or new thrombosis were seen. There are no neurological sequels.

Without substitutive treatment infant has AT 46%. A mutation in SERPINC1 gene was detected (exon 3, C481c>t). Five of nine (55%) affected members of the family have developed VTE. Two of them under 10 years old. Only 2 of them showed clear additional risk factors

Conclusions: An early diagnosis of ATD is essential for an adequate management of VTE in children.

Treatment in neonates with ATD must be tailored. Satisfactory clinical evolution in our patient is the result of the maintenance of AT around 100%, that allows adequate anticoagulation controlled with anti-Xa and avoids treatment failure or hemorrhage complications.

It´s difficult to correlate so different phenotype with the same genotype in this family

PB 532 | Venous Thromboembolism Prevention in Children

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Background: Venous thromboembolism (VTE) in children has a low prevalence, but prophylaxis in young patients is an emergent issue for increased survival of children at high risk (prematurity, congenital heart disease), wider use of high risk procedures (surgery, central lines) and of prothrombotic drugs

Aims: In adults idiopathic VTE is prevalent, in children VTE is often secondary to transient or permanent risk factors (congenital heart or kidney disease, devices, cancer). These factors should guide us in the choice of the right prophylaxis.

Methods: Between April 2013-March 2014 we evaluated a selected population of diplegic children who underwent orthopedic corrective surgery (fasciotomy, osteotomy). We adopted an internal protocol to uniform the patient/procedure VTE risk and assess when to perform prophylaxis. We considered individual factors (age, puberal development, overweight/obesity, congenital conditions such as thrombophilia), preexistent conditions at risk (previous VTE, chronic inflammatory bowel disease, nephrotic syndrome), active cancer, ipomobility, either preexistent or induced, prothrombotic drugs and devices. Prophylaxis with enoxaparin was adopted when at least 3 factors were present

Results: . We studied 25 children (12 M, 13 F, mean age 14.3 ys) who underwent VTE medium-high risk surgery (osteotomies 36%, fasciotomies 56%) followed by a long immobilization period/cast. We decided to use enoxaparin in 20/25 patients, mechanical prophylaxis in 5. In all the patient on enoxaparin antiXa activity was measured on day 2 to define the right dose: in only 2 children heparin dose had to be modified, mean antiXa levels were 0,345, (in prophylactic range). Follow-up at 1 and 6 months didn‘t show haemorrhages or thrombosis.

Conclusions: Our numbers are too small to be statistically relevant, and our algorithm should be tested on a larger scale to stratify the thrombotic risk in children. Nonetheless we held that VTE prophylaxis should be performed in this setting without a significant bleeding risk.

PB 533 | Development of a Thrombus in Giant Coronary Aneurysm of a Child with Kawasaki Disease - An Unusual Clinical Manifestation

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Background: Kawasaki disease (KD) is the most common vasculitic disorder in children. Coronary artery abnormalities (CAA) can develop in up to 15-25% of patients with KD if appropriate treatment is not administered in time. Occasionally, giant coronary aneurysms (diameter>8mm) can also develop. Coronary thrombosis is an unusual, but dreaded, complication that can sometimes be seen in such patients. We describe one such case.

Aims: To describe the case of a young boy with KD and giant coronary artery aneurysm who developed thrombosis on follow-up.

Methods: A 7 year old boy was diagnosed with KD at 3 years of age when he was admitted elsewhere with a febrile illness and left cervical lymphadenopathy. On day 6 of illness, he developed erythematous maculopapular rash, swelling over dorsum of hands with conjunctival congestion. He went on to develop periungual peeling of skin 3 days later. Two dimensional echocardiography revealed ectasia of left main coronary artery. (Table 1). He was treated with intravenous immunoglobulin and aspirin. The fever responded promptly. Follow-up echocardiography revealed a giant aneurysm in distal segment of left anterior descending (LAD) coronary artery (Table 1). Anticoagulation with acenocoumarol was initiated at this stage but the parents discontinued this therapy on their own. He remained clinically well and was referred to us at 7 years of age.

Results: We performed 128 Slice Dual Source CT coronary angiography. It revealed a fusiform aneurysm in mid segment of LAD coronary artery (width 8 mm and length 13.5 mm) with an eccentric hypodense filling defect (3 mm width) in the aneurysm suggestive of coronary thrombosis. He was initiated on low molecular weight heparin (1 mg/kg/ dose twice daily) and aspirin was continued.

Conclusions: CT angiography is an important investigation for detailed assessment of coronary artery complications in patients with KD. Patients with giant coronary artery aneurysms need to be maintained on long-term anticoagulation to prevent thrombosis.

TABLE 1 Two dimensional echocardiography findings

Diameter of coronary arteries	At admission	1 week later	6 months later	3 years
LMCA	5 mm with ectasia of mid portion (> +3Z)	4.2 mm (> +3Z)	-	-
LAD	4 mm (> +3Z)	-	8.7 mm (>+10Z)*	6 mm (>+10Z)*
RCA	3 mm	5 mm (> +3Z)	3.1 mm (+2.8 Z)	-
LCx	3 mm	-	-	-

*Diameter of the aneurysmal segment; Abbreviations- LAD- left anterior descending coronary artery; LCx- left circumflex coronary artery; LMCA- left main coronary artery; RCA- right coronary artery

PB 534 | Management of Anticoagulation Therapy in Patients with Thromboembolism in The Context of Renal Diseases: Case Series and Practical Suggestions

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Background: Current guidelines for anticoagulation therapy (ACT), including low molecular weight heparin (LMWH), do not address dosing and monitoring in the context of various renal diseases. LMWH is renally cleared, hence renal dysfunction can cause accumulation of the drug, potentially resulting in increased bleeding risk.

Aims: To describe challenging cases of ACT management in patients with thromboembolism in the context of renal diseases.

Methods: Patient records were reviewed for demographics, details of thromboembolic (TE) diagnosis and therapy, details of renal condition, and bleeding episodes.

Results: A 12 year old girl with steroid resistant nephrotic syndrome developed inferior vena cava thrombus and pulmonary embolism. After initial thrombolytic treatment, she received ACT with enoxaparin, which required multiple dose adjustments based nephrotic syndrome remission or relapse (Table 1).

A 5 year old girl with repaired congenital heart disease developed common femoral and external iliac vein thrombus while on prophylactic enoxaparin for mechanical tricuspid valve. In this case, increasing anti-Xa levels were the first sign of acute kidney injury (AKI). Enoxaparin doses were adjusted until improvement in AKI (Table 2).

Anephric 3 year old boy developed line related superior vena cava thrombus while on hemodialysis. Enoxaparin is renally cleared, hence it was administered once daily post-dialysis and adjusted based on combined peak and trough anti-Xa levels.

Conclusions:

1- Anti-Xa levels should be monitored closely with any disease activity change in nephrotic syndrome patients.

Figure 1. Enoxaparin dosing and monitoring in a hemodialysis patient.

Table 1. Enoxaparin dose adjustments and corresponding anti-Xa levels in a nephrotic syndrome patient with thromboembolism.

Day of Treatment	Enoxaparin	Anti-Xa (U/ml)	Albumin (g/L)	Comment
1	60 mg BID		28 T*	
20	**63 mg BID**	**1.18**	**25**	
21	55 mg BID	1.27		Lower LMWH doses in nephrotic remission
22	50 mg BID	1.13		
27	45 mg BID	0.70	33	
42	**50 mg BID**	**0.39**	**17**	**Higher LMWH doses required in nephrotic syndrome relapse due to lower anti-thrombin levels**
43	**60 mg BID**	**0.33**		
44	**80 mg BID**	**0.44**	**20**	
63	65 mg BID	1.07		

*Post Albumin Transfusion

Table 2. Enoxaparin dosing and monitoring in a patient with thromboembolism who later developed acute kidney injury.

Day of Treatment	Enoxaparin	Anti-Xa (U/ml)	Urea (mmol/L)	Creatinine (umol/L)	eGFR	Comment
7	26 Mg BID	0.82	11.4	50	76.6	
16	29 Mg BID	0.85	9.4	45	85.1	
17	32 Mg BID	0.89				
54	**30 Mg BID**	**2.10**				**Accumulation of LMWH with decreased renal function**
59	**22 Mg BID**	**1.68**				
61	**18 Mg BID**	**1.18**	**36.8**	**77**	**49.7**	
65	**18 Mg BID**	**0.79**	**32.2**	**66**	**58**	
99	20 Mg BID	0.61	15	66	58	

2- Significant changes in anti-Xa levels should prompt investigation of renal function.

3- Enoxaparin can be dosed once daily to maintain a target peak anti-Xa level of 0.5-1.0 U/ml and trough of 0.1-0.3 U/ml in a patient on hemodialysis requiring ACT (Figure 1).

PB 535 | Successful Use of Low Molecular Weight Heparin (LMWH) in Intracardiac Thrombus of a Premature Infant: Case Report

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Background: Treatment guidelines for Pediatric Atrial Thrombosis are limited. Treatments in neonates include observation, thrombectomy and anticoagulation. Thrombolytic therapy are increasingly being

used to treat intracardiac and arterial thrombosis, but bleeding risks make them unpopular. Currently, optimal treatment modalities are not known.

Aims: We report a premature infant with an Atrial Intracardiac Thrombus which had indication for thrombolysis treatment, because of gestation age, weight and Grade I Ventricular Hemorrhage antecedent, treatment with low molecular weight heparin (LMWH) was used.

Methods: 31 weeks gestation baby, weighted 1002 gr, presented with neonatal respiratory distress syndrome, required INSURE therapy and noninvasive ventilation for 28 hours, venous and arterial umbilical catheter were placed. Grade I Ventricular Hemorrhage was diagnosed at 4 days old. He presented with clinical deterioration and had positive cultures. Echocardiogram showed a 6 x 7 mm mass in the right atrium adhered to the interatrial septum. Treatment was initiated with antibiotics. Follow up echocardiogram showed an increase in size and a new atrial vegetation trough branches of the pulmonary artery were seen. Treatment with LMWH was started (1.5 mu/kg/day). The mass was monitored with serial echocardiograms. After 8 days of treatment, vegetation size is reduced, and by day 15 it disapeared. 5 weeks of anticoagulation and 4 weeks of antibiotics were completed, No hemorrhagic complications presented during the treatment. Interventricular Hemorrhage resolved.

Results: .

Conclusions: Treatments for Thromboembolism in preterm infants are still based on expert opinion. Unfractionated heparin has been the standard treatment in preterm infants with clinically significant and large thrombi that aren´t life-threatening. LMWH is frequently used in post-acute treatment. As with this case, LMWH could be an effective and safe treatment for Acute Right Atrial Thrombosis in preterm Infants.

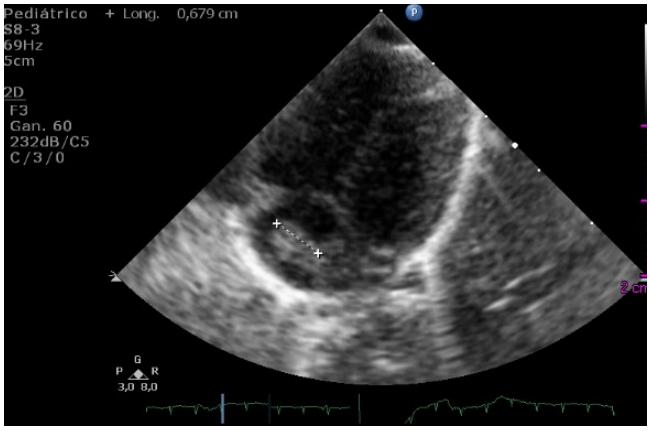

FIGURE 1 echocardiogram showing the intracardiac thrombus in the right atrium.

PB 536 | Petechial Rash and Thrombocytopenia with Unfractionated Heparin (UFH) Use: Heparin Induced Thrombocytopenia (HIT) in a Child with Non-vegetative Infective Endocarditis

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Background: HIT, a life-threatening complication of heparin therapy, is rare in pediatric patients despite the frequent use of heparin for anticoagulation.

Aims: We present a unique case of HIT in a 10-year old boy being treated with an UFH infusion for multiple venous thrombi.

Methods: Case report.

Results: A 10-year old boy with non-vegetative infective endocarditis and extensive line associated thrombi developed thrombocytopenia and petechial rash after less than 2 days of cumulative exposure to an UFH infusion, approximately one week after initial exposure to heparin. He presented with a rapidly progressive diffuse petechial rash, concurrent drop in platelets (326 to 48x10^9/L over 36 hours), and stable thrombi. Pretest probability of HIT, by the 4T's score, was intermediate. HIT ELISA was positive (optical density of 1.743, inhibition of 87%). Change to bivalirudin, a direct thrombin inhibitor (DTI), was recommended, but not immediately available. Due to the risk of thrombosis progression associated with sudden discontinuation of heparin, UFH was continued for under 12 hours until argatroban was started. Argatroban was titrated from 0.75 mcg/kg/minute to a goal PTT of 50-90 seconds. Within 48 hours, his platelets normalized and rash improved. Anti-platelet factor 4/heparin enzyme immunoassay (IgG EIA) was 1.534, indicating high probability for HIT. A serotonin release assay was indeterminate. The patient required surgery for his underlying cardiac valve disease; once available, bivalirudin was used intra-operatively. Argatroban was restarted post-operatively. He was later switched to warfarin for the remainder of his three-month treatment course. Subsequent ultrasound showed complete thrombi resolution.

Conclusions: Recognition of HIT and management with DTIs rapidly improved the patient's clinical status. DTIs are an effective anticoagulant alternative intra-operatively. Although rare, it is important that clinicians keep HIT on their list of potential risks associated with using heparin in children.

PB 537 | Resolution of Cerebral Sino-venous Thrombosis (CSVT) without Anticoagulation in a Neonate with Co-existing Protein C and Antithrombin Deficiency: A Case Report

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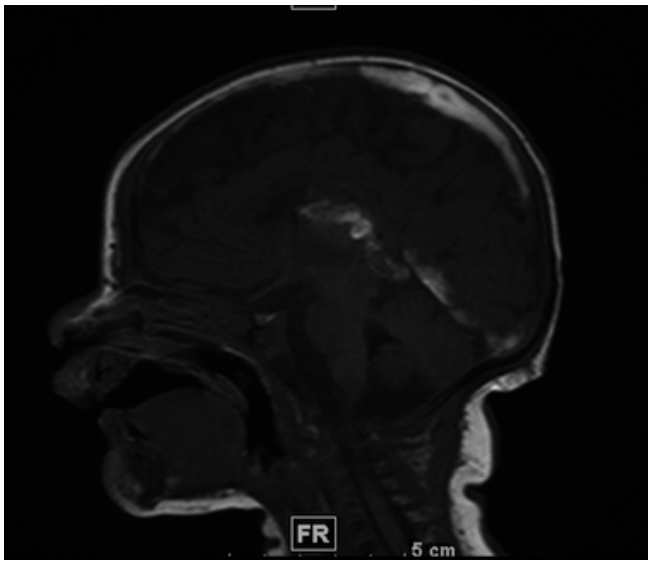

FIGURE 1 Sagittal view of extensive CSVT and bilateral thalamic hemorrhage. Patient 16 days old

Background: There is variability in management of CSVT by age. Evidence supporting anticoagulation (ACT) for adults and older children is established, however conflicting guidelines exist for management of neonatal CSVT. Observational studies have identified reassuring safety profile for ACT in neonates with CSVT, even with pre-existing intra-cranial or intra-parenchymal hemorrhage. However, certain systemic issues (large hemorrhage, infection, coagulopathy) may cause the clinician to manage conservatively.

Aims: We present a novel case of neonatal CSVT with excellent clot resolution despite conservative management in a patient with inherited thrombophilia (protein C and anti-thrombin deficiency).

Methods: Case study verified by healthcare team. Detailed literature review performed using Google Scholar and PubMed from 2000-2017.

Results: The neonate was born at term via vacuum-assisted delivery. At 2 weeks of age, she presented with focal seizure. Head imaging (Figure 1) revealed extensive CSVT. Due to co-existing extensive thalamic and intra-ventricular hemorrhage, the decision for conservative management was made. Follow-up imaging at one year showed complete resolution of CSVT (Figure 2).

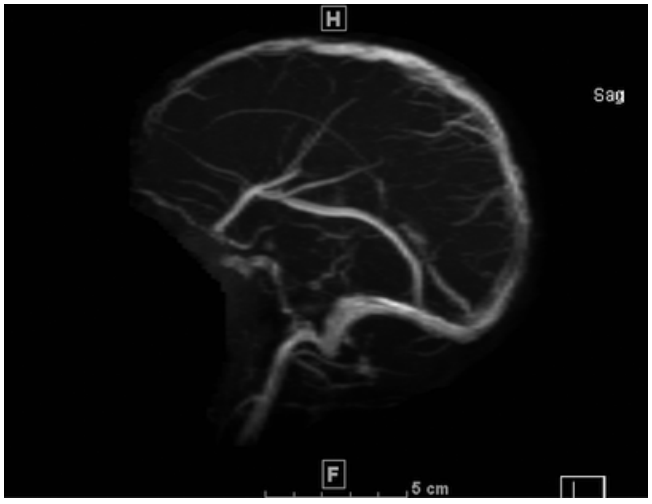

FIGURE 2 Magnetic resonance venography (time of flight) at 1 year old. Shows complete resolution of thrombus

The neonate's father had protein C and anti-thrombin deficiency. Genetic testing at age 10 confirmed same mutation in the patient. The patient is currently 13 years old and has not experienced further thrombotic events.

Conclusions: Neonatal CSVT may resolve completely in some patients without anticoagulation. This highlights the unique thrombogenicity in a neonate as well reassures clinicians who may be pressed to treat a patient conservatively due to above mentioned reasons. However, we acknowledge that resolution of thrombus still remains to be established as a marker of good clinical outcome.

PB 1244 | The Incidence and Assessment of Post-thrombotic Syndrome in Critically Ill Children Two Years Post-central Venous Catheter Placement

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Background: Central Venous Catheters (CVCs) are associated with over 50% of thromboses in children. Post-thrombotic Syndrome (PTS) is a long-term complication of thrombosis in children, which can impact quality of life. There is limited data regarding the epidemiology of PTS in children with CVC-related thrombosis, which is further confounded by the wide variance in published PTS rates within pediatric populations (6-64%).

Aims: To determine the frequency and severity of PTS two years following CVC placement in critically ill children and to optimize the scoring criteria for the severity of PTS in children.

Methods: 205 children admitted to a pediatric intensive care unit (PICU) with a CVC in situ participated in a prospective cohort study. Institutional ethics approval and informed consent were obtained. Approximately 24 months after PICU admission and CVC placement, a PTS assessment was performed. Two validated pediatric PTS tools were used independently in conducting PTS assessments in each child: the Manco-Johnson instrument (MJI) and the Modified Villalta scale (MVS).

Results: 126 Children underwent PTS assessment. The same 13 children were classified as having PTS using the MJI and MVS. Two children met the criteria for clinically significant PTS using the MJI, whilst the MVS assessed all PTS cases as mild. The Cronbach's alpha for the MVS and MJI was 0.989 and 0.935, respectively.

Conclusions: The incidence of clinically significant PTS was 1.6% in this cohort, despite 10.3% of children having some degree of PTS. However, only the MJI assessed children to have clinically significant

PTS. This raises the possibility that published estimates of PTS incidence in pediatric populations may overcall the clinical significance of this outcome measure. Based on the findings of this study, MJI may overcall the incidence of clinically significant PTS in younger children. As the MVS is less dependent on measuring pain, it may be more suitable for use in younger children.

PB 1245 | Venous Thromboembolism (VTE) in Pediatric Recipients of Hematopoietic Stem Cell Transplant (HSCT): Principal Findings from a Multicenter Cohort Study

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Background: HCT is the standard of care for a variety of benign and malignant disorders. Placement of central venous catheters (CVC), prolonged hospitalization, infections and graft versus host disease (GvHD) create a pro-thrombotic environment in recipients of HCT. A high incidence of VTE has been described in adult recipients of HCT. Similar data for children is lacking.

Aims: The objective of this retrospective, multicenter cohort study was to estimate the incidence of VTE and describe associated risk factors in recipients of HCT across children's hospitals in the US.

Methods: This study was deemed to be exempt by the IRB at Nationwide Children's Hospital. Data source for this study was the Pediatric Health Information Systems (PHIS), an administrative database that contains clinical and resource utilization data for inpatient and ambulatory surgery patient encounters from 49 free standing children's hospitals in the US. ICD-9 codes were used to identify eligible subjects, VTE, post-HCT complications and CVC placement. All patients who underwent a HCT at one of the PHIS hospitals between 01/01/2010 - 09/30/2014 were eligible for inclusion. Data were analyzed using non-parametric methods.

Results: 4158 unique subjects (3015 allogeneic and 1143 autologous HCT recipients) were included in the analysis. Mean age (±SD) at HCT was 8.8 (±6.5) yrs.1704 (41%) subjects were female. Post HCT, 290 subjects (6.9%) developed VTE. Pre-transplant predictors of VTE are summarized in table 1. GvHD (acute and/or chronic) was the only post-transplant complication associated with VTE [OR (±95% CI): 1.65 (1.2-2.1)]. Among allogeneic transplant recipients, VTE was associated with an increased risk of invasive bacterial and fungal infections. As summarized in table 2, VTE diagnosis in HCT recipients was associated with a worse prognosis.

TABLE 1 Pre-transplant risk factors associated with VTE development

Characteristic	Odds Ratio (95% CI)	p-value
Age ≥ 13 years (compared to < 13)	1.38 (1.08-1.77)	0.0115
Allogeneic (vs Autologous)	1.60 (1.19-2.15)	0.0021

TABLE 2 Morbidity associated with VTE development

Variable	VTE present	VTE absent	P value
Median length of hospital stay	81 days	54 days	p < 0.001
Median length of ICU stay	18 days	12 days	p < 0.001
1-year mortality	13.9% (n=78)	5.9% (n=212)	p < 0.001

Conclusions: Prevalence of VTE in subjects undergoing HCT at tertiary care pediatric hospital's in the US in about 7%. Older age, allogeneic transplants, development of GvHD are associated with VTE.

PB 1246 | Chronic Blood Transfusion Therapy Corrects Abnormalities of Coagulation and Fibrinolysis in Children with Sickle Cell Disease

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Background: Sickle cell disease (SCD) has been described as a hypercoagulable condition. The components and mechanisms of the hypercoagulability of SCD continue to be characterized.

Aims: This study attempts to characterize a specific subset of sickle cell disease patients, patients on chronic transfusion programs for primary or secondary stroke prevention, with thromboelastography (TEG), prior to and following a red blood cell transfusion.

Methods: Thromboelastography profiles prior to and following transfusion, and coagulation parameters (aPTT, INR, fibrinogen, d-dimer) prior to transfusion, were completed in 25 children with SCD presenting for regular transfusion. A retrospective review of clinical history was completed. Neurovascular disease progression was defined as Magnetic Resonance Imaging of brain with increased vessel tortuosity, increased vessel stenosis, or new parenchymal infarct. Descriptive statistics, Student t test and Pearson correlation co-efficient were calculated. Study was approved by institutional review board.

Results: Thromboelastography was completed on 13 males, 12 females, mean age 10 years (5-15 years). Changes in the thromboelastography profile following transfusion were compatible with decreased hypercoagulability (increased K, decreased angle, decreased maximal amplitude). Maximal amplitude on TEG profile prior to transfusion, (p=0.017), d-dimer value pre-transfusion (p=0.048) were significantly associated with neurovascular disease progression.

Conclusions: Transfusion with the intended goal of altering the red blood cell population, significantly altered the hypercoagulable thromboelastography profile in children with sickle cell disease. Thromboelastography may offer an additional monitoring investigation or therapeutic target in the management of chronically transfused patients with sickle cell disease.

TABLE 1 Thromboelastography values prior to and following transfusion in 25 children with sickle cell disease

TEG parameter	Pre-transfusion Median	95% CI	Post-Transfusion Median	95% CI	P value
R(min) Clotting time	7.2	7.2-8.5	7.5	6.7-9.1	NS
K(min) Clot kinetics	1.6	1.5-1.8	1.8	1.7-2.4	P < 0.05
Angle(deg) Clot Kinetics	68	65.6-69.4	65	57.9-66.7	P < 0.05
MA(mm) Maximum Amplitude	71	66.7-71.2	65	31.0-66.6	P < 0.05
TMA(min) Time to Maximal Amplitude	33	31.4-35.5	30	15.1-34.1	NS
G(d/sc) Clot Elasticity	11 958	10407.0-12740.5	9295	248.8-7738.2	P < 0.05
E(d/sc) Clot Elasticity	239	208.1-254.8	186	86.8-195.5	P < 0.05
TPI(/sec) Thrombodynamic Potential Index	73	64.4-88.6	23	25.6-62.6	P < 0.05

PB 1247 | Evaluating the Relationship between von Willebrand Factor, ABO Blood Group and Circulating Lymphoblasts in the Development of ALL-associated Thromboembolism in Children

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Background: Non-O blood group is known to be an independent prothrombotic risk factor in children with acute lymphoblastic leukemia (ALL), but the exact pathogenesis of its prothrombotic effect is unknown. Von Willebrand factor (vWF) has been contemplated as a pathogenetic mechanism in ABO blood group-related prothrombotic risk. An association of vWF with circulating blasts has been previously shown.

Aims: As a part of a larger study defining risk factors for symptomatic TE (sTE) in children with *de novo* ALL, this sub-study is evaluating the relationship of ABO blood groups, circulating blasts and vWF antigen levels at diagnosis.

Methods: Consenting patients (1-≤18 yrs.) with *de novo* ALL enrolled on the Dana-Farber Cancer Institute 05-01 trial were included and demographic data (ABO blood group, complete blood count, therapy and development of sTE) were collected. Factor VIII:C and vWF antigen were analyzed centrally prior to starting therapy. Regression analyses evaluated relationship between blood group, vWF antigen level and circulating blasts.

Results: Of 131 patients [mean age (range) 6.4 (1-17) yrs.; 70 boys], 21 (16%) developed sTE. Fifty-one patients had blood group O and 76 non-O blood group. Older age [Odds Ratio (OR 1.9, p=0.029] and non-O blood-group (OR 4.27, p=0.028) were identified as independent predictors for development of sTE. Patients with peripheral blasts had higher odds of developing sTE (OR 7.79; p=0.059). There was no interaction between ABO blood group and circulating blasts on vWF levels (p=0.723, Table 1).

TABLE 1 Comparison of vWF levels in patients with and without circulating blasts in patients with O vs. Non-O blood group

		vWF levels at diagnosis (mean [SD]) (range)			
Blood Group	N	Overall	With Blasts	Without Blasts	P value#
O	51	1.697 (0.806) (0.670, 4.430)	1.787 (0.820) (0.780, 4.430)	1.370 (0.688) (0.670, 2.760)	0.130
Non-O	76	1.575 (0.689) (0.710, 4.450)	1.647 (0.745) (0.710, 4.450)	1.344 (0.397) (0.710, 1.970)	0.034
All Participants	127	1.629 (0.731)	1.706 (0.769)	1.368 (0.514)	0.008
P value*		0.3695	0.3876	0.9094	

Conclusions: vWF levels at ALL diagnosis were significantly higher in patients with circulating blasts and among them, in non-O blood group. The relationship between blood group and vWF may be affected by dominant effect of circulating blasts on vWF. However, we showed no interaction between ABO blood group and circulating blasts on the vWF levels. The prothrombotic effect of ABO blood group patients with ALL is thus not yet clear and may deserves consideration in future studies.

PB 1248 | Central Venous Catheter Associated Thrombosis in Congenital Heart Disease Patients: A Preliminary Analysis of the Children's Hospital Acquired Thrombosis (CHAT) Registry

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Background: Central venous catheters (CVCs) are associated with hospital acquired venous thromboembolism (HA-VTE). Congenital heart disease (CHD) patients often have CVC(s) due to multiple surgeries and prolonged hospitalizations.

Aims: To analyze CVC-VTE in hospitalized CHD patients.

Methods: This retrospective study identified and reviewed CHD patients with VTE within the Children's Hospital Acquired Thrombosis (CHAT) registry hospitalized from 2012-2016. CVC VTE was defined as VTE surrounding the CVC, at the CVC tip, or in the same vein where the CVC was placed.

Results: 141 of the 646 HA-VTE pts were found to have CHD. 131 pts had at least one CVC placed during admission. 327 CVCs were placed in CHD pts and most were PICCs, temporary femoral, or temporary jugular lines (Figure 1). Most CVCs were placed in the upper extremity (54%). 106 VTEs were CVC-related with 32% of CVCs developing VTE (Table 1). Multi-lumen CVCs had a higher number of VTE compared to single lumen CVCs. The first CVC placed in each patient had a higher number and percentage of VTE (72 and 54.9%, respectively) compared to subsequent lines (34 and 17.3%, respectively). PICCs had the highest number of VTE (59) while the highest percentage of VTE were with temporary femoral lines (53.7%). 34 patients developed CVC-related VTE despite mechanical, chemical, or combined VTE prophylaxis (Table 1). Of the 85 CVCs placed in these patients, 24.7% developed CVC-VTE (21/85).

Conclusions: CVCs were a main contributor to VTE formation in CHD patients. The most CVC-VTE cases were with the initial CVC, PICCs, and multi-lumen CVCs. Those receiving mechanical and/or chemical prophylaxis had a lower VTE to CVC percentage than the overall cohort, suggesting some protective effect from these prevention strategies. Future analysis will include control patients for direct comparison of purported risk factors.

Figure 1.

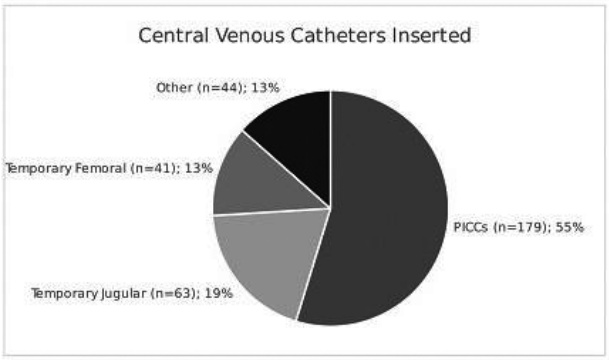

Figure 1. A total of 327 Central Venous Catheters (CVCs) were placed in congenital heart disease patients who developed VTE. The majority of CVCs placed were peripherally-inserted central catheters (PICCs) followed by temporary jugular lines and temporary femoral lines.

FIGURE 1 Central venous catheters placed in congenital heart disease patients who developed VTE

TABLE 1 CVC Related VTE Relating to CVC type, Number of Lumens, and VTE prophylaxis in subjects with congenital heart disease

	CVC Related VTE	Number of CVCs Placed	VTE per CVC percentage
All Lines	106	327	32.4%
PICC Lines	59	179	33%
Temporary Femoral Lines	22	41	53.7%
Temporary Jugular Lines	17	63	27%
Other	8	44	18.2%
Single Lumen	17	70	24.2%
Multi-Lumen	89	258	34.5%
Mechanical and/or Chemical Prophylaxis	21	85	24.7%
No Prophylaxis	85	242	35.1%

PB 1249 | Neutrophil Extracelular Traps (NETs) and Inflammatory Biomarkers in Patients with Hereditary Spherocytosis. Influence of Splenectomy

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Background: NETs formation is part of the immune response but it has been related to thrombotic processes. In patients with hereditary spherocytosis (HS), splenectomy increases risk of sepsis by encapsulated bacteria and probably with a long-term increase in thrombotic risk.

Aims: To analyze NETs (cell-free-DNA, citrullinated histone 3 (CitH3)), and inflammatory markers (RANTES, CXCL5 and HMGB1), in children diagnosed with HS with and without splenectomy, as potential factors involved in potential thrombotic risk increase.

Methods: Children (n=48) were recruited, male 60.5%; mean age 11.1 years (SD 3.9). They were classified into 3 groups: healthy control (n=16), children with HS non-splenectomized (n=16) and children with HS who were splenectomized (n=16). NETs and inflammatory markers were analyzed in plasma by ELISA. Parametric test (ANOVA), non-parametric test (Kruskal-Wallis) and Pearson correlation were used. $P < 0.05$ was considered significant. The study was approved by the ethical Committee of the Hospital La Fe. All patients or their proxy gave their written informed consent.

Results: DNA, HMGB1 and RANTES were significantly elevated in HS vs. controls (Table 1). HS splenectomized patients had higher CitH3 and inflammatory markers when compared to HS patients without splenectomy, except for DNA.

TABLE 1 NETs and inflammatory markers in HS. * HS vs control. # HS+splenectomy vs control. †HS+splenectomy vs HS

		Controls	Patients		P
			HS	HS+ splenectomy	
NETs	DNA	424,5	798,1	645,8	0.0001*#
	CitH3	0,168	0,157	0,207	0.057
Inflammatory markers	CXCL5	89,4	82,5	304,5	0.082
	HMGB1	1,43	1,7	2,38	0.0019#†
	RANTES	242,1	286,9	411,9	0.0087#†

Conclusions: NETs are significantly elevated in patients with HS, particularly in those with splenectomy. There is a link between NETs and inflammatory markers in our patients. The elevation of these biomarkers could be related to the thrombotic risk in patients with HS. Grants. FIS13/00016. ACIF/2016/465. RETICS networks INVICTUS (RD12/0014/0004) and INVICTUS+ (RD16/0019/0008) Instituto de Salud Carlos III.

PB 1250 | Paget-Schroetter Syndrome in Children and Young Adults: The Bane of Athletes of Either Gender

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Background: Paget-Schroetter syndrome (PS) or thoracic outlet (TO) venous thrombosis occurs in approximately 2/100,000 individuals and is provoked by excessive overhead activity. Typically, PS occurs in the dominant arm of young athletic males (2:1) 18-30 years of age.
Aims: To review the presentation, management, and outcomes of PS in young individuals < 19 years of age treated at Mayo Clinic between 2000-2016 with a minimum follow-up of 10-weeks.
Methods: Retrospective chart review and analysis.
Results: Of 30 eligible patients, 28 (93%) were athletes or had excessive overhead activity. Median age was 16.8 years (range 12-18.7 years). Gender distribution was nearly equal with similar characteristics.

TABLE 1 Gender-based patient characteristics

Characteristics	Males (n=16)	Females (n=14)	p-value
Median age in years (range)	16.6 (15.0-18.4)	17.2 (12.0-18.7)	0.308
Median body mass index (range)	23.0 (19.1-43.7)	22.5 (19.1-43.7, n=12)	0.403
Dominant hand thrombus (%)	14 (87.5%)	11 (78.5%)	0.051
Athlete or excessive overhead activity (%)	16 (100%)	12 (85.7%)	0.073
Median duration of anticoagulation in weeks (range)	16.5 (7-43.1)	19.1 (4.1-48.3)	0.442
Thoracic outlet decompression surgery (%)	10 (62.5%)	11 (78.6%)	0.333
Median months from thrombosis (range)	1.9 (0.2-7.7)	3.5 (0.3-22.1)	0.098
Median duration of follow-up in months (range)	12.0 (2.64-101)	12.2 (2.82-113.6)	0.819
Complete resolution of thrombus (%)	5 (31.2%)	9 (64.3%)	0.068
Recurrent thrombosis (%)	2 (12.5%)	5 (35.7%)	0.130
Post-thrombotic syndrome (%)	7 (43.7%)	8 (57.1%)	0.463

Symptoms included edema (100%), pain (63%), and discoloration (30%). All patients were anticoagulated (unfractionated/low molecular weight heparin and/or warfarin/rivaroxaban) and 23 (77%) underwent thrombolysis (catheter-directed n=18, pharmacomechanical n=3, systemic n=2) while 21 (70%) underwent TO decompression surgery resulting in complete thrombus resolution in 14 (47%). Median duration of follow-up was 12 months (range 2.6-113.6 months). Recurrent thrombosis noted in 7 (23%) at median 2.25 months (range 0.29-15.6 months) and 15 (50%) had post-thrombotic syndrome. Of the 20 (67%) that had thrombophilia workup, 6 (20%) revealed heterozygous factor V Leiden (n=5), heterozygous prothrombin gene mutation (n=1), or elevated fibrinogen (n=1). Median duration of anticoagulation was longer in known thrombophilia patients at 33.7 weeks (range 16.7-48.2 weeks) compared to those without (14.8 weeks, range 4.1-43.1 weeks), p=0.010.
Conclusions: Young female athletes are prone to develop PS with presentation, course, and outcomes indistinguishable from males. Successful outcomes can be achieved with early recognition and multimodal treatment including anticoagulation, endovascular intervention, and surgical TO decompression.

PB 1251 | The Association between Central Venous Catheter Placement and Central Venous Catheter Related Thrombus

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Background: Over 50% of thromboses in children are related to central venous catheters (CVCs). Recently, an increased focus upon concomitant risk factors for CVC-related thrombosis has emerged.

Aims: To describe the contribution site of CVC placement has upon the incidence and sequelae of CVC-related Venous-Thromboembolism (VTE) in acutely unwell children during their pediatric intensive care unit (PICU) admission.

Methods: A prospective cohort study recruited children admitted to a PICU requiring a CVC in the jugular or femoral veins. The study was approved by the hospital ethics committee and informed consent was obtained. Each child had a (blinded) ultrasound of the blood vessel in which the CVC was placed during their admission (Phase I). A second ultrasound was performed approximately 24 months following CVC placement (Phase II).

Results: 21.9% of the children in our cohort developed a CVC-related VTE of which 6.2% (n=2) were symptomatic (both femoral CVCs). This study showed a significantly higher incidence of VTE in children with a femoral CVC at phase II (Table 1). The incidence of clinically significant Post Thrombotic Syndrome (PTS) in was 1.6% (n=2), occurring only in children with femoral CVCs. Despite 18.5% of children with jugular CVCs having radiologically confirmed thrombosis at Phase 1 (all asymptomatic), none developed clinically significant PTS.

TABLE 1 Femoral or Jugular CVC placement and CVC-related Thrombus at Phase I and Phase II

	Normal	Thrombus	P Value (Chi Square)	Odds Ratio (95% CI)
Phase I				
Jugular	92	21	0.72	1.27 (0.16 to 9.9) p=0.8
Femoral	22	11	0.72	1.27 (0.16 to 9.9) p=0.8
Phase II				
Jugular	87	8	0.002	26.17 (1.5 to 443.2) p=0.02
Famoral	17	8	0.002	26.17 (1.5 to 443.2) p=0.02

Conclusions: Children with a femoral CVC have a greater risk of residual CVC-related VTE approximately 2 years post-CVC placement. The study demonstrated no risk of clinically significant PTS in children with asymptomatic jugular vein thrombosis. These findings suggest that children with femoral CVCs present a higher risk of clinically significant thrombosis and thrombosis-associated morbidity at follow up 2 years post CVC.

PB 1252 | Acute Ischemic Stroke in Children on Left Ventricular Assist Device. Is There a Role For Endovascular Treatment?

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Background: Left ventricular assist devices (LVADs) are being increasingly used as a bridge to heart transplant in children with end-stage heart failure. However, about 30 to 40% of the children on LVADs develop acute ischemic strokes (AIS). Children with severe neurological deficits are being excluded from a heart transplant. As a consequence, they will die after retrieval of the LVAD. In adults, endovascular treatment has improved outcome in patients with AIS and has become standard of care in patients with an occlusion of a cerebral artery. In children, only case-reports and small case-series are available.

Aims: Despite lack of evidence, endovascular treatment can be considered in children with severe AIS on LVADs to improve outcome.

Methods: A local guideline on endovascular treatment in children on LVAD was developed in the Sophia Children's Hospital ErasmusMC, the nationwide pediatric cardiac transplant center, with 4 children on LVADs a year, in order to quickly detect, diagnose and treat AIS.

Results: Since 2015 3 children on LVAD were identified with severe AIS and underwent endovascular treatment.

Patient 1, was an 11 year old girl, on LVAD day 13 she developed an AIS with an occlusion of the left MCA (M1 and M2). A mechanical thrombectomy was performed with complete neurological recovery. 8 days later she developed a massive intracerebral hemorrhage and as a result she died.

Patient 2, was a 3 year old girl, on LVAD day 17 she developed an AIS with an occlusion of the distal intracranial carotid artery. A mechanical thrombectomy was performed with complete neurological recovery. The following days she developed multiple strokes as a result of a sepsis and died.

Patient 3, was a 14 month old girl, on LVAD day 40 she developed an AIS with an occlusion of the left MCA (M1). A mechanical thrombectomy was performed without any signs of recovery .

Conclusions: Endovascular treatment in children with severe AIS on LVAD is feasible but should be further investigated in international prospective studies.

PB 1253 | Thrombotic and Hemorrhagic Complications in Pediatric Recipients of Hematopoietic Stem Cell Transplant (HSCT): Mayo Clinic Experience

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Background: Hemorrhagic (HE) and thromboembolic event (TE) incidences in adult HSCT recipients have been reported; however, these complication rates in pediatric HSCT population are unknown.

Aims: To characterize HE and TE events in pediatric HSCT patients.

Methods: Clinical characteristics, HE, TE and follow up data up to one year were abstracted from medical records on patients aged < 21 years undergoing HSCT between July 2000-June 2015.

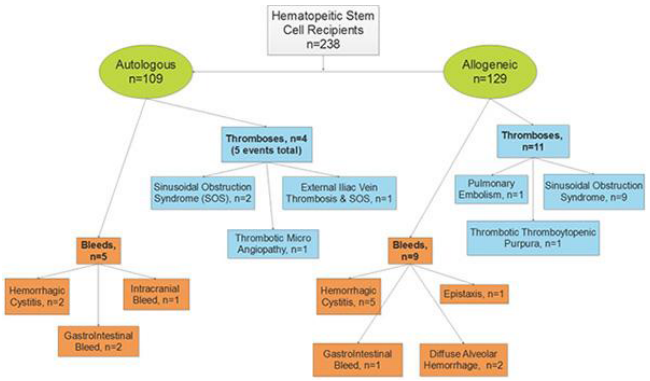

FIGURE 1 Types of Hemorrhagic and Thrombotic Events

Results: During study period, 238 pediatric patients underwent HSCT. Type of HE/TEs are shown in Fig. 1.

Incidence of "HE or TE" was higher in allogeneic HSCT compared to auto HSCT (p=0.02, Table 1). Median time from HSCT to acute TE was 11 days (range; -2 to 59) and 25 days (range; -7 to 46) for acute HE. The proportion of affected patients with ***both*** HE and TE was higher in autologous group (p=0.028). Median time to platelet engraftment for allogeneic and autologous populations was 27 and 29 days, respectively. All patients who had HE had platelets < 50,000 $x10^9$/L and one had platelets < 20,000 $x10^9$/L at time of HE. All patients with hemorrhagic cystitis (n=7) had received cyclophosphamide (Cy). For patients with sinusoidal obstruction syndrome, conditioning had included either busulfan (Bu)/Cy (n=5) or Cy with total body irradiation (n=4), or Thiotepa (n=2). Amongst allogeneic HSCT recipients, 60% of HE and 92% of patients with TE had underlying myeloid neoplasms. HE and TE were managed through supportive medical care. Median time to resolution of symptoms was 8 days (range; 1-53). One patient with thrombotic microangiopathy required renal transplant.

TABLE 1 Patient and Transplant Characteristics (Total Study Population = 238)

Characteristics in Subgroup	Autologous Recipients, n=109	Allogeneic Recipients, n=129	Analyses of Significance
Median Age in yrs. (range)	14.8 (0.9-20.9)	12.3 (0.6-20.9)	
Male sex (%)	66 (60.1%)	75 (58.1%)	
Underlying Diagnoses (%)			
Hematologic	32 (29.4%)	120 (93%)	
Solid tumor	77 (70.6%)	9 (7%)	
Any hemorrhagic or thrombotic complication -	6 (5.5%)	19 (14.7 %)	p= 0.02 for difference in frequency
Hemorrhagic	2 (1.8%)	8 (6.2 %)	
Thrombotic	1 (0.9%)	10 (7.8 %)	
Both Bleed & Thrombosis	3 (2.8%)	1 (0.7 %)	p=0.028 for difference in distribution

Conclusions: Allogeneic pediatric HSCT patients have higher risk of hemorrhagic and thrombotic complications compared to autologous HSCT, perhaps due to intensity of the preparative regimen, especially in the setting of myeloid neoplasms. True incidence of thrombotic complications might be underestimated especially in presence of central venous lines as radiographic surveillance for thromboses is not routine for asymptomatic patients.

PB 1254 | Venous Thromboembolism in Children with Hodgkin´s Lymphoma

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Background: Data about venous thromboembolism (VTE) epidemiology and risk factors in children with cancer, especially Hodgkin´s Lymphoma (HL) is lacking.

Aims: To estimate frequency and evaluate risk factors of VTE in children with HL.

Methods: Retrospective analysis of medical chart records of 58 inpatients up to 18 yo (3-17.5) with HL, since 1 January 2013 to 1 September 2016 had been performed. All patients had finished treatment according to GPOH-HD-2002 protocol. All episodes of DVT were confirmed by standard radiological methods and ultrasound investigation. Screening for asymptomatic thrombosis was performed before planting and removing of central venous catheters (CVC). All patients had CVCs. Influence of sex, age, stage, B-symptoms, treatment group, involvement of mediastinum, bulky mediastinum had been analyzed using Chi-square test with Yates correction where available (The R Foundation software, The R Project, Vienna, Austria).

Results: During the analyzed period, twelve (20.7%) of 58 patients developed VTE. VTE was symptomatic in 41.7% (n=5) of cases. Most frequently VTE occurred in the basin of v. cava superior (VCS, 66.7%, n=8), less of the patients had VTE in the basin of v. cava inferior (VCI, 16.7%, n=2). In one case there was combined VTE VCI+VCS and one patient had VTE in right atrium. One patient with VCI thrombosis had 2 episodes of PE.

Most of VTE episodes occurred during induction (58,3%, n=7) and consolidation (25%, n=3) of remission period of treatment. One (8,3%) episode was diagnosed at the primary diagnostic and one (8,3%) - during radiation therapy.

VTE was CVC-associated in 75% (n=9) cases. In the rest of 25% (n=3) all episodes were symptomatic and were strongly associated with vein compression by tumor.

None of risk factors, except therapeutic group (p=0.05), had influence on developing of VTE episodes.

Conclusions: VTE is a frequent complication of HL. We consider that advanced stages with big tumor burden can be potential risk factors for big prospective studies.

PB 1255 | Thrombin Generation in Venous Thromboembolism, Arterial Ischaemic Stroke and Bleeding Events in Children

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Background: Venous thromboembolism (VTE), arterial ischaemic stroke (AIS) and bleeding in children are associated with high morbidity and mortality, and significant long-term side effects that impact on quality of life. Whilst previous studies investigated individual haemostatic parameters in these paediatric cohorts, thrombin generation (TG) as a global assay of haemostasis has not been studied in this setting.

Aims: To measure TG in children with clinically relevant thrombotic or haemorrhagic events.

Methods: Blood was collected 6 to 12 mths post first manifestation from children with thromboembolism (VTE/AIS) and inherited bleeding disorders. Asymptomatic siblings (VTE/AIS) or healthy subjects (bleeding) served as controls. Frozen aliquots of platelet free plasma were used for the TG assay (Innovance® ETP test, Siemens) on the BCS-XP analyser (Siemens). The endogenous thrombin potential (ETP) and maximum concentration of thrombin generated (CMax) were calculated.

Results: The total number of participants in each cohort was: controls, n=256; VTE, n=63; AIS, n=56; bleeding, n=50. Samples from siblings were matched for 17 VTE and 18 AIS patients. Figure 1 summarizes the main results. One or both TG parameters were decreased in the setting of VTE, AIS and bleeding, compared to controls. There was no difference in TG in children with VTE compared to their siblings, but CMax was decreased in children with stroke compared to their siblings and when compared to the VTE cohort. ETP was decreased in children with VTE treated with heparin and both TG parameters, were reduced in those treated with VKA, compared to controls.

Conclusions: Clinical events such as VTE, AIS and bleeding have an impact on TG parameters in children. Matching clinical cases to their siblings can be used to study the pathophysiology of thrombosis in children. Whilst more detailed studies are needed, TG has the potential to be used as a clinical tool for identification of children at risk of VTE, AIS or bleeding and/or to guide anticoagulant therapy.

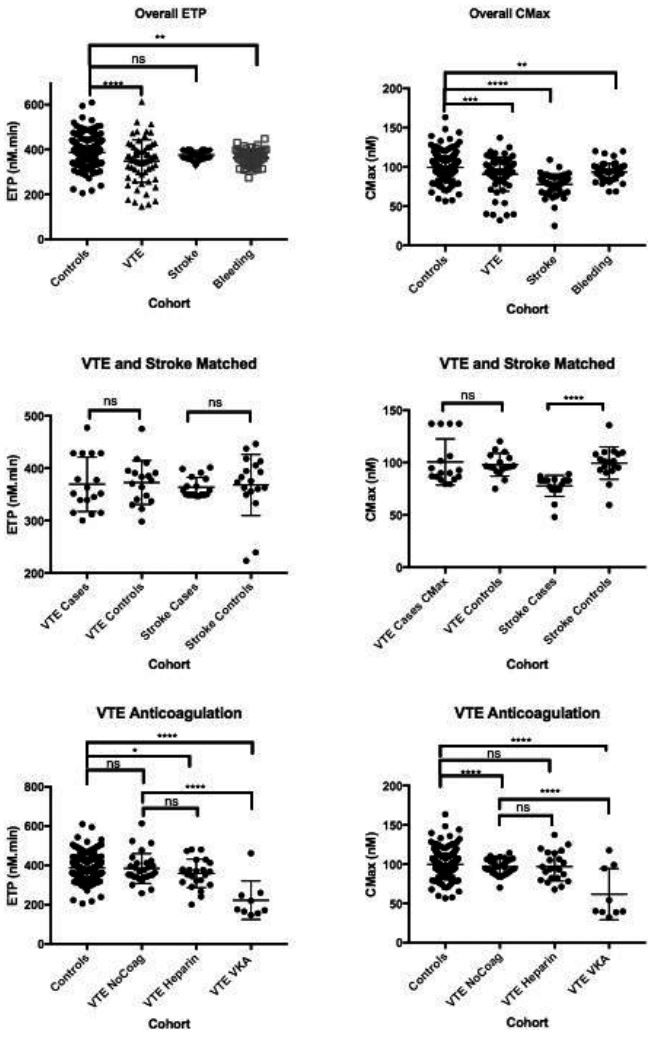

FIGURE 1 Summary of the main results (ETP and CMax)

PB 1256 | Paroxysmal Nocturnal Hemoglobinuria Spectrum among Pediatric Age Group of Patients: Single Center Experience without Any Thrombotic Events

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is rare in childhood and children with PNH usually presents in the form of aplastic anemia phenotype or overlapping phenotype. Whereas the classical hemolytic form of the disease is less common. PNH is a disease that may cause thrombotic complications and the size of the clone has not been related to predict the risk of thrombotic events.

Aims: PNH is one of the acquired thrombophilic states and the data on the presentational findings and thrombotic events in these patients is limited among children.

Methods: Local Ethical Committee approval is present. Between 2013 and 2016, 14 acquired aplastic anemia patients, 4 patients with undefined hemolytic anemias and 1 patient with recurrent thrombosis of more than 3 times were screened with FLAER for the presence of PNH clones. Two of the patints with aplastic anemia were tested for PNH clones after complete response (CR) to immune-supressive treatment (IST).

Results: None of the patients with undefined hemolytic anemias and the patient with recurrent thrombosis were found to be positive for PNH clones. On the other hand, of the 14 patients with aplastic anemia, 8(57%) were found to be PNH clone positive. The median age of the PNH clone positive patients were 12 years(8-17). CR to IST was 85.7% among clone positive patients, whereas 67% among clone

negative patients. Three patients underwent stem cell transplantation. Of the PNH clone positive 8 patients, 2(25%) were classified as overlapping PNH syndrome and these patients had typically higher clone sizes compared to those with isolated aplastic anemia (median erythrocyte, neutrophil, monocyte clone sizes 17%, 52.5%, 46.5 vs 0.35%, 1.39% vs 0.7%) . None of the patients with clone positivity developed thrombotic events, but one patient who persistently had high clones despite IST response , developed hemolytic attacks in the course and eculizimab was initiated for thrombo-prophylaxis.

Conclusions: In childhood, classical PNH presentations and thrombotic events are rare.

PB 1257 | Incidence and Risk Factors for Childhood Thrombosis: Ten Years Experience of a Paedatric Hematology Center from Turkey

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Background: Either incidence or knowledge about childhood thrombosis is increasing within the last decades.

Aims: This study evaluated incidence, risk factors, age, gender, family history, symptoms, location and treatment of thrombosis among hospitalized children.

Methods: Clinical and laboratory results of 150 patients diagnosed with thrombosis between 2005-2015 were retrospectively examined.

Results: The annual frequency of thrombosis among hospitalized patients was found 40/10.000. Male/female ratio and the mean age were 60/40 and 83 months (0-338 M), respectively. Of them, 8.6% was neonates. Majority of the events were venous 88.7% and the rest was arterial 11.3%. Thrombosis frequently occurred in cranial 48.7% site followed by lower extremities 26.7% and cardiac 10.7%. Mutation analysis sent from 129 patients. FV Leiden was the most common mutation 20.2%. Congenital and environmental risk factors were simultaneously present in 30.2% of children. No congenital mutation was observed in 8.5% of the cases. Infection 37.6%, catheter 34.1% and congenital heart disease 32.9% were the most frequent environmental risk factors. The majority of children 81.1% were treated with low molecular weight heparin. Four patients with cardiac thrombosis were successfully treated recombinant tissue plasminogen activator. Three neonatals (2.7%) with intraventricular cardiac thrombosis required thromboembolectomy. Treatment-related complication of which was bleeding occurred despite of anti-coagulant therapy. Thrombosis-related mortality was not detected. However, 3% patients were lost due to the underlying disease.

Conclusions: Thrombosis is a multifactorial disorder. In the current data, acquired, respectively. They were combined in 30.2% of children. However, 20.9% of children had none of these factors. Thrombotic disorders during childhood are better defined and treated in our decade with the new approaches.

PB 2162 | ISTH DIC Score is Associated with Mortality in Children with Disseminated Intravascular Coagulation

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Background: Disseminated intravascular coagulation (DIC) is an acquired consumptive coagulopathy and intravascular fibrin formation and deposition. DIC is associated with high mortality rate in children. The international society on thrombosis and haemostasis (ISTH) developed ISTH DIC scoring system for diagnosis and prognosis of DIC. However, few of ISTH DIC scoring data was studied in pediatric setting.

Aims: To compare the mortality rate of children who diagnosed with DIC by using ISTH DIC score.

Methods: A retrospective study was performed in children age from 28 days to 15 years who diagnosed with DIC from 2005 to 2014 at Siriraj hospital in Thailand.

Results: Two hundred and forty-four patients (118 boys, 126 girls) were enrolled. One hundred and seventy nine (73.4%) and 65 (26.6%) patients were overt DIC and non-overt DIC, respectively. The common etiologies of DIC were infections (84.4%) followed by tissue injuries (7%) and malignancies (2.9%).The mortality rate of overt DIC patients (76%) was statistically significant higher than non-overt DIC patients (15.4%) ($p< 0.001$). The higher DIC score was correlated with higher mortality rate ($R^2 = 0.89$).

The clinical manifestations that associated with high mortality rate were bleeding and multi-organ dysfunction ($p< 0.001$) but thrombosis was not associated with mortality rate ($p = 0.98$). The laboratories including low platelet count, prolonged prothrombin time and high D-dimer were significantly associated with high mortality rate ($p< 0.01$) but fibrinogen level was not effect on mortality rate ($p = 0.14$). The patients who were admitted in ICU and treated with inotropic drugs, ventilator support, dialysis, albumin and blood component transfusion were significantly increase the mortality rate ($p< 0.001$).

Conclusions: Children with overt DIC had higher mortality rate than children with non-overt DIC. The higher ISTH DIC score was associated with higher mortality. The ISTH DIC score can be used to predict prognosis of DIC in children.

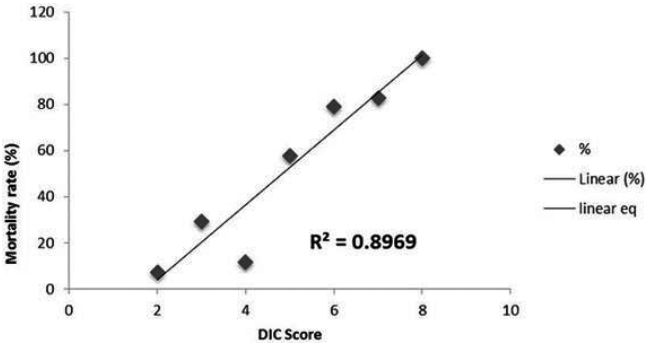

FIGURE 1 Correlation between ISTH DIC score and mortality rate.

PB 2163 | Out of Sight, Out of Mind - The Acquired von Willebrand Syndrome is a Bleeding Cause in Disguise during Congenital Heart Disease Surgery

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Background: Cardiac surgery of the newborn and infant with complex CHD (congenital heart disease) is afflicted by a high rate of intraoperative bleeding complications. In the context of (congenital) cardiac disease, acquired von Willebrand syndrome (AVWS) has previously been associated with patent ductus arteriosus (PDA) or severe valvular stenosis. There is no published data on its intraoperative incidence after cardiopulmonary bypass (CPB) in infants with CHD.

Aims: To investigate the pre- and intraoperative incidence of AVWS in newborn and infants with CHD and its impact on intraoperative bleeding.

Methods: We screened 12 consecutive newborn and infants undergoing complex cardiac surgery for AVWS before and immediately after CPB intraoperatively. The investigated parameters included FVIII-Levels, vWF:Ag, vWF:CB, vWF:RCoF and multimer analysis. The coagulation therapy followed a ROTEM-based algorithm. The intraoperative bleeding was assessed indirectly by comparing the amount of substituted coagulation factors and blood products.

Results: AVWS was detected in 10 out of 12 (83%) patients before surgery. Although vWF:Ag, vWF:CB and vWF:RCoF increased significantly after CPB, still 8 out of 12 patients (66%) were diagnosed with AVWS. The patients with and without intraoperative AVWS did not differ in the volumes of transfused blood products and fibrinogen concentrate, but the ones with AVWS needed significantly higher amount of Prothrombin Complex Concentrate. Nevertheless, all patients were massively transfused due to strong intraoperative bleeding, so it was not possible to detect significant differences in the small cohort.

Conclusions: This data reveal a new possibility to treat intraoperative bleeding during high risk cardiac surgery of the newborn by substitution of von Willebrand Factor concentrate. The high incidence of AVWS is probably due to shear stress caused by valvular or vascular stenosis. Further causes still need to be investigated in a larger cohort.

PB 2164 | International Survey of the Management of Disseminated Intravascular Coagulation among Pediatric and Neonatal Health Care Practitioners

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Background: ISTH developed a diagnostic scoring system for disseminated intravascular coagulation (DIC) which has been used widely in adult intensive care units. However, controversies exist in pediatric DIC care regarding choice of diagnostic tests.

Aims: To evaluate current practice in pediatric/neonatal DIC management among neonatologists (NICU), pediatric intensivists (PICU), hematologists/oncologists (PHO), emergency physicians, and general pediatricians.

Methods: An online survey (LimeSurvey software) was developed; 3 sections: Respondent Demographics; DIC Diagnosis; DIC Management. It was internationally disseminated via professional societies. Study coordinated by Hamilton (Canada) and Melbourne (Australia) in collaboration with Manchester (England) and members of ISTH Pediatric and DIC SSC. Data collected January - September 2016.

Results: 211 responses: 160 full, 51 partial. 133 (63%) respondents from PHO, 45 (21%) NICU, and 23 (11%) PICU. Geographic distribution: 96 (46%) North America, 56 (27%) Asia, 25 Australia (12%), 24 Europe (11%). The most frequent cause of DIC was sepsis (163/211; 77%), mostly bacterial. To investigate DIC, 79 (42%) respondents use clinical experience only, 52 (28%) use the ISTH guidelines, 28 (15%) use local institutional guidelines. Of those who use only clinical experience, 96% order a platelet count and over 80% order INR, PT, aPTT, fibrinogen, and D dimer. Fibrin degradation products and smears for schistocytes are ordered by 28% and 41%, respectively, while thromboelastography (TEG) is ordered by 6% of respondents. Transfusion of platelets (69%), plasma (64%) and cryoprecipitate (47%) were the most common treatments, while antithrombin (18%), fibrinogen concentrate (16%), antifibrinolytics (9%), unfractionated heparin (9%) and LMWH (5%) were less common. Barriers to using standard DIC guidelines were identified.

Conclusions: Survey reveals variable practices in pediatric/neonatal DIC. Adult guidelines are not widely accepted in pediatrics. Pediatric DIC guidelines are needed.

PB 2165 | Efficiency and Safety of Off-label Use of Recombinant Activated Coagulation Factor VII (rFVIIa) in Pediatric Oncological and Hematological Patients

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Background: Recombinant FVIIa is often used off-label as a rescue therapy when conventional measures failed. But data on efficiency and safety of off-label rFVIIa use are scarce.

Aims: To analyze efficiency and safety of off-label use of rFVIIa in critically ill children.

Methods: Data were collected retrospectively. Efficacy was scored subjectively on a 3-point scale (complete, partial, or no response). Second endpoints were measurement of transfusion of red blood cells (RBC), platelets, fresh frozen plasma (FFP) in the 24 hours prior to and following use of rFVIIa. All thrombotic events within 7 days from last rFVIIa dose were recorded as adverse events.

Results: Final analysis included 92 cases of rFVIIa use in 65 patients aged from 2 months to 18 years.

TABLE 1 Patient primary context and 30-day mortality

Underlying diagnosis	No. (%)	30-day mortality, No. (%)
Acute leukemia and lymphomas	22 (34)	6 (27)
Primary Immunodeficiency	9 (14)	2 (22)
Congenital and acquired bone marrow failure	8 (12)	5 (63)
Solid malignant tumors	13 (20)	2 (15)
Blood vessel tumors and malformations	4 (6)	1 (25)
Others	9 (14)	1 (11)
HSCT recipients		
Yes	16 (25)	8 (50)
No	49 (75)	9 (18)

In 18 cases rFVIIa was applied to terminate surgical bleeding. Others most frequent indications were gastrointestinal (21 cases), pulmonal (12) and nasal bleeding (12). The median initial therapeutic dose was 75mcg/kg (IQR 44-98). Complete response was achieved in 52% of cases and in 21% there was partial response. There was not statistically significant correlation between severity of thrombocytopenia and efficiency of rFVIIa. In multivariate analysis, only severe hemodynamic instability before administration was independently associated with poor response to first dose. The need of transfusion of RBC declined (13,6 ml/kg VS 0 ml/kg, p=0,0011) after rFVIIa use. But there were no reduction in use of platelets and FFP. Four (6%) of 65 patients developed a thrombotic adverse event (TAE) up to 7 days after rFVIIa application. Initial and total doses of rFVIII in these patients did not exceed the corresponding values in those without thrombosis (p=0,71 and p=0,68, respectively). All patients with TAE were younger than 3 years and had multiple additional risk factors of thrombosis.

Conclusions: Use of rFVIIa is effective and generally safe for management of severe bleeding in children including patients with profound thrombocytopenia.

PB 2166 | Intracranial Hemorrhage in Children with Immune Thrombocytopenic Purpura: A Systematic Review

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Background: Immune thrombocytopenic purpura (ITP) in children is usually a self-limiting disorder however intracranial hemorrhage (ICH) is its rare but most serious complication which leads to significant morbidity/mortality. The incidence, characteristics, predictive factors and outcome of ICH in children with ITP has not been fully established.

Aims: Systematic review of literature to identify the incidence,age of onset, mean platelet count, predictive factors, clinical characteristics and outcome of ICH in children with ITP.

Methods: Literature search (1954-2016) on ICH in children with ITP was performed by two independent reviewers using PubMed, Embase and Cochrane. Articles were included if they described ICH in children with ITP. Pooled estimates expressed as percentage (%) with 95% Confidence Interval (CI) were generated using the inverse-variance weighted method in random-effect models.

Results: 21 articles met our inclusion criteria (5 institutional surveys, 7 case-series, 1case-control study, 8 case reports). Total 143 children with ICH were identified with an occurrence of 0.9% (95% CI= 0.4-1.3).Median age was 11 years (range = 0-17 years). Mean platelet count at the time of ICH was 9 ± 3 X 10^{9}/L. A total of 34% (95% CI= 20-51) had acute ITP, 19% (95%CI= 8-27) persistent ITP and 47% (95% CI=28-67) had chronic ITP. Only 28 %(95% CI= 18-36) of children had identifiable risk factors, the commonest was head trauma reported in 19% (95% CI= 8-30) of total cases of ICH in childhood ITP. Despite being on treatment ICH was fatal in 37% (95% CI= 19-55) of the children, 12% (95% CI=4-19) had significant neurological sequelae while 51% (95% CI= 36-70) children recovered fully with no neurological sequelae.

Conclusions: ICH in children with ITP tends to occur more frequently during chronic ITP. Large-scale prospective trials are needed to assess possible predictive factors. This systematic review constitute an essential step to identify ICH and the rationales for further clinical studies on ICH in children with ITP.

PB 2167 | Comparison of 3 Current Disseminated Intravascular Coagulation (DIC) Scores in Pediatric Intensive Care Unit (PICU)

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Background: DIC is frequently found in ICU. The DIC score defined by International Society on Thrombosis and Haemostasis (ISTH) is used worldwide. Besides ISTH score, Japanese Association for Acute Medicine (JAAM) and Japanese Ministry of Health and Welfare (JMHW) scores have been published and mainly used in adults. The studies of using those 3 scores in children were restricted.

Aims: To compare 3 DIC scores in PICU at Chiang Mai University (CMU) Hospital.

Methods: 100 subjects, aged 1 mo-18 yrs, admitted to PICU >24 hours with underlying diseases predisposing to DIC were enrolled. After the consent, complete blood count (CBC), prothrombin time (PT), fibrinogen and D-dimer were tested in all subjects at 24-48 hrs of admission. DIC was diagnosed when score ≥5 (overt) by ISTH score, ≥4 (acute phase) by JAAM scores and ≥4 and ≥7 in subjects with and without hematopoietic tumors, respectively, by JMHW score. This study was approved by IRB/EC.

Results: Sixty-two per cent were male and mean age (±SD) was 3.6 (±4.4) yrs. The prevalence of DIC by ISTH, JAAM and JMWH were 22, 54 and 16%, respectively. The prevalence of DIC was 56% when 3 scores were combined. Only 12% were diagnosed DIC by all 3 scores and 8/12 had cancers as the underlying diseases. Subjects diagnosed with DIC by ≥1 score had more bleeding symptoms (16/56 vs 4/44, *P*=.0.02) and required more transfusion (37/56 vs 19/44, *P*=.0.02) but did not have higher episodes of thrombosis than non-DIC group. Platelet count was lower (189 vs 278x10^3/uL, *P*= 0.01) and PT was prolonged in subjects with DIC (20 vs 13 s, *P*= 0.01) than in non-DIC group. Subjects with DIC did not have higher chance of death (11/56 vs 6/44, *P*=.0.43) but increase length of stay (LOS)
(99 vs 62 d, *P* = 0.01) than non-DIC group.

Conclusions: JAAM score seemed to be more sensitive than ISTH and JMWH scores for diagnosing DIC in children. Cancers were the most common cause of DIC in this cohort. DIC tends to cause bleeding, blood product use and LOS in children.

PB 2168 | Predictors of Postoperative Bleeding in Children Undergoing Cardiopulmonary Bypass: A Preliminary Italian Study

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Background: Several characteristics such as demographics, pre-existing conditions, surgical procedure, perioperative coagulopathy may predispose children undergoing cardiopulmonary bypass (CPB) to bleeding complications. As yet, studies on risk factors for postoperative bleeding have brought mixed results.

Aims: The purpose of our study was therefore to retrospectively evaluate the parameters able to predict postoperative bleeding in a group of consecutive children undergoing cardiac surgery involving CPB.

Methods: We collected demographic and perioperative laboratory data, as well as intraoperative transfusion requirements and blood loss during the first 24 hours after surgery in a group of consecutive children (aged ≥ 1 month) scheduled for cardiac surgery with CPB at Padua University Hospital between June 2014 and April 2015. Cases were patients who experienced a 24-h postoperative blood loss ≥ 80th percentile.

Results: Eighty-three children (M:F 38:45; age range 1-168 months) were enrolled. In a multivariate logistic regression analysis taking into consideration surgical features, postoperative laboratory data and intraoperative transfusion requirements, age below 7.7 months (p 0.015), postoperative platelet counts lower than 109 x10^9/L (p 0.003) and postoperative D-dimer ≥2350 µg/L (p 0.007) were the variables most significantly and independently associated with excessive 24-h postoperative blood loss.

Conclusions: Although preliminary, our study identified younger age, lower postoperative platelet count and higher D-dimer plasma levels as possible risk factors for postoperative bleeding. As for coagulation parameters, our results suggested consumptive coagulopathy may cause a strong predisposition to postoperative bleeding in children. Further large-scale prospective cohort studies would provide vital insight into the early diagnosis and treatment of CPB-related coagulopathies.

PB 2169 | Clinical Utility and Mechanism of Thrombin Generation in Children with Liver Disease

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Background: Changes in pro- and anti-coagulant factors in children with liver disease result in adverse haemorrhagic and thrombotic events in these patients. Routine coagulation tests provide an incomplete assessment of the bleeding risk, as they are not sensitive to changes in anticoagulant factors. Thrombin generation assay (TGA), as a global measure of haemostasis could be suitable for risk prediction in children with liver disease.

Aims: To: Assess the accuracy of the TGA in predicting the risk of bleeding in children with chronic liver disease; Determine the mechanism of TG in this population, focusing on prothrombin conversion and thrombin inhibition.

Methods: Patients were categorised as having: Severe (PELD > 15) and mild (PELD < 15) disease, or a portal vein obstruction or shunt. Age and gender matched healthy controls were used. The TGA was performed on plasma samples from patients and controls with and without thrombomodulin (TM) and the results were incorporated into a computational model of thrombin decay.

Results: A total of 42 patients (severe, n=5; mild, n=29, obstruction/shunt, n=8) and 20 controls were included. Whilst there was no

correlation between TGA parameters and PELD score, the endogenous thrombin potential (ETP) correlated to the bleeding score in the presence of TM (p = 0.038) across all patient groups. The rate of prothrombin conversion, thrombin-AT formation and the thrombin decay capacity, with and without TM were reduced in severe liver disease, whilst prothrombin conversion and thrombin decay capacity were increased in patients with obstruction/shunts compared to controls.

Conclusions: ETP is an accurate marker of bleeding risk in both liver disease and portal vein obstruction/shunt. The mechanism of TG in children with liver disease is largely dependent on the concentration of prothrombin and it's availability to be converted to thrombin, as well as on the availability of AT as an inhibitor in this process.

PB 2170 | Carotid Intima Media Thickness and Obesity Phenotypes among Children and Adolescents

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Background: Obesity phenotypes and subclinical atherosclerosis are premature complications of excess weight.

Aims: In the current study, we sought to determine the association obesity phenotypes and carotid intima media thickness (cIMT) in overweight and obese pediatrics.

Methods: Sixty-eight overweight and obese children and adolescents (41 boys and 27 girls, age 10.4±1.7, range 7-13 years) were recruited. For the evaluation of arterial IMT, carotid scans were performed by operator according to a standardized scanning protocol for carotid arteries. Obesity phenotypes was defined based on BMI and metabolic status: metabolically healthy obese (MHO): overweight or obese (BMI ≥ 85th percentile) and ≤ 2 parameters of cardio-metabolic risk factors; metabolically unhealthy obese (MUO): overweight or obese (BMI ≥ 85th percentile) and ≥ 3 parameters of cardio-metabolic risk factors.

Results: Forty children were obese (BMI≥95th percentile) and the 28 others were overweight. The mean±SD of cIMT was 0.44±0.05 mm. The prevalence of MHO and MUO were 55.9% and 44.1%, respectively. After adjusting BMI, insulin concentration was higher among MUO phenotype (16.9±1.05 μU/mL) compared to MHO one (12.3±0.93 μU/mL, $P< 0.05$). The BMI-adjusted cIMT was higher in MUO subjects [0.46 (95% CI: 0.43-0.48)] compared to those with MHO phenotype [0.43 (95% CI: 0.41-0.45), P=0.07].

Conclusions: Metabolically unhealthy obese children and adolescents are at higher risk of subclinical atherosclerosis than metabolically healthy obese ones.

PB 2171 | Acquired von Willebrand Syndrome (aVWS) in Severely Ill Pediatric Patients with Bleeding Complications

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Background: Von Willebrand factor is a multimeric protein which is required for platelet adhesion and plays an important role in haemostasis. Generally, acquired von Willebrand syndrome (aVWS) is associated with a recognizable underlying disorder. In the general population, aVWS is reported with a prevalence of 0.04 to 0.13%. According to the ISTH registry, lymphoproliferative (48%), cardiovascular (21%), myeloproliferative (15%), other neoplastic (5%), and autoimmune disorders (2%) are most common.

Aims: Acquired VWS seems to be an underdiagnosed condition in pediatric patients.

Methods: We report the cases of 14 critically ill paediatric patients who suffered from severe bleeding. In all patients, aVWS was diagnosed. Underlying disorders included dyskeratosis congenita (one boy), posttransplantation lymphoproliferative disease (two girls), sepsis with multiorgan failure and uremia (three patients), congenital heart failure (CHD) with stenosis (6 patients), unknown immunodeficiency (one girl), and progressive familial intrahepatic cholestasis (one boy).

Results: Analyses were performed because of bleeding. In most cases the treatment of bleeding was very difficult, and Treatment with vWF /FVIII concentrates was not sufficient to control bleeding. Then, substitution with rVIIa concentrates was necessary.

Conclusions: Acquired VWS seems to be an underdiagnosed condition in critically ill paediatric patients. The most common causes of avWF in children are congenital heart failure with stenosis, uraemia and lymphoproliferative disease or lymphoma. Microangiopathy, autoimmune disorder, leukocytosis or thrombocytosis were seen as further underlying conditions. Therefore, if there is unexpected bleeding in a critically ill pediatric patient, aVWS should be suspected. Specific treatment can be a challenge, however.

PB 2172 | Thrombopoietin Receptor Agonists for Children with Refractory Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which antibodies develop against platelets (plts) and dysregulation of cellular immunity result in premature destruction of plts and impaired plt production. For most children, ITP is

a self-limiting disease. Near 10% childs eventually develop refractory ITP (RITP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative to Rituximab (RTX) and splenectomy.

Aims: We present 3 different children with RITP treated with TPO-RA.

Methods:

Case 1: A 5-year-old girl admitted to the hospital due to ITP with mucocutaneous bleeding. She was refractory to corticoids, immune globulin (Ig) and anti-D Ig. RTX was started. After the 3rd dose, she responded temporarily along with fever, renal insufficiency and arterial hypertension probably related to Ig A deficiency, not previously diagnosed. Romiplostim was indicated, reaching response after 2 doses and stopped after the 4th dose. Nowadays, plt count remains within normal limits (pic.1A).

Case 2: A 5-year-old boy was diagnosed of ITP with mucocutaneous bleeding. He received treatment with corticoids and Ig with short response. RTX was indicated. After 4th dose, severe thrombocytopenia and mucocutaneous bleeding persisted. Eltrombopag was started with response after 6 weeks of treatment and bleeding were resolved (pic.1B).

Case 3: A 4-years-old boy with RITP was referred to our hospital. Eltrombopag was begun as 2nd line treatment. He developed response after 4 weeks of treatment. Six weeks later, he presented 600,000plts/μL so the drug was stopped. We observed a quick descent in plts levels and Eltrombopag was restarted with progressive response (pic.1C).

Results: In all cases, splenectomy was avoided due to long-term risk of sepsis, as well as immunosuppressive agents like RTX in 3rd case. In 1st case, TPO-RA was able to stop with sustained response as described in some publications.

Conclusions: In our experience, TPO-RA appear to be efficacy and well tolerated in children.

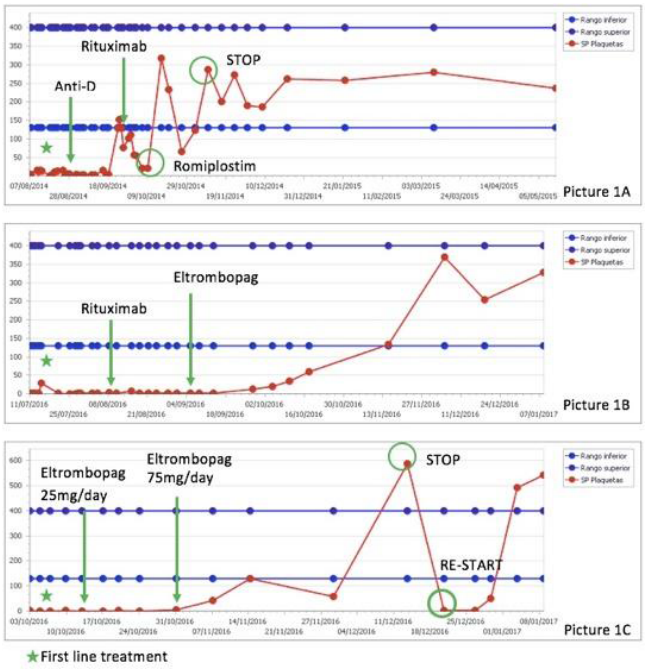

FIGURE 1 Evolution of platelet count

PB 2173 | An Unusual Cause of Bleeding in a Young Girl with Systemic Lupus Erythematosus: Lupus Anticoagulant Hypoprothrombinemia Syndrome

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Background: Lupus anticoagulant hypoprothrombinemia syndrome is an extremely rare clinical entity characterized by isolated acquired Factor II (Prothrombin) deficiency in patients with Lupus Anticoagulant (LA) positivity.

Aims: To describe the case of a young girl with systemic lupus erythematosus (SLE) and LA hypoprothrombinemia syndrome.

Methods: An 8 year old girl presented with 10 days history of fever, arthralgia, swelling over body, chest pain, respiratory distress, epistaxis, melena and bleeding from gums. On examination, she had pallor, anasarca and sub-conjunctival bleed. Chest examination revealed signs of a pleural effusion.

Results: Laboratory investigations revealed anemia, thrombocytopenia, deranged coagulogram with prolongation of both Prothrombin time (PT) and activated partial thromboplastin time (aPTT), and hypoalbuminemia (Table 1). Her blood culture was sterile, IgM dengue serology was negative, IgM scrub typhus serology was negative, serum Widal titres were normal, human immunodeficiency virus serology was negative and no malaria parasite was seen on peripheral smear. She was initiated on intravenous ceftriaxone and cloxacillin and fresh frozen plasma. However, there was no improvement in the coagulopathy and fever. Mixing studies revealed partial correction of PT and specific factor assay revealed very low levels of Factor II (Table 2). Work-up for SLE revealed positive ANA, high anti-dsDNA titres, low complements and positive antiphospholipid antibodies (Table 2). She was diagnosed as SLE with LA hypoprothrombinemia syndrome and was initiated on pulse intravenous methylprednisolone (30 mg/kg/day X 5 days). This led to prompt improvement in her clinical condition and resolution of fever and coagulopathy.

TABLE 1 Laboratory investigations

Investigation	Results
Hemoglobin	58 gm/L
White cell count	69×109cells/L (Polymorphs 71%, Lymphocytes 22%)
Platelet count	101×109/L
Prothrombin time	89 seconds (Normal: 12-14 seconds)
Activated partial thromboplastin time	More than 2 minutes (Normal: 25-32 seconds)
Serum fibrinogen	3.7 gm/L (Normal:2-4)
D- dimer	540
C-reactive protein	33 mg/L (Normal: <6)
Procalcitonin	0.9 ng/mL (Normal <0.5)

TABLE 2 Laboratory investigations

Investigations	Results
Antinuclear antibody	4+ homogenous pattern (indirect immunofluorescence)
Anti-dsDNA antibody titre	1394 IU/mL (Normal <60)
C3;C4	<27 mg/dL; <3 mg/dL
i)Lupus anticoagulant; ii)Anti β2 GPI (IgM and IgG); iii)Anti cardiolipin (IgG and IgM)	i)Positive; ii)Positive (IgG); iii) Positive IgM and IgG
24 hour urine protein	40 mg/m2/hour
Direct Coomb's test	Negative
Factor II assay	6% (60-160%)
Factor V assay	85.5% (Normal: 60-160%)
Factor X assay	105.3% (Normal: 60-160%)

Conclusions: LA hypoprothrombinemia syndrome is an uncommon condition and may be associated with SLE. Mixing studies and specific Factor II assay will establish the diagnosis. Immunosuppressant medications lead to prompt improvement of coagulopathy.

PB 2174 | Cost-benefit Relationship of the Collagen-binding Assay on von Willebrand Disease Study in Children

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Background: Von Willebrand disease (vWD) is the most frequent inherited bleeding disorders in children. In our institution the diagnosis is made by quantification von Willebrand factor (vWF:Ag), factor VIII (FVIII:c), Ristocetin cofactor (CoR) and collagen binding assay (vWF:CB), defined as complete vWD study (EC-vWD). Data published in adults show that vWF:CB contributes 23% to the diagnosis of vWD. In our country, vWF:CB is not codified by social health system which increases the cost of the study.

Aims: To evaluate whether EC-vWD improved capacity of diagnosis in children between 0 and 18 years evaluated in the hemostasis laboratory of our institution.

Methods: A retrospective study that reviewed the database of EC-vWD assays from 2008 to 2012. We reviewed a total of 464 records. The results were classified according to the diagnostic criteria of vWD (Quiroga, 2014): Normal (N), Normal Low (NL), Possible vWD (P-vWD) and vWD. Data was analyzed with Graph Pad Prims 6®. Statistical analysis: Fisher´s exact test for differences between criteria and Spearman-rank tests for correlations. Statistical significance: $p < 0.05$.

Results: Of the 464 EC-vWD analyzed, the mean age was 8.4±4.7 years. The proportion of women was 49.1%. EC-vWD results were:

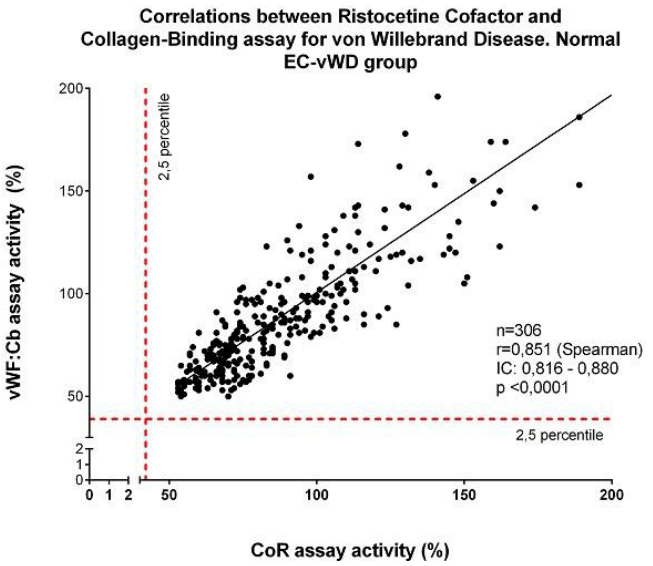

FIGURE 1 Correlations between CoR and vWF:CB assays. Normal children group

N: 65.9%; NL: 15.9%; P-vWD: 8.8% and vWD: 9.4%. The correlation between CoR and vWF:CB was significant in all groups (N r=0.85; NL r= 0.70; p-vWD r= 0.93 & vWD r= 0.82, all $p < 0.001$; fig 1 & 2.

To comparing contribution of CoR and vWD:CB for the all diagnostic categories there were no differences in any categories.

Conclusions: According to the analysis vWF:CB alone did not increase the research of vWF cases in children. These results differ from those of Quiroga et al., who showed that vWF:CB improves the vWD diagnosis on 23% in adults. This allows us to point out that for the diagnosis of vWD in children the initial approach may omit the vWF:CB

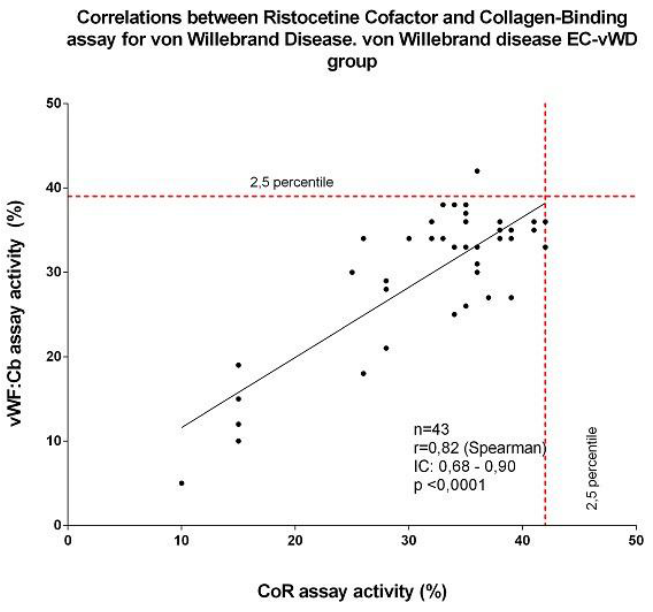

FIGURE 2 Correlations between CoR and vWF:CB assays. groupvon Willebrand Disease children group

assay, which does not alter the diagnostic capacity of vWD and reduces the cost of study vWD. EC-vWD could be used as a second-line study.

PB 2175 | Severe Factor VII Deficiency in Lebanon: The Impact of Molecular Studies, Prenatal Diagnosis and Primary Prophylaxis

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Background: Congenital deficiency of Factor VII is rare but is the most common autosomal recessive hemorrhagic disorder. The clinical phenotype varies from asymptomatic forms to lethal hemorrhagic diathesis characterized by central nervous system (CNS) and gastrointestinal (GI) hemorrhages occurring commonly during the neonatal period. Severe phenotypes account for 10-15% of symptomatic FVII deficient patients. Some mutations are consistently associated to the severe clinical phenotype, mostly specific missense and invariant AG or GT splice site mutations.

Aims: We are presenting a case series of several Lebanese offsprings of consanguineous marriages homozygous for the same *F7*:c.291+1G>C splice site mutation known to be associated with life-threatening bleeding phenotype and evaluating the impact of prenatal diagnosis and primary prophylaxis on the outcome of these children.

Methods: In our series, five children from four different families, presented as a first symptom with GI bleeds (n=4) or CNS bleed (n=1) within the first two months of life (range Day 1 to Day 40 of life). Among them, three could not receive the assigned rFVIIa prophylactic regimen and CNS bleeds recurred in all of them leading either to death (n=2) or sequelae (n=1).

Results: Three children from affected families were diagnosed prenatally via amniocentesis analysis at 16 weeks of maternal gestation, and in homozygous patients prophylaxis with rFVIIa was started at the dose of 30 µg/kg 2-3 times a week right after birth with excellent outcome in terms of bleeding prevention. When continued, this regimen was very well tolerated, and was shown to avoid any life-threatening bleed allowing the patient to lead a normal life.

Conclusions: Early diagnosis and, if possible, prenatal diagnosis is of paramount importance in these severe variants and primary prophylaxis starting at birth should be considered the management of choice in this clinical setting, to avoid life-threatening bleeds and disability, at least till gene therapy becomes available.

PB 2177 | Genetic Confirmation and Identification of Novel Mutations in Glanzmann Thrombasthenia and von Willebrand Disease Families by Diagnostic Exome Sequencing

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Background: Congenital platelet function disorders and von Willebrad disease (vWD) are very heterogeneous group resulting in primary hemostatic defects. Physicians generally have difficulty to confirm them due to complicated diagnostic technique.

Aims: We intended to apply diagnostic exome sequencing (DES) for genetic confirmation and finding causative variants in children with primary hemostatic problems.

Methods: Library preparation was performed with TruSight One sequencing panel (Illumina, USA), which enriches about 4,800 genes with clinical relevance. Massively parallel sequencing was conducted with NextSeq (Illumina). Variants were annotated with population databases (1000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium) and disease databases (OMIM). For missense variant, in-silico analysis was done with SIFT, PolyPhen-2, and MutationTaster. Candidate variants were confirmed by Sanger sequencing and family study. For *VWF* gene, multiplex ligation dependent probe amplification assay was also done.

Results: Twelve children with easy bruising, frequent epistaxis, or menorrhagia and their family members were enrolled. Two unrelated children were confirmed as Glanzmann thrombasthenia (GT). One proband had compound heterozygous variants of c.1913+5G>T and c.1451G>T (p. Gly484Val) in *ITGB3*. The former was pathogenic which results in aberrant splicing and the latter is novel. The other proband had homozygous variant of c.1913+5G>T in *ITGB3*. Three unrelated children were confirmed as vWD. One proband had compound heterozygous variants of c.2574C>G (p.Cys858Trp) and c.3390C>T (p.Pro1127_Gly1180delinsArg) in *VWF*, especially the latter synonymous variant previously confirmed to be resulted in exon 26 skipping. Another proband had a novel variant, c.2008C>T (p.Arg670Cys). The last proband had a known *VWF* pathogenic variant of c.1728G>T (p.Met576Ile).

Conclusions: DES is a valuable method to confirm GT or vWD. Further study is needed to find out unidentifiable mutations by this strategy.

PB 2178 | Application of Different Technologies during Prenatal Diagnosis in Glanzmann´s Thrombasthenia

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Background: Glanzmann´s thrombasthenia is a bleeding disorder caused by qualitative and/or quantitative defects of platelet

membrane glycoprotein (GP) IIb/IIIa complex. The disease is inherited in an autosomal recessive manner.

Aims: This report describes the prenatal diagnosis performed using flow-cytometry and DNA sequencing technologies.

Methods: The high-throughput capture sequencing technique and PCR-Sanger sequencing were used to detect pathogenic mutations in the proband of this family and analyze the whole family at the genomic level. After the genetic cause was clarified, fetus blood sample was collected by direct puncture of the umbilical vein from the proband´s mother who was pregnant for 22 weeks. Both DNA sequencing and GPIIb/IIIa complex quantified by flow-cytometry were applied for prenatal diagnosis.

Results: The proband carried compound heterozygous mutations of p.Tyr220Cys and p.Leu872fs in the GPIIb gene. Pedigree analysis showed that the two mutations were inherited from the mother and father, respectively. DNA sequencing showed that the fetus inherit the mutation p.Tyr220Cys from mother and had a new mutation which had not been reported. Using flow-cytometry, GPIIb/IIIa complex on fetus platelets was quantified, which is about half of normal controls. The fetus was proved to be a carrier of Glanzmann´s thrombasthenia.

Conclusions: Combined DNA sequencing and flow cytometry can attain more reliable and accurate results of prenatal diagnosis in Glanzmann´s thrombasthenia.

PB 2179 | Continuous Infusion of Recombinant Factor IX in a Neonate with Severe Hemophilia B and A Vascular Ring Requiring Surgical Correction

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Background: Infusion of continuous coagulation factor in patients with hemophilia undergoing major surgery is an effective strategy for maintaining consistent hemostatic levels. Very little data exists for the use of continuous infusion in neonates, in particular those with hemophilia B receiving recombinant Factor IX (rFIX).

Aims: To report the case of a neonate with severe hemophilia B who required a high infusion rate of continuous rFIX during and after surgery for correction of a vascular ring.

Methods: The subjects medical and laboratory record were reviewed and relevant data extracted.

Results: A one week old infant with severe hemophilia B was diagnosed with a congenital vascular ring with planned surgical correction on the tenth day of life. Factor concentrate replacement via continuous infusion was planned for the perioperative period. A goal factor level of 1.0 IU mL^{-1} was set. Continuous rFIX infusion was started at a dose of 6 IU/kg/hr after a 150 IU/kg bolus. The infusion was begun 24 hours prior to surgery. Four hours after beginning the infusion, a factor IX of 0.6 IU mL^{-1} was obtained. Over the subsequent 18 hours the patient´s rate was adjusted based on serial examination of factor IX levels. A preoperative level of 0.94 IU mL^{-1} was obtained on a dose of 15 IU/kg/hr. No bleeding complications were noted during or after surgery and the factor level remained above 1.0 IU mL^{-1} at this rate until intermittent dosing was started on POD 2. Factor IX inhibitor titers measured 4 and 11 months later were negative.

Conclusions: Coagulation factor replacement by continuous infusion has advantages for hemophilia patients undergoing major surgery. Our experience suggests that neonates with hemophilia B require higher infusion rates in comparison to older patients and supports individualized dosing based on serial factor evaluations in the preoperative period. Using this approach, hemostatic levels of factor IX can be achieved and maintained in the newborn.

PB 2180 | Tailored Prophylaxis in Children with Hemophilia A: A New Approach for the Future

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Background: Primary prophylaxis started early at dosage of 25-40 IU/kg three times/week is considered the gold standard for the treatment of children with severe hemophilia, improving their quality of life (QoL) and reducing bleeds. In the last years a new approach to prophylaxis based on annual bleeding rate (ABR), presence of target joints, pharmacokinetics (PK) or lifestyle of each patient has begun to be adopted in hemophilia treatment.

Aims: To evaluate whether in a group of children with severe or moderate hemophilia A (HA) a tailored approach may be used to replace the standard therapy.

Methods: PK evaluation was carried out in eight children with severe or moderate HA referred to Hemophilia Center of Padua and previously receiving recombinant factor VIII, octocog alfa (20-80 IU/kg), using a computing program (Bayesian approach). For each patient a tailored prophylaxis was estimated with the same program considering a trough level of 1% (severe HA) or 3% (moderate HA).

Results: PK evaluations of six children with severe HA (FVIII < 1%) previously on prophylaxis and of two children with moderate HA (FVIII 2-3%) and ABR >5, previously treated on-demand, were carried out. Bayesian curve was created for each child. Afterwards a tailored prophylaxis was assessed individually employing PK data: clearance and half-life of FVIII, steady state volume, time to reach +1% from basal FVIII. Following our results, the previously on-demand treated pts were placed on prophylaxis and their ABR decreased significantly. Based on trough level data, the weekly frequency of infusions may be reduced in three patients with severe HA, while may be increased in two children. As to the remaining child, it may be to change only the dosage (Fig. 1).

Conclusions: A therapeutic approach based on PK and clinical characteristics of each patient could change the standard treatment. Based on our results tailored prophylaxis could be another effective option for children with HA, improving their QoL.

			Previous treatment		Pharmacokinetics				Tailored prohylaxis	
ID	Age (yr)	Weight (kg)	Dose (IU/kg)	Frequency	Clereance (dl/hr/kg)	Hal-life (hr)	Steady State Vol. (dl/kg)	Time (hr) to reach +1 %	Dose (IU/kg)	Frequency
*PD1	8	30.0	50.0	on demand	0.029	12.3	0.5	80	37.8	every other day
PD2	7	28.0	35.7	3 times/wk	0.031	11.9	0.5	78	37.2	every 72 hr
PD3	9	37.0	27.0 54.0	2 times/wk + 1 time/wk	0.039	10.5	0.5	56	15.3 74.1	2 times/wk + 1 time/wk
PD4	2	11.5	43.5	3 times/wk	0.052	8.9	0.6	53	29.7	every other day
PD5	7	29.0	34.5	3 times/wk	0.039	10.6	0.6	72	69.7	every 72 hr
PD6	7	22.0	22.7	3 times/wk	0.042	9.5	0.5	59	20.3	every other day
*PD7	9	33.0	51.7	on demand	0.027	12.9	0.5	83	51.9	every 72 hr
PD8	6	20.0	25.0	3 times/wk	0.034	11.6	0.6	63	44.0	every 72 hr

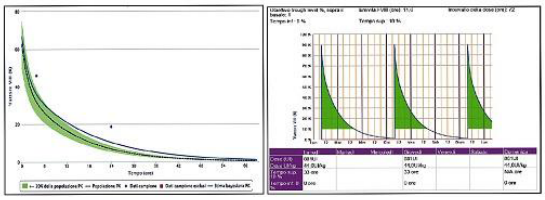

FIGURE 1 Previous treatment, PK, tailored prophylaxis for each child and an example of Bayesian curve of a patient (PD8) * Patients with moderate HA

PB 2181 | Normal aPTT in Children with Mild Factor XI Deficiency

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Background: Hemophilia C is a bleeding disorder caused by factor XI deficiency. Unlike hemophilia A or B the bleeding pattern is variable.Patients are screened for factor XI deficiency because of abnormal bleeding and/or an abnormal aPTT. It has been suggested that patients with factor XI deficiency may have a normal aPTT. Unfortunately, there are few data in the medical literature to confirm this notion.

Because clinicians rely on the aPTT in diagnostic algorithms when evaluating patients with abnormal bleeding, it is essential to determine if a normal aPTT eliminates the need for factor XI activity testing. This is especially true in children where phlebotomy and specimen volume constraints can be significant.

Aims: To determine if children at our center with factor XI deficiency have ever had a normal aPTT.

Methods: We queried our bleeding disorders database for all children diagnosed with factor XI deficiency (defined as more than one abnormal result). The subject's electronic medical record (EMR) was then extracted for bleeding symptoms/scores, demographic information, treatments given, and all available aPTT and factor XI measurements. This study was deemed exempt research by the Institutional Review Board of Saint Louis University.

Results: Our pediatric bleedings disorder center is located in the central United States with a referral region covering 3 million people. Seven children were identified as having factor XI deficiency. All had mild deficiency (0.21 to 0.39 IU/ml[1]). Their median bleeding score was 5. Four of the subjects received antifibrinolytics or plasma for surgery or abnormal bleeding. Three of the 7 subjects had a normal aPTT at the time of diagnosis, and 4 of 7 ever had a normal aPTT recorded.

Conclusions: The majority (57%) of subjects with mild factor XI deficiency had a normal aPTT. This supports the hypothesis that children with mild factor XI deficiency can have a normal aPTT. Pediatricians should incorporate this evidence in their diagnostic algorithm for children with abnormal bleeding.

PB 2182 | Impact of SIPPET [Survey of Inhibitors in Plasma-product Exposed Toddlers] Study on Clinical Practice in United States: A Survey of Hemophilia and Thrombosis Research Society [HTRS] Members

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Background: Factor VIII product class (recombinant versus VWF containing plasma derived factor VIII) and its role in inhibitor development among PUPs with hemophilia A was debated over years. SIPPET study addressed this question and showed that recombinant factor VIII (rFVIII) almost doubled the risk of inhibitor development compared to plasma derived factor VIII (PDFVIII) after adjusting for known high risk factors. These findings have created confusion among health care providers.

Aims: Understand the impact of SIPPET results on clinical practice in United States.

Methods: An anonymous web-based survey of HTRS members.

Results: Forty-nine percent [61/130] of pediatric hemophilia treaters completed the survey. Majority(94%) worked at HTCs. All providers treated racially diverse population with inhibitors. Majority (80%) used rFVIII while remaining customized their product choice based on family preference and a priori risk of inhibitor. Eighty-five percent were uncertain about the protective role of VWF against inhibitor development. Sixty percent [37/61] expressed concerns about study design primarily related to ethnicity differences followed by product choice of first and second generation concentrates versus third generation in US. Fifty percent shared SIPPET study with all PUPs while only a third discussed the results with all patients seen in comprehensive clinic. Seventy percent [48/61] admitted that the results of SIPPET study will influence their clinical practice and 35%[17/48] will consider using plasma derived concentrates to all PUPs while 54%[26/48] will use it for high-risk PUPs. About 80% of them will subsequently switch to rFVIII after 50 exposures. For PUP with minimal exposure to rFVIII and genetic predisposition for inhibitor, only 40% will switch to PDFVIII while 30% were uncertain and remaining 30% will continue with rFVIII.

Conclusions: Despite the concerns about SIPPET study design, the results of the study will influence the clinical practice of majority of pediatric hemophilia treaters in US.

PB 2183 | Intraosseous Infusion of Recombinant Factor IX

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Background: Obtaining vascular access can be challenging in the neonate. The use of the intraosseous route has been well described as a viable way to administer a variety of medications. To our knowledge, the administration of recombinant Factor IX (rFIX) by intraosseous infusion has not been reported.

Aims: To report a case of a neonate with severe hemophilia B requiring and safely receiving intraosseous infusion of rFIX.

Methods: The subjects medical and laboratory record were reviewed and relevant data extracted.

Results: The 2 day old infant presented with persistent post circumcision bleeding as well as persistent bleeding from a heel stick site. Laboratory values included a PTT >100s, Factor IX< .01 IU mL^{-1}, and a hemoglobin decline from 15 g/dL to 10 g/dL within a 24 hour period. Attempts at obtaining vascular access including umbilical and peripheral vessels were unsuccessful. A left tibial intraosseous line was placed to facilitate factor replacement. A single infusion of 100 IU/kg of rFIX was administered which resulted in excellent hemostasis. The intraosseous line was removed within 24 hours and no complications were noted. Factor IX inhibitor titers measured 4 and 11 months later were negative.

Conclusions: We report the successful administration of rFIX via the intraosseous route. We believe this represents the first report of rFIX concentrate being administered in such a fashion. Although we could not determine the recovery of rFIX, the rapid cessation of bleeding indicates adequate factor IX recovery. Our experience suggests that intraosseous infusion of factor concentrate is a viable option in emergencies.

PB 2184 | The Differential Diagnosis of Acute Rheumatic Fever in a Hemophilic Child

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Background: Acute rheumatic fever (ARF) is a pediatric age disease. Most occurred ages is between 5-15 years. The incidence of ARF is 30-50/100.000 in developing countries.Migratory nature of arthritis is specific for ARF. Hemophilia-A and ARF togetherness is rare.

Aims: Discriminative diagnosis of acute rheumatic fever from hemophilic arthritis.

Methods: Ten years old boy diagnosed as severe Hemophilia-A was on factor VIII prophylaxis. The patient was admitted with systemic fever, swollen right ankle. Factor VIII was applied due to spontaneously swollen left knee two days ago. His joint condition seemed to improve but, his right ankle started to swell and became painful. On admission right ankle was found swollen and warm. His left knee symptoms was resolved.

Results: His laboratory results; white blood cell: 22.500 /$mm^{3,}$ absolute neutrophil count: 18.700/mm^3, fibrinogen: 740 mg/dl, C-reactive protein: 24.7 mg/dl, erythrocyte sedimentation rate: 53 mm/hour, and Antistreptolysin-O:1420 U/ml . Antibiotic therapy and factor replacement were given concurrently. He was found inhibitor free. His left ankle started to swell under treatment. ARF was diagnosed according to Modified Jones Criteria with no carditis. Fever and arthritis were resolved and acute phase reactants decreased after adding prednisolone as anti-inflamatuar.

Conclusions: The main challenge for ARF diagnosis in hemophilic patients is to differentiate arthritis from acute hemorrhage. Prednisolone and oral penicillin prophylaxis can be preferred instead of ASA and intramuscular penicillin due to high risk of bleeding.

PB 2185 | Proteomic Analysis of Changes Induced by Infant Cardiopulmonary Bypass

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Background: Bleeding and thrombotic events cause significant morbidity and mortality in children on extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass (CPB). Existing laboratory studies fail to reliably predict and guide management of these events.

Aims: To better characterize the complex and overlapping interactions that make the pediatric coagulome and its response to vascular injury, we performed an unbiased proteomic analysis of infants undergoing CPB.

Methods: Blood samples were collected from infants (0-12 months) at initiation of CPB, and at 1, 4, and 24 hours after initiation. 2D-difference gel electrophoresis (2-DIGE) was utilized to identify changes in plasma protein concentrations across time points. Inflammatory cytokines and vascular injury markers were assessed by ELISA.

Results: Ten infants with congenital cardiac anomalies were enrolled with mean age 125±121 (range 3-330) days (Table 1). No immediate complications were observed. Using 2D-DIGE, >1400 individual protein spots were observed, and 89 proteins demonstrated significant concentration change (>30%, $p < 0.02$). 70/89 proteins were upregulated at one or more time points after initiation of CPB, while 19/89 were downregulated. 29/89 protein spots were identified by mass spectrometry (Table 2). These included multiple proteins not previously described as hemostatic proteins, with at least 7 identified as novel proteins with unknown functions. Of the cytokines analyzed with ELISA, IL-2, IL-8 and IL-10 were elevated at 4h after initiation of bypass and IL-6 was elevated at both 4 and 24 hours after initiation of bypass.

TABLE 1 Characteristics of infants undergoing CPB

Diagnosis	Operation	Age (days)	Sex	BSA (m^2)	CPB time (min)	Circulatory arrest time (min)	Survival
HLHS	Glenn shunt, PA plasty	161	M	0.2	176	53	Yes
TGV	Arterial switch	3	M	0.27	174	0	Yes
VSD	VSD closure	195	F	0.3	67	34	Yes
AVSD	AVSD repair	120	F	0.34	125	0	Yes
DORV	Bidirectional Glenn shunt	330	M	0.36	85	0	Yes
TGV	Arterial switch	3	F	0.2	129	0	Yes
Pentalogy of Cantrell	Modified Blalock-Taussig shunt/PA plasty	5	F	0.16	158	0	Yes
ASD	ASD repair	288	M	0.34	62	0	Yes
AVSD	AVSD repair	135	F	0.27	152	0	Yes
Pulmonary Atresia	RV-PA conduit	6	M	0.2	40	0	Yes

HLHS = Hypoplastic left heart syndrome,; TGV = Transposition of the great vessels; VSD = Ventricular septal defect; AVSD = Atrioventricular septal defect; DORV = Double outlet right ventricle; ASD = Atrial septal defect

TABLE 2 Changes in Plasma Protein Concentrations at Time Points From Baseline at Initiation of CBP.

Protein Name	1h	4h	24h	Function
Antithrombin III	---	48%	42%	Anticoagulant
Fibrinogen gamma chain	---	88%	106%	Hemostasis
α-2 HS glycoprotein	---	-40%	-29%	Acute phase reaction protein
α 1-acid glycoprotein	-284%	-409%	-174%	Acute phase reaction protein
α 1-antichymotrypsin	---	---	266%	Acute phase reaction protein
α1-B glycoprotein	---	37%	---	Acute phase reaction protein
Apolipoprotein E	---	-59%	-42%	Maintenance of lipoprotein particle
Apolipoprotein A1i	-55%	---	---	Maintenance of lipoprotein particle
Hemopexin precursor	59%	61%	---	Heme scavenger
Amyloid P component, serum	---	55%	112%	Cell free DNA scavenger
SPT2 chromatin protein domain containing 1	---	---	242%	Histone chaperone
Rho guanine nucleotide exchange factor 11	---	---	-263%	Second messenger signaling
Serine/threonine-protein kinase PDIK1L	-23%	-41%	---	Protein kinase
LBH domain-containing protein-1 LBHD1	---	-68%	-49%	Unknown
Integrator complex subunit 7 INTS7	---	56%	39%	Unknown
Transmembrane protein 78 TMEM78	---	-73%	-53%	Unknown
Novel protein DKFZp779N0926	---	---	82%	Unknown
Novel protein CAF16400	---	51%	43%	Unknown
Novel protein CAE91331	---	---	107%	Unknown
Novel protein CAE98668	---	---	48%	Unknown

Conclusions: In response to CPB in infants, changes are observed in the concentrations of numerous plasma proteins. Associating these plasma protein changes with clinically significant bleeding and thrombotic events that occur during the course of support may provide predictive biomarkers for better management of bleeding and thrombosis in these infants.

PB 2186 | Spatial Clot Propagation in Neonates and Adults

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Background: The hemostatic balance of neonates is more fragile than that of adults due to lower levels of clotting factors but also inhibitors. Nonphysiologically high tissue factor concentrations in conventional clotting assays result in apparent hypocoagulation with neonatal samples, but with low levels of tissue factor neonatal and adult hemostasis is almost comparable. This finding can be explained by lower concentrations of inhibitors resulting in rapid clot propagation after onset of clot formation. The Thrombodynamics assay uses immobilized tissue factor and video analysis for separate determination of initial and stationary rate of clot formation, thus, modelling the initiation and propagation phase of coagulation.

Aims: We aimed to test neonatal, cord, and adult samples with this assay and to compare results with data from Calibrated Automated Thrombography (CAT).

Methods: Plasma from cord blood (N=30), and venous blood of healthy neonates (N=10) as well as adults (N=20) was measured with the Thrombodynamics assay. All samples except venous blood from neonates were also measured with CAT.

Results: Initial (Vi) and stationary rates (Vs) of clot formation in the Thrombodynamics assay were significantly higher in neonatal than in adult samples (Vi: 69.54±5.32 µm/min vs. 54.93±5.47 µm/min P< 0.001; Vs: 52.17±12.93 µm/min vs. 31.14±5.62 µm/min, P< 0.001). No differences were observed between cord blood and neonatal venous blood. CAT parameters showed shorter lag times in cord blood samples than in adult samples (4.79±0.78 min vs. 6.94±1.5 min, P< 0.05), but a significantly lower enodgenous thrombin potential (714,8±130,7 nM*min vs. 1643±187 nM*min, P< 0.001).

Conclusions: Although the thrombin formation capacity is significantly lower in cord samples than in adult samples, the rate of clot propagation in the Thrombodynamics assay is faster. Hence, the Thrombodynamics assay, depicts the clinical picture of neonatal coagulation better than CAT and conventional clotting assays by mimicking a damaged vessel wall.

PB 2187 | Platelet-leukocyte Aggregates as Possible Biomarkers of Thrombotic Risk in Children with Hereditary Spherocytosis and Splenectomy

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Background: Hereditary spherocytosis (HS) is a chronic hemolytic disease caused by a pathologic structure of the erythrocyte membrane that causes their splenic sequestration. Splenectomy is the treatment used in severe forms, but implies the risk of encapsulated bacterial sepsis and recently has been suggested a long-term increased risk of thrombosis.

Aims: To study platelet activation markers (platelet-leukocyte (PL) and platelet-monocyte (PM) aggregates) as potential factors involved in the risk of thrombosis.

Methods: Forty-eight children were included in the study, 60.5% male, mean age 11.1 years. Control (n=16), with HS non-splenectomized (n=16) and with HS who were splenectomized (n=16). The 3 groups had similar sex and age. PL and PM aggregates were analyzed

by flow cytometry. ANOVA, Kruskal Wallis, Pearson correlation and multiple linear regression analysis were used. The study was approved by the ethical Committee of the Hospital La Fe. All patients or their proxy gave their written informed consent.

Results: When compared with the control group, the PL aggregates were 2-fold increased in the HS group, and 3-fold in the HS group with splenectomy (Table 1). The PM aggregates were also elevated in the patients (Table 1).The number of platelets was significantly correlated ($p < 0.05$) with the formation of PL and PM. The multiple linear regression adjusted by platelet number, showed a significant increase in PL aggregates in both groups, which was greater in the splenectomized group. For each increase of 100,000 platelets/ml, a 2.7% increase in PL aggregates was observed ($P < 0.05$).

TABLE 1 PL and ML aggregates in HS

	Control	HS	HS+ Splenectomy	P
PL aggregates (%)	7.3 (SD 3.5)	14.6 (SD 5.9)	21.9 (SD 9.2)	0.0001
PM aggregates (%)	25.1 (SD 21.3)	50.4 (SD 23.3)	55.4 (SD 13.4)	0.0006

Conclusions: Platelet activation is elevated in children with HS, which is greater in children with HS who were splenectomized, adjusted by the number of platelets. PL aggregates may be a useful biological marker of thromboembolic risk in patients with HS. Grants. FIS13/00016. ACIF/2016/465. RETICS networks INVICTUS (RD12/0014/0004) and INVICTUS+ (RD16/0019/0008) Instituto de Salud Carlos III.

PB 2188 | Pediatric Chronic Kidney Disease: Assessment of Endothelial Function and Hemostasis

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Background: Chronic kidney disease/CKD is associated with cardiovascular diseases and impairment of patient´s quality of life. These problems are more serious in childhood because affect not only the course of CKD but also triggering complication in adult life. Endothelial dysfunction plays a critical role in the development of atherosclerosis, which may be a common pathogenesis pathway for CKD and cardiovascular disease.

Aims: To evaluate in CKD patients, the endothelial activation through the biomarkers levels, E-selectin, Vascular/VCAM-1, Intercelular adhesion molecule-1/ICAM-1, Vascular Endothelial Growth Factor/VEGF and the hemostasis by D-Dimer levels.

Methods: This study was conducted in accordance with the declaration of Helsinki and local committee of ethics. It included 38 CKD pediatric patients (6-18 years old) and 31 healthy volunteers match for age and sex. Soluble E-selectin, VCAM-1, ICAM-1, VEGF and D-dimer plasma levels were evaluated by ELISA. Mann-Whitney test was used for comparison between groups and Spearman for biomarkers and glomerular filtration rate (GFR) correlation.

Results: Higher endothelial activation was found in CKD patients comparing to volunteers; sE-selectin 40.39(32.95) vs 32,87(15.16) P=0.005; sVCAM 925.6(340.5) vs 511.7(145.2)P< 0.0001); sICAM 254.1(128.5) vs 202(109,2)P=0.007; VEGF 253.7(155.1) vs 111(86.15) P=0.0001. As well, a hipercoagulability was found in CKD patients vs healthy volunteers; D-Di 343.7(329.9) vs 287.9(158.4) P=0.022. Considering all participants, correlations were found among the endothelial activation biomarkers each other, as well as between D-Di and GFR.

Conclusions: Our findings show endothelium dysfunctional in CKD pediatric patients. High D-Di levels reinforce the early predisposition to hypercoagulability in these patients. The studied markers can be useful for pediatric CKD management .Clinical measures are needed to prevent or postpone atherosclerosis and cardiovascular disease in these patients.

PB 2189 | Derivation of a Clinical Prediction Rule to Use with D-dimer to Exclude Pulmonary Embolism in Children

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Background: Pulmonary embolism (PE) rarely occurs in children. Prior work suggests a frequent delay in diagnosis. D-dimer testing offers non-high pretest probability to exclude PE in adults, but little evidence exists regarding utility in children.

Aims: We sought to derive criteria for a clinical prediction rule (CPR) to guide D-dimer use in children with suspected PE.

Methods: Records of children aged 5-17 years who had D-dimer or pulmonary vascular imaging (PVI) were collected by query of admin databases from Jan 2004 to Dec 2014 from a large multi-center US hospital system. Chart review confirmed that D-dimer was sent to evaluate PE with a random sample (>15%) to examine interobserver variability (Cohen's K). Criterion standard for venous thromboembolism+ (VTE+) required diagnostic PVI or deep vein thrombosis on ultrasound. Predictor variables were examined by univariate analysis and retained variables used to test the provisional CPR without and with D-dimer ordered as part of standard care.

Results: The search identified 525 children aged 5-17 tested for PE with D-dimer or PVI (K=0.95 for random sample of 100); 51 had PE and 5 had isolated DVT (56/525 VTE+ (10.6%, 95% CI 8-14%)). Children with PE+ had higher mean heart (HR) and respiratory rate (RR) and a lower pulse oximetry (SaO2%) and hemoglobin concentration. Five conditions were more frequent in PE+ versus no PE: surgery, central line, limb immobility, cancer, and prior PE or DVT; and, 23.5%

of patients who were PE+ were currently prescribed an anticoagulant. Without D-dimer, a provisional CPR(-) requiring HR< 100, RR< 22, SaO2%>94, and no cancer, central line, recent surgery, limb immobility, prior VTE+, or leg swelling had diagnostic sensitivity of 93% (83-97%) and specificity of 44% (40-48%). The CPR(-) with D-dimer normal had a sensitivity of 100% (91-100%) and specificity of 39% (35-44%).

Conclusions: We found VTE+ rate among children to be high, and D-dimer is frequently used. A preliminary CPR with normal D-dimer was 100% sensitive and 39% specific.

PB 2190 | Thrombin Generation in Pediatric Patients with Generalized Infectious Diseases and Multiple Organ Dysfunction Syndrome

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Background: Severe sepsis is associated with systemic activation of coagulation and increased thrombin generation, which can result in thrombosis. Coagulation parameters, such as prothrombin time (PT) or aPTT, poorly reflect the activation of blood coagulation. The Calibrated Automated Thrombography (CAT) is a more useful tool for the testing of the haemostatic system. It can be performed with thrombomodulin (TM) addition, and in this case, the method becomes sensitive to the protein C system disorders.

Aims: The aim of our study was to evaluate the possibility of CAT using for assessment of the hypercoagulability in pediatric patients with multiple organ dysfunction syndrome (MODS).

Methods: The study involved 5 patients with MODS and 30 healthy adults. PT, aPTT, fibrinogen level and antithrombin activity were measured in all patients. The thrombin generation (TG) was measured by CAT according to Hemker et al. with PPP plasma +/- rh-TM reagent. We analyzed the following parameters: endogenous thrombin potential (ETP, nM*min), thrombin peak height (Peak, nM), and such parameter as sensitivity to TM - the reducing rate of the ETP and Peak thrombin after the addition of rh-TM.

Results: It is known that children have lower TG than adults. The results of CAT without rh-TM showed that ETP in all children did not differ from the adults, and Peak was increased in 3 of 5 patients. In the study of CAT with rh-TM ETP was increased in 4 of 5 cases, and Peak - in all samples. The sensitivity to TM was markedly reduced in all patients, which reflects the serious disturbances in the protein C system (table 1).

At the same time, the usual coagulation parameters were within the normal range in 4 of 5 patients. Only one child demonstrated decreased antithrombin activity (32%).

Conclusions: Our results show that CAT performed with and without TM is the good instrument for the early detection of hypercoagulability. One of the causes of hypercoagulability in pediatric patients with MODS is a disturbance in the system of a protein C.

PB 2191 | A New ADAMTS13 Mutation and Pharmacokinetic Properties in Congenital Thrombotic Thrombocytopenic Purpura (TTP)

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Background: Congenital TTP is a rare and potentially fatal disease secondary to constitutional deficiency of ADAMTS13. TTP is characterized by platelet aggregation in the microvasculature that results in microangiopathic hemolytic anemia and isquemia in various organs.

Aims: To report the case of a patient with a new ADAMTS13 mutation and evaluate pharmacokinetic (PK) properties after plasma infusion.

Methods: ADAMTS13 activity was measured by ELISA Technozym test and genetic study was done by gene sequencing (Illumina HiSeq/Nextera Exome Capture).

Results: A three-year-old girl, the first offspring of non-consanguineous parents, was hospitalized presenting as pale and apathetic, with petechiae and ecchymosis, vomiting and fever. Laboratory evaluation showed schistocytic hemolytic anemia, thrombocytopenia and

TABLE 1 The thrombin generation parameters in pediatric patients with MODS

CAT parameters	Normal range in healthy adults (Me, 95% CI)	Patient 1, 12 years	Patient 2, 3 years	Patient 3, 3 years	Patient 4, 3 years	Patient 5, 4 years
ETP without rh-TM, nM*min	1756,0 (1220,6-2159,9)	1941,5	2016,5	1722.5	1269.0	1717,0
ETP with rh-TM, nM*min	878,0 (538,8-1381,0)	1846,5	1924,0	1698.0	1252.5	1627,0
Sensitivity of ETP to TM, %	51,5 (22,9-64,4)	5,0	4,0	2,0	1,0	5,0
Peak without rh-TM, nM	293,9 (198,7-376,1)	396,5	427.7	387.9	324,9	341,9
Peak with rh-TM, nM	174,5 (113,8-310,0)	341,9	416.0	386.1	325,4	336,7
Sensitivity of Peak to TM, %	35,8 (14,8-53,1)	2,0	3,0	0,4	0	2,0

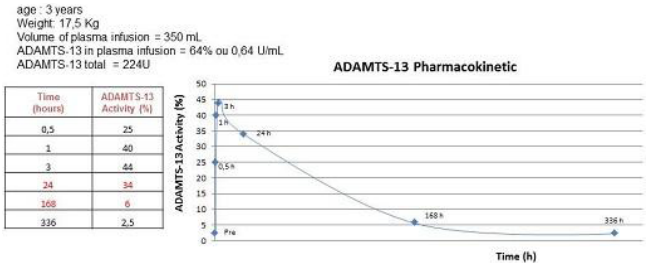

FIGURE 1 ADAMTS13 Phamacokinetics

renal dysfunction. Hemoglobin 9.4g/dl (11.7-14.4); Platelets 8,000/µL (150,000-450,000); Reticulocytes 8.9% (0.8-2.1); BUN 103mg/dl (11-38); Creatinine 1.47mg/dl (0.31-0.47); LDH 715U/L (160-370); ADAMTS13 activity < 5% (≥70%). Patient evolved with seizure and was treated with plasma infusions, dialysis and supportive care. After patient recovery, ADAMTS13 PK study after plasma infusion showed a half-life of 57.5 hours (Fig.1) and a recovery of 146%. Genetic analysis detected a compound heterozygous status: Chr9:136.307.625C>T(p.Arg692Cys) and Chr9:136.314.904G>GT(p.Trp955Leu fs*33) a new mutation. Patient is going well on prophylaxis with plasma infusion each two weeks without relapses.

Conclusions: This case report showed a high recovery and long half-life of ADAMTS13 in a Brazilian child with congenital TTP that presented a new ADAMTS13 gene mutation. These findings are important to individualize plasma infusion prophylaxis and for considering future treatment with recombinant ADAMTS13 concentrate.

PB 2192 | Thromboelastographic (TEG) Parameters in Newly Diagnosed Pediatric Patients with Acute Lymphoblastic Leukemia (ALL) and Central Venous Catheters (Cvcs)

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Background: One of the most common adverse events in children with newly diagnosed ALL is VTE. Many of these patients have CVCs, which are the most common risk factor in children for developing VTE. TEG is a global hemostasis assay that measures various parameters of coagulation including clot initiation and propagation. This study prospectively monitored TEG parameters in patients with newly diagnosed ALL and CVCs during Induction.

Aims: To investigate if TEG can detect and monitor hypercoagulability in patients with newly diagnosed ALL and a CVC and predict VTE development.

Methods: This study was approved by the IRB. Patients with newly diagnosed ALL and CVCs who consented/assented had blood sampled at baseline (pre-treatment/Day 0) and then weekly throughout Induction (Days 7, 14, 21, 28) unless they presented with a symptomatic VTE. TEG was performed on citrated blood that was not activated. Patients who did not have symptomatic VTEs had an ultrasound performed on the extremity with the CVC at the end of Induction. Random-effects interval-data regression models were used to assess whether there was a significant difference in TEG parameters between VTE+ patients and VTE- patients, or among blood samples collected at different time points.

Results: Patient demographics are shown in Table 1. TEG parameters of R time, K time, and MA all showed statistically significant decreases over time ($p < 0.001$ for all TEG parameters, Figure 1). There were no differences between VTE+ and VTE- patients in any of the TEG parameters.

Conclusions: TEG parameters of R time, K time, and MA all showed statistically significant decreases over time in patients with newly diagnosed ALL and CVC during Induction, indicating that TEG can detect and monitor hypercoagulability. There were no differences in TEG parameters between patients who did and did not develop VTE, though the number of subjects was small. Other labs collected (markers of thrombin generation) may indicate differences.

PB 2193 | Look, Feel, Move: A Case of Idiopathic Femoral Artery Thrombosis in a Neonate

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Background: True idiopathic thrombosis is extremely rare in children. Most thrombi occur secondary to iatrogenic causes. We present a case of successfully treated idiopathic thrombosis with low molecular weight heparin (LMWH).

Aims: To highlight the features of spontaneous femoral artery thrombosis resulting in peripheral limb ischaemia in a neonate.

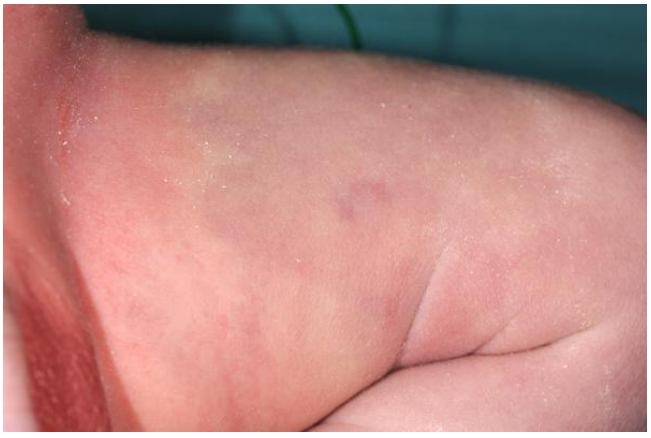

FIGURE 1 The appearance at birth of demarcated left upper leg

Methods: An infant boy was born at term via vaginal delivery and immediately after birth was noted to have a pale left leg. The left limb was also cold to touch, had a capillary return of six seconds and the left femoral pulse was weak while distal pulses were absent. Leg movement was normal. There was an irregular area of demarcation distal to the femoral arterial region.

Urgent Doppler ultrasound studies were undertaken which showed reduced arterial flow within the left femoral, popliteal and posterior tibial arteries. Reduced blood flow was also noted within the venous system of the left lower limb.

Results: LMWH injections were commenced twice daily as per haematology. A repeat ultrasound three days later showed chronic thrombosis of the left femoral artery with dilated collaterals in the inguinal region.An echocardiogram did not detect any abnormalities.

Enoxaparin was continued twice daily until discharge whilst monitoring the Factor Xa levels to keep within a target range of 0.5-1 IU/ml lower limb perfusion improved gradually. The left leg had a normal appearance by day 5 but was still cooler to touch and peripheral pulses remained difficult to palpate.

Following discharge LMWH was continued for three months and a follow up scan performed at this time showed a normal Doppler flow with no evidence of thrombosis.

A thrombophilia screen showed the boy to be heterozygous for FV Leiden and not thought to be a causative factor.

Conclusions: Our case highlights the importance of careful inspection of the neonate post-delivery and of appropriate investigation of abnormal findings.

PLATELETS – BASIC

PB 538 | Selected Expression and Functional Importance of alpha4a-Tubulin in Platelet Biogenesis

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Background: Platelets are produced from mature megakaryocytes (MK) following a profound cellular reorganization. This includes the assembly of microtubules (MT) into a unique submenbranous coiled structure, the marginal band (MB). This process is thought to depend on a specific αβ-tubulin isotype repertoire. Whereas the MK-restricted-β1-tubulin is already known to be important for platelet biogenesis, the implication of other tubulin isotypes is currently unknown.

Aims: Our goal was to establish the αβ-tubulin repertoire in platelets and during megakaryopoiesis and to evaluate the implication of selected isotypes in platelet formation.

Methods: We used a combination of RT PCR and proteomic analyses in human platelets and in human CD34+-derived MK to establish an exhaustive and quantitative list of the tubulin isotypes. The function of a selected α isotype (α4A) in MK and platelets was explored in an ENU mutant mouse model.

Results: β-6, β-5 and α1c -tubulin transcripts were already present in CD+34 cells and decreased during the final stages of megakaryopoiesis. β-1, α4A- and α-8tubulin transcripts were only observed later during MK differentiation and in platelets. Quantitative LC-SRM mass spectrometry revealed an important expression of the α4A -isotype in platelets. In mice carrying a point mutation in *tuba4a* (V260E) we observed a thrombocytopenia (25%) and enlarged platelets with a decreased number of MT coils (1-3/plt). α4A -tubulin was not detected in these platelets and MT were hyper-tyrosinated. Molecular modelling predicted that the mutation would result in a steric clash with neighboring residues.

Conclusions: This study reveals new information on the evolution of the tubulin isotype repertoire in platelet formation pointing to a role of less-widely expressed α-isotype.

PB 539 | Redundant and Non-redundant Roles of RhoA and Cdc42 in Platelet Biogenesis and Function

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Background: Small GTPases of the Rho family, such as RhoA and Cdc42, are critically involved in the regulation of cytoskeletal rearrangements during platelet activation. We have previously shown that megakaryocyte (MK)/platelet-specific RhoA deficiency leads to moderate thrombocytopenia and impaired platelet responses downstream of G-protein coupled receptors, whereas lack of Cdc42 was associated with moderate thrombocytopenia and enhanced agonist-induced platelet granule release. Despite partially overlapping downstream signaling pathways, little is known about the specific roles and functional redundancy of both proteins in platelet biogenesis and function.

Aims: We investigated functional redundancies of RhoA and Cdc42 in platelet production and function.

Methods: MK/platelet-specific (Pf4-Cre) RhoA/Cdc42 double knockout (DKO) mice were generated by intercrossing the respective single KO mice. MK and platelet morphology was analyzed by different microscopy techniques. Platelet functions were assessed by biochemical methods in vitro, or in vivo in models of arterial thrombosis and by hemostatic assays.

Results: Combined deficiency of RhoA and Cdc42 led to severe macrothrombocytopenia associated with pronounced alterations in MK morphology and generation of platelets of heterogenous size and granule content. The life span of circulating RhoA/Cdc42 DKO platelets was markedly reduced due to enhanced platelet clearance, whereas the rate of platelet production after antibody-induced platelet depletion was unaltered compared to wild-type littermates. RhoA/Cdc42-deficient platelets exhibited an overall reduction of agonist-induced integrin activation, but unaltered granule secretion. Despite severe hemostatic defects, $FeCl_3$-induced formation of occlusive thrombi was largely unaffected in RhoA/Cdc42 DKO mice.

Conclusions: These results implicate the existence of both distinct and overlapping roles of RhoA and Cdc42 in platelet production and function.

Research was funded by the DFG.

PB 540 | PDK1 is Crucial to Proplatelets Formation and Thrombopoiesis

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Background: Megakaryopoiesis is a complex process including megakaryocytes (MKs) maturation and differentiation. Finally, MKs form proplatelets within the bone marrow (BM) and release platelets in BM sinusoids. PDK1 has been shown to be highly expressed in platelets and megakaryocytes and is required for Ca^{2+}-dependent platelet activation, but its role in the regulation of platelet biogenesis has not been investigated so far.

Aims: The present study explored the role of PDK1 in the regulation of thrombopoiesis and platelet survival in a platelet- and megakaryocyte (MK) specific knockout approach.

Methods: PDK1-floxed mice were crossed with PF4-Cre mice to generate platelet-specific PDK1 deficient mice (*pdk1*$^{-/-}$) and wildtype littermates (*pdk1*$^{fl/fl}$); FACS analysis; Western Blot; RT-PCR; ploidy assay; proplatelets formation assay; immunohistological analysis.

Results: MK/platelet-specific PDK1-deficient mice (*pdk1*$^{-/-}$) developed a significant macrothrombocytopenia as compared to wildtype (*pdk1*$^{fl/fl}$) mice. As revealed by immunohistological analysis, *pdk1*$^{-/-}$ mice further displayed a remarkable MK hyperplasia as well as a significantly increased extramedullary thrombopoiesis. However, the size of spleen and liver was comparable between *pdk1*$^{-/-}$and *pdk1*$^{fl/fl}$ mice. While platelet lifespan was not significantly affected, *pdk1*$^{-/-}$ MKs have less contact to bone marrow sinusoids and displayed a drastic increased number of MKs with a significant lower size within the bone marrow. However, there was no significant difference in plasma TPO levels in *pdk1*$^{-/-}$ mice as compared to *pdk1*$^{fl/fl}$ mice. Further examination of bone marrow MKs revealed no significant difference in the ploidy of *pdk1*$^{-/-}$ MKs as compared to *pdk1*$^{fl/fl}$ MK, though *pdk1-null* MKs produced significantly less proplatelets indicating that PDK1 is required for proplatelet formation.

Conclusions: The present observations unraveled PDK1 as an important signaling molecule in proplatelet formation and thrombopoiesis.

PB 541 | Inhibition of MAP Kinase-interacting Kinase-1 (Mnk1) Regulates Platelet Functional Responses and Protein Synthesis in Megakaryocytes

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Background: MAPK-interacting kinases (Mnks) are serine/threonine kinases that control protein synthesis in neurons and neutrophils. The expression and function of Mnks in megakaryocytes (MKs) and platelets is unknown.

Aims: To evaluate the role of Mnk1 in MKs and platelets.

Methods: We used ETP-45835 (Mnk1 specific pharmacological inhibitor) to evaluate the role of Mnk1 in MKs and platelets.

Results: Mnk1, but not Mnk2, is expressed in human and murine MKs and platelets (Fig. A). Inhibiting Mnk1 activation in MKs with ETP-45835 reduced proplatelet formation. As protein synthesis is essential for proplatelet formation, we next examined the mechanisms by which Mnk1 regulates protein synthesis in MKs. We identified that CYFIP1, which repress translational in neurons through eIF4E, is expressed in MKs. Activation of Mnk1 in MKs was sufficient for eIF4E phosphorylation and release of CYFIP1 from eIF4E, thus permitting translation. Mnk1 inhibition reduced global protein synthesis by ~30%

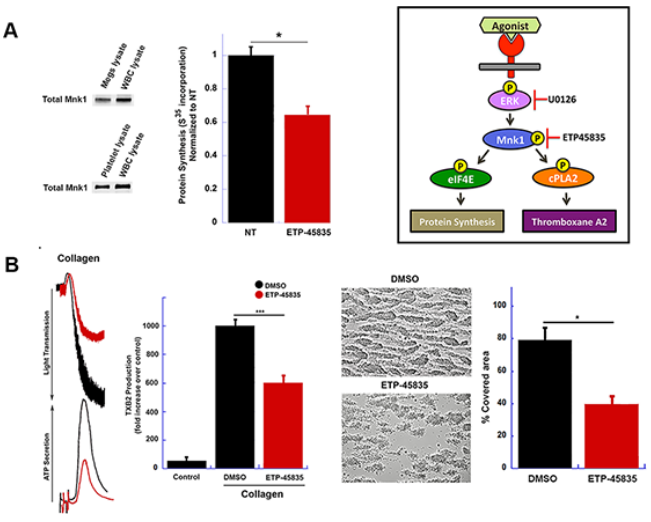

FIGURE 1 Mnk1 regulates platelet activation and protein synthesis in megakaryocytes

(Fig. A), as assessed by S[35]methionine, and prevented CYFIP1 dissociation from eIF4E. We next determined the role of Mnk1 in platelets. Mnk1 was activated in stimulated platelets (e.g. collagen, ADP). Mnk1 activation was abolished with U0126, an Erk1/2 specific inhibitor, indicating that Erk regulates Mnk1 activation in platelets. When Mnk1 activity was specifically inhibited with ETP-45385 *ex vivo*, platelet aggregation, secretion, TxB2 generation, and adhesion to collagen under flow was inhibited (Fig. B). Concordantly, treating mice with ETP-45385 *in vivo* prevented collagen and epinephrine induced pulmonary embolism. The addition of arachidonic acid to ETP45385-treated platelets *ex vivo* fully rescued platelet aggregation and TxB2 generation, demonstrating that Mnk1 activation in platelets regulates the arachidonic acid pathway.

Conclusions: Mnk1 controls proplatelet formation and protein synthesis in MKs. In platelets, Mnk1 regulates platelet activation responses and thrombosis through thromboxane dependent mechanisms.

PB 542 | On-track to Producing Commercially-Feasible cGMP-Compliant Human Platelets from induced Pluripotent Stem Cells at Clinical Scale

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Background: Platelets are the principal blood cells responsible for clot formation at sites of active bleeding, and are critical for treatment of cancer, trauma, and surgery. Nevertheless, donor-derived platelet transfusions are significantly limited by: donor shortages and recipient access; usable shelf life of only 1.5 - 2 days due to a 5-day storage limit at room temperature and a 2-day screening period for microbial growth and donor-specific pathogens; risk of bacterial and viral transmission; and wide functional variability among units and donors, leading to over-transfusion to insure effective bleeding control limit the effectiveness of donor-derived platelets.

Aims: The production of lab-generated human platelets is necessary to extend platelet transfusions beyond major cities in first-world countries and meet present and future transfusion needs.

Methods: We are advancing a cGMP-compliant; 1) serum- and feeder-free cell culture protocol to differentiate human induced pluripotent stem cells into megakaryocytes; and 2) automated scalable microfluidic device that supports continuous media perfusion and parallelization, maximizes cell zonal distribution and trapping, and allows independent control of pressure, shear stress, and stretch force exposure to trigger platelet production.

Results: We have developed a cGMP-compliant process that can produce >3x10^8 human platelets per business card-sized bioreactor, and is amenable to clinical and commercial scale up to produce an apheresis-equivalent platelet unit for < $5,000. Morphology, ultrastructure and function of our megakaryocytes and bioreactor platelets is ongoing, and consistent with donor platelets.

Conclusions: We can generate functional human megakaryocytes and platelets from cGMP-compliant human induced pluripotent stem cell cultures at costs/yields necessary to make production of a platelet transfusion unit clinically and commercially feasible.

PB 543 | Disruption of the Cholesterol Efflux Transporters ABCA1 and ABCG1 Alters Megakaryocyte Proplatelet Production

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Background: Platelets, produced by megakaryocytes in the bone marrow, play an important role in the onset and development of atherosclerosis and atherothrombosis. Platelet numbers and functionality are affected by hypercholesterolemia in mice and humans.

Aims: We investigated whether disruption of cholesterol efflux influences megakaryocyte proplatelet production.

Methods: Proplatelet production was studied in bone marrow explants from mice lacking ATP-Binding cassette transport A1 (ABCA1 KO), G1 (ABCG1 KO) or both (ABCA1/ABCG1 double KO (dKO)) and wildtypes.

Results: Explant cultures of both ABCA1 KO and ABCG1 KO bone marrow showed lower amounts of megakaryocytes at the periphery of the explants (-52±4% ($p < 0.01$) and -32±3% ($p < 0.001$) resp.), indicating that reduced cholesterol efflux capacity negatively influences either megakaryocyte maturation from progenitor cells, or their migratory capacity. Interestingly, ABCA1 and ABCG1 deletion had differential effects on proplatelet formation. ABCA1 deficiency inhibited proplatelet formation (-67±16%, $p < 0.001$). In contrast and despite the lower amount of megakaryocytes, a clear increase in proplatelet formation was observed in ABCG1 KO explants (+243±77%, $p < 0.001$). In line with the notion that ABCA1 and ABCG1 independently modulate megakaryocyte maturation and/or migration, the amount of visible megakaryocytes was even further reduced in bone marrow explants from ABCA1/ABCG1 dKO mice (-75±3%, $p < 0.001$). In further support, the marked increase in proplatelet formation resulting from ABCG1 deficiency was diminished upon additional deletion of ABCA1, leading to only a mild increase in proplatelet formation (+42±15%, $p < 0.05$) in dKO compared to wildtype megakaryocytes.

Conclusions: In conclusion, absence of cholesterol efflux transporters ABCA1 and ABCG1 alters proplatelet production by megakaryocytes. Moreover, our studies suggest that modulation of individual cholesterol efflux transporter function may translate into an overall differential effect on platelet production.

PB 544 | Environmental Chronic Stress Induces Abnormal Megakaryopoiesis Predisposing to Thrombosis: Protective Effects of Apocynin

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Background: Environmental chronic stress (ECS) has been identified as a trigger of acute coronary syndromes (ACS). Changes in redox balance, enhanced reactive oxygen species (ROS) production, and platelet hyper-reactivity were detected in both ECS and ACS. However, the mechanisms by which ECS predisposes to thrombosis are not well known.

Aims: We analysed the impact of ECS on megakaryopoiesis and platelet activation in mice and the effect of Apocynin, an inhibitor of NADPH oxidase, in this experimental setting.

Methods: Forced swimming for 4 days (5 min twice/day) and FeCl3 arterial injury were used as model to induce ECS and to assess thrombosis in mice, respectively. Megakaryocytes and platelets were analyzed by flow cytometry. Apocynin was administrated 2.4 mg/ml in drinking water for 4 days.

Results: We show that ECS leads to an abnormal megakaryopoiesis increasing the number of BM megakaryocytes (MKs) and affecting circulating platelets. MKs of stressed mice show an advanced maturation state (e.g. expression of CD42d), and an enhanced ability to produce ROS. Interestingly, a higher number of large and reticulated platelets with marked functional activation (e.g. integrin $a_{IIb}b3$ and P-selectin expression, and platelet/leukocyte aggregates) is detected after ECS. In addition, Apocynin decreases the total number of MKs and prevents their ability to generate ROS without affecting the percentage of $CD42d^+$ cells.

Finally, Apocynin reduces the hyper-activation of platelets and the enhanced susceptibility to $FeCl_3$-induced arterial thrombosis in stressed mice.

Conclusions: Apocynin treatment, reducing ROS generation in MKs, restores the physiological bone marrow megakaryopoiesis and platelet behaviour, and it prevents the detrimental effect of chronic stress on atherothrombosis. These data suggest a potential use of NADPH oxidase inhibitors in the occurrence of thrombosis associated with chronic stress. Studies in human will verify the clinical impact of these findings.

PB 546 | CCalcium/calmodulin-dependent Protein Kinase II (CamKII) Regulates Platelet Formation

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Background: CamKII is a known regulator of actin polymerization. Calcium fluxes result in phosphorylation of CamKII and activation of downstream Rho family small GTPases such as Rac-1 causing actin polymerization. While CamKII has been demonstrated to regulate dendrite formation in neurons, it's role in platelet formation has never been studied.

Aims: We hypothesized that calcium signaling through CamKII would regulate proplatelet formation in megakaryocytes.

Methods: We examined the Platelet RNA And eXpression-1 (PRAX-1) data set to determine if *CamKII* expression correlated with platelet counts in humans. RNA-seq was performed on murine megakaryocytes (MKs) and platelets to assess *CamKII* expression. Calcium fluxes were inhibited in cultured murine Mks and CamKII phosphorylation levels and proplatelet formation was determined. Mice were treated *in vivo* with KN93, a specific CamKII inhibitor, and platelet counts were followed over time.

Results: Human and murine MKs and platelets express both isoforms of *CamKII* (gamma and delta). *CamKII delta* expression in humans positively correlated with platelet counts ($p=0.03$). Concordantly, *CamKIIdelta* expression significantly increased during human MK maturation and proplatelet formation *in vitro*. The calcium chelators EDTA and BAPTA significantly inhibited CamKII phosphorylation ($p< 0.01$) and reduced proplatelet formation by 45% ($p< 0.01$). Specifically inhibiting CamKII with KN-93 significantly blocked CamKII phosphorylation, proplatelet formation by 90% ($p< 0.001$), and decreased Rac1-GTP activity, a downstream substrate of CamKII, two-fold. Activation of CamKII through brain-derived neutrophic factor increased proplatelet formation by 50%. Inhibiting CamKII *in vivo* with KN-93 significantly decreased platelet counts by 42% ($p< 0.05$).

Conclusions: Our data demonstrate, for the first-time, calcium signaling regulates proplatelet formation through the activation of CamKII and its subsequent interactions with Rac-1.

PB 547 | Presence and Absence of Platelet Proteins in Differentiating Megakaryocytes

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Background: Generation of functional platelets from in vitro cultured megakaryocytes (MKs) remains a challenge. The function and protein content of MKs at their final stage of differentiation should closely reflect that of platelets. Insight into differences between cultured MKs and platelets may therefore contribute to the development of protocols to obtain functional platelet-generating MKs.

Aims: We aim to compare the proteome and function of platelets with in vitro maturating MKs.

Methods: Hematopoietic progenitor cells from peripheral blood were differentiated along the MK lineage until formation of proplatelets was observed. The changes in protein levels were assessed using label-free quantitative mass spectrometry analysis, and compared with the proteome of normal platelets. In addition, maturing MKs were stimulated with platelet agonists to assess the release of granule content.

Results: MK stimulation with platelet agonists induced surface expression of P-selectin and secretion of VWF already at an early stage of MK differentiation. This shows that the secretory machinery is already developed in immature MKs. Proteomics data of the immature MKs also revealed the presence of platelet proteins that contribute to signaling, degranulation, and coagulation. These proteins were strongly upregulated upon further differentiation along the MK lineage. Several proteins were identified in platelets but not in cultured MKs, including Factor V (FV), EGF, and VEGFC. Absence of FV may be expected as it is endocytosed by MKs and the culture media did not contain FV. Other proteins may not be identified because they originate from endocytosis as well, or because their transcription trigger is absent in cultured MKs.

Conclusions: We show that cultured MKs resemble platelets in that they support granule release and express the required proteins for this. Platelet proteins that are not detected in MKs represent attractive targets for future investigations directed towards obtaining functional platelet-generating MKs.

PB 548 | Platelet Factor 4 (PF4) Levels Correlate with Platelet Count and Ability to Inhibit Megakaryopoiesis in Pediatric Patients with ITP

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Background: Immune Thrombocytopenia (ITP) is a relatively rare disorder resulting in significant thrombocytopenia. Mechanisms that determine degree and duration of thrombocytopenia are not well understood. Our previous work has shown that platelet (p)PF4 levels are inversely correlated with platelet count and that PF4 released by megakaryocytes in a damaged bone marrow inhibits megakaryocyte proliferation. We hypothesized that PF4 levels may influence meg biology and platelet count in patients with ITP.

Aims: Determine the relationship between pPF4 levels and platelet count in pediatric ITP patients as well as to determine whether this would influence lymphocyte profiles.

Methods: As a pilot study, we collected blood on 15 patients with ITP and examined the relationship between platelet count and pPF4 level using a PF4 ELISA kit. We also cultured megakaryocytes to determine whether PF4 levels would influence cell growth and differentiation using murine PF4 knockout bone marrow.

Results: Patient platelet count did not correlate with megakaryocyte growth from bone marrow. However, there was an inverse relationship between pPF4 level and megakaryocytes cultured from murine bone marrow (R^2=0.45, p= 0.04). In contrast, pPF4 levels correlated with platelet count (R^2=0.71, p=0.003) in contrast to control populations where PF4 levels were inversely correlated with PF4 levels (historical data). Finally, patients with ITP had significantly higher numbers of CXCR3B (receptor for PF4 on lymphocytes) positive lymphocytes in the peripheral blood compared to controls.

Conclusions: Platelet PF4 levels in pediatric patients with ITP determine megakaryocyte proliferation from bone marrow. In contrast to the general pediatric population, platelet PF4 levels in this patient population correlate with platelet count. The PF4 level may influence various lymphocyte markers and may help modulate the immune response. Further studies will need to examine whether platelet PF4 levels influence bleeding or response to therapy.

PB 549 | Role of NOD-like receptors (NLRs) in Megakaryo/thrombopoiesis

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Background: Toll-like receptors are expressed in megakaryocytes and platelets, providing a link between the inflammatory response and megakaryo/thrombopoiesis. Recently, the expression and functionality of other pattern recognition receptors (PRRs) such as the NOD-like receptors (NLRs): NOD2 and NLRP3, were described in platelets, however its role in megakaryocytes remain unknown. NOD2 is an intracellular receptor for muramyl dipeptide (MDP), the minimal bioactive peptidoglycan motif common to all bacteria. The NLRP3 activation is triggered by endogenous damage signals such as monosodium urate crystals (MSU) and also by PRRs agonists. NLRP3 recruits

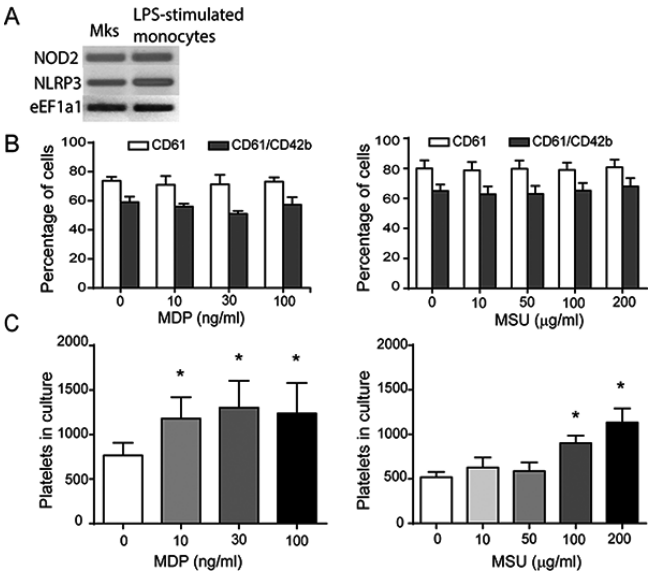

FIGURE 1 *p<0.05 vs. without agonist, n=5

caspase-1 to assemble the inflammasome and process Interleukin-1 beta.

Aims: We here aimed to study the expression and functionality of NOD2 and NLRP3 inflammasome in human megakaryo/thrombopoiesis.

Methods: NOD2 and NLRP3 expression were determined by semi-quantitative RT-PCR in human $CD34^+$-derived megakaryocyte progenitors and LPS-stimulated monocytes as positive control. Surface expression of CD61 and CD42b as indicators of megakaryocyte differentiation and maturation, respectively, and the number of cultured-derived platelets were determined by flow cytometry. Data was analyzed by ANOVA.

Results: We found that megakaryocytes progenitors constitutively expressed NOD2 and NLRP3 (Fig. 1A). Stimulation of cells with MDP failed to affect megakaryocyte survival, proliferation, differentiation or maturation (Fig. 1B). Similar results were observed after MSU stimulation. However, both MDP and MSU significantly increased platelet generation in culture (Fig. 1C).

Conclusions: These findings show that the activation of two NOD-like receptors regulate thrombopoiesis without altering megakaryopoiesis, suggesting that these pathways could be involved in reactive thrombocytosis caused by bacterial infections.

PB 550 | *In vitro* Generation of Functional Megakaryocytes Derived from Induced Pluripotent Stem Cells of Parahaemophilia Patients

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Background: Congenital factor V (FV) deficiency (parahaemophilia) is a rare autosomal recessive bleeding disorder. The ability of obtaining induced Pluripotent Stem Cells (iPSCs) derived megakaryocytes (MK) from parahaemophilia patients represents a powerful technology to study the mutant gene function, to characterize platelet FV and consequently to develop a gene therapy to correct the genetic defects.

Aims: For the development of patient-specific *in vitro* MK cell model for FV deficiency, by using iPSCs, and for the investigation of genotype-phenotype correlates of the disease.

Methods: Peripheral mononuclear blood cells were isolated and cultured to expand into erythroblast cells and then were transduced with Sendai Virus vectors or episomal plasmids technique, expressing the factors OCT4, SOX2, KLF4 and cMyc. To verify the differentiation potential of iPSCs, the Embryoid Bodies test was performed and gene expression of markers belonging to the three germ layers was analysed by RT-PCR. The iPSCs obtained from healthy and parahaemophilia subjects were differentiated into the MK lineage. iPSC-MK were analysed for the expression of CD41 and FV by immunofluorescence technique.

Results: iPSC-MK were capable of platelet formation *in vitro*, showing no major differences in morphology and function with respect to blood-derived MK. iPSC-MK cells were stained with CD41 and the confirmed that these iPSC-MK were indeed MK-like cells. iPSCs-MK from healthy subjects were positive for the expression of FV. In the contrary, iPSC-MKs from parahaemophilia patients were negative.

Conclusions: The ability of obtaining iPSCs-MK from parahaemophilia patients represents a useful tool to study the underlying mechanisms of disease, for drug screening tests, as well as a source of pluripotent cells, that could be modified with gene editing tools to rescue the phenotype of the disease, the mutant gene function, to characterize platelet FV and consequently to develop a gene therapy to correct the genetic defects.

PB 553 | The Application of High throughput Sequencing to the Diagnosis of Thrombocytopenia

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Background: Inherited platelet disorders are a heterogeneous group of rare diseases associated with altered platelet structure and/or function, reduced platelet count and mucocutaneous bleeding of varying severity. The published frequency of 1/10000 individuals is likely to be an underestimate as there is currently no consensus approach to diagnosis. We have performed molecular analyses in 68 patients diagnosed with macrothrombocytopenia or familial thrombocytopenia in whom BSS and GT had been excluded. We analysed a subset of genes known to be associated with platelet function and were able to identify a causative mutation in 34 cases. This leaves 50% of patients without a molecular diagnosis.

Aims: To date over 50 genes have been implicated in the aetiology of platelet disorders. Screening this number of genes using conventional methodologies is neither practical nor cost-effective. Therefore we have set up a pilot study to investigate the application of high throughput sequencing to the molecular diagnosis of thrombocytopenias.

Methods: We used the Agilent SureSelect focused exome to investigate 12 patients analysed previously (6 with an identified mutation and 6 without). The Agilent NGS Bravo platform was used for library preparation and target enrichment. Sequencing was performed on an Illumina MiSeq. VCF files were generated using the Broad Institute Best Practice guidelines. The Ingenuity Variant Analysis Package was used to identify and prioritise potential disease-causing variants.

Results: All 6 previously identified mutations were recapitulated. We were also able to identify potential causative mutations in a number of patients for whom diagnosis had previously been unavailable. These are being investigated further in family members.

Conclusions: We have shown that high throughput sequencing using a focused exome allows rapid molecular diagnosis of patients with inherited thrombocytopenia even in the absence of detailed phenotypic data. This information will improve patient treatment and prognosis.

PB 554 | PEAR1 Methylation Is a Determinant of Platelet and Plasma P-selectin Levels, Platelet-leukocyte Aggregates and Platelet Distribution Width

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Background: Platelet Endothelial Aggregation Receptor 1 (PEAR1) is a transmembrane receptor involved in platelet activation and megakaryopoiesis. *PEAR1* variants were associated with differential platelet response to activation with possible implications for cardiovascular disease onset. *PEAR1* expression during megakaryopoiesis is driven by DNA methylation.
Aims: To investigate *PEAR1* DNA methylation changes as determinants of platelet phenotype and function variability in a cohort of large families with or without myocardial infarction (MI) at young age.
Methods: Moli-family is an Italian family-based cohort including 732 subjects (aged 43±19 SD, 44% men) from 54 extended pedigrees (23 families with familiarity for MI at young age and 31 matched control families). *PEAR1* methylation (32 CpG sites) was investigated for 605 Moli-family participants, for whom plasma P-selectin levels and blood counts were available. Whole blood platelet function was assessed through platelet P-selectin and platelet-leukocyte mixed aggregates measurements. We performed principal factor analysis (PFA) to identify groups of CpG sites with similar methylation estimates (eigenvalue > 1.0), and multivariable linear regression analysis (using age, sex, smoking, and familiarity for MI as covariates) to assess their relation with each of the platelet specific variables measured.
Results: We discovered a methylation factor (PEAR1-F1) characterized by positive loadings of previously identified megakaryocyte-specific CpG sites (Izzi et al. Blood 2016) including CpG 7_8, 19_20, 15_16, 25_26 and 31_32. PEAR1-F1 was inversely significantly associated with platelet ($p< 0.0001$) and plasma ($p=0.0004$) P-selectin and both platelet-monocyte ($p=0.0002$) and platelet-PMN ($p=0.0065$) aggregates in whole blood, while it was positively associated with PDW ($p=0.0022$).
Conclusions: This large *PEAR1* epigenetic analysis, performed in the framework of the Moli-family epidemiological study, for the first time links methylation changes to platelet function variability.

PB 555 | Single Cell Transcriptome Analysis in Living Human Platelets

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Background: Platelets (PLT) are involved in other functions besides hemostasis, ranging from inflammation, immunity to angiogenesis and tumor metastasis. It is reasonable that within circulating PLTs there are subsets committed to play these different functions by means of a specific proteome and transcriptome. Transcriptome analysis on the whole platelet population does not allow to characterize subsets.
Aims: To identify PLT subsets taking advantage of Smartflare technology that allows the identification of mRNA in single living PLTs by flow cytometry.
Methods: Single cell expression of 50 mRNA involved in inflammation and hemostasis was assessed in PLTs isolated from 10 healthy subjects aged 30±10 years. Experimental conditions were optimized in order to reach an uptake of the control probe in more than 90% of PLTs. PLTs were then incubated with nanoparticles conjugated to an oligonucleotide complementary to a specific mRNA. Analysis was performed by imaging flow cytometer in order to assess the fluorescence intensity related to mRNA expression only in PLTs with internalized probes.
Results: Among the analyzed mRNA, 10 were undetectable, while 3/4 of transcripts involved in hemostasis, like P-selectin, and only 1/4 of genes involved in inflammation, like C-C motif chemokine ligand 5, were found in more than 70% of PLTs (86±7 and 97± 4 %). Conversely, 3/4 of genes related to inflammation, like COX1, and 1/4 of mRNA related to hemostasis, like tissue factor, were found in a reduced percentage of PLTs (49±8 and 44±10%). Housekeeping genes like GAPDH, 18S and actin-β were expressed in more than 90% of PLTs.
Conclusions: These results show for the first time that in healthy subjects a differential enrichment of specific mRNA occurs in platelet subsets. It is tempting to speculate that the relative abundance of both mRNA as well as the platelet subsets may change in pathological condition.

PB 556 | Uncovering GEnetic NEtworks Underlying Platelet Response to Thienopyridines by EXome Sequencing of Extreme Phenotype Patients: Discovery and Validation Results

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Background: The genetic determinants of the platelet reactivity (PR) phenotype in clopidogrel-treated subjects play an important role (heritability, $h^2=0.73$) but previous GWAS approaches have identified very few variants, explaining only 5-12% of PR variability. Pilot studies relying on whole exome sequencing have identified candidate genes and pathways.
Aims: To validate, in independent cohorts, genes and pathways that contribute to the variability of PR observed in response to clopidogrel treatment.
Methods: Based on previous whole exome sequencing (WES) findings in 96 extreme PR phenotype patients from a 534 clopidogrel-treated

outpatients a network was built. Genes and pathways were tested in an independent population of 96 extreme PR phenotype patients Compliance to clopidogrel was confirmed by the quantification of clopidogrel carboxylic acid in the serum in both populations. Average coverage of the exome bait region was 70-fold, with more than 98% of bases having at least 10x read-depth coverage.

Results: Exome analysis samples of the discovery cohort yielded 585 variants in 418 genes with a different frequency between LPR and HPR patients (adjusted p-value< 0.05). Principal component analysis showed complete discrimination of the two extreme PR groups, which was not dependent on the known CYP2C19*2 variant. Interestingly, the previously identified B4GALT2 c.909C>T variant was not detected in the discovery cohort. Based on literature search and gene annotation a network of 40 genes was built with several known CYP-P450 2C19, 2C19, 2C18 genes, Thrombospondin, apoptosis related genes (BAX, CASP9, SORBS3 and BIRC6) and CLASP2 (Cytoplasmic linker associated protein 2) involved with stabilization of microtubules. In the validation population the network genes from the top of the list were confirmed, mostly Cytochrome genes.

Conclusions: The candidate genes and pathways identified in the WES population network were confirmed in the validation population but most of the information is restricted to Cytochrome genes.

PB 557 | Poorly Controlled Diabetes Mellitus Is Associated with Decreased Aspirin-mediated Acetylation of Platelet Cyclooxygenase-1 (COX-1) at Serine 529

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Background: Diabetes is a major risk factor for cardiovascular diseases. Although aspirin is considered as a cornerstone of the prevention and treatment of the atherothrombosis-related ischemic events, this antiplatelet drug appears to be less effective in poorly controlled diabetic patients. It has been suggested that glycation of platelet proteins plays a pivotal role in poor responsiveness to aspirin. However, a direct effect on the critical residue (serine 529, or Ser529) of the catalytic pocket of cyclooxygenase 1 (COX-1) has never been demonstrated.

Aims: This study aimed to elucidate the impact of hyperglycaemia on the aspirin acetylation of platelet COX-1 from poorly controlled diabetes mellitus.

Methods: We used targeted mass spectrometry to measure the level of acetylation of platelet cyclooxygenase-1 (COX-1) at serine 529 on control (HbA1c < 6%) and diabetic (HbA1c ≥ 8%) subjects.

Results: Using a targeted mass spectrometry approach, we found that hyperglycaemia only had a direct impact on the level of acetylation of the Ser529 residue, whereas the overall COX-1 acetylation level remained unchanged. Also, the functional aspirin-induced inhibition of COX-1 was dose-dependently impaired as glucose concentrations increased.

Conclusions: These results provide new insights into the interplay between glucose and aspirin and their effects on platelet COX-1; they provide a mechanistic explanation for the phenomenon of poor response to aspirin in diabetic patients.

PB 559 | A Combination of Biochemical and Functional Approaches Shows a Hyperactivation of Src Family Kinases-mediated Signaling Pathways in Platelets from Obese Patients

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Background: Central obesity constitutes a relevant risk factor for the development of atherothrombosis and coronary artery disease. Platelets play a fundamental role in this increased cardiovascular risk since they show hyperactivation and lower sensitivity to antiplatelet therapy in obese patients.

Aims: The main goal of this study was to identify platelet biomarkers related to the risk of suffering atherothrombosis in obese patients without cardiovascular disease, confirm the platelet activation levels in these patients, and identify those activation pathways more altered.

Methods: The study was done by a combination of proteomic and functional analyses. For the former, platelets were obtained from ten obese patients (BMI>40), with no associated comorbidities or chronic treatments, and ten age- and sex-matched lean controls. Proteome analyses were based on two-dimensional differential in-gel electrophoresis (2D-DIGE) and mass spectrometry. Validations were by western blotting and functional studies by aggregation assays.

Results: Following the proteomic analysis, we detected 55 differences that varied between obese and lean groups. From those, 38 were successfully identified by MS, corresponding to 28 open-reading frames. Most proteins were involved in platelet activation and aggregation, such as integrin alpha-IIb, alpha actinin-1, actin, and vinculin. Since proteomic data pointed towards alteration in Src family kinases (SFKs)-mediated signaling pathways, we explored the levels of the active form of Src (pTyr418) in a cohort of obese patients and lean controls, and found this form was up-regulated in the obese group. Moreover, aggregation studies showed platelets from obese patients were hyper-reactive in response to collagen and collagen-related

peptide (CRP) pointing towards GPVI signaling as one of the altered pathways.

Conclusions: In conclusion, our results suggest a higher activation state of SFKs-mediated signaling pathways in platelets from obese patients, with a primary involvement of GPVI signaling.

PB 560 | First Quantitative Proteomic Approaches of Platelet Protein Expressions and Networks in Human MYH9-Related Disorders

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Background: MYH9-Related Disorders (MYH9-RD) - rare autosomal dominant disorders characterized by decreased platelet count but giant platelets, leukocyte inclusions, and, in some cases, various combinations of hearing loss, nephropathy and cataracts - are due to mutations in *MYH9* gene coding the non muscle myosin-IIA heavy chain (MYH9). A proteomic approach of the molecular role of wild-type (WT) MYH9 has been reported in rare extra-platelet studies, (Hays 2014, Zhang 2015). The consequences of *MYH9* mutations on platelet proteins expression is poorly known.

Aims: To identify modifications of MYH9 partners expression and networks within MYH9-RD platelets compared to healthy donors using different proteomic approaches.

Methods: Platelets from MYH9-RD patients (n=4, platelet count mean= 54G/L, large platelets mean : 21%, all mutations located in the tail domain) and from healthy donors (n=4) were isolated from Platelet Rich Plasma. Proteomic study of platelet crude extracts was performed by 2D-DIGE and Label Free analysis. Bioinformatic analysis of the results was performed using Ingenuity and Pathway Studio software.

Results: From 1,756 platelet proteins identified and quantified, Label Free analysis showed altered expression of 128 proteins in patients while the 2D-DIGE analysis highlighted 23 altered proteins (ANOVA $p< 0.05$). We identified an association between mutated MYH9 and an altered expression (either up- or down- fold changes) of proteins co-acting with cytoskeleton signaling - such as FLNA, VCL, TLN, TUBB, MSN - and main functional networks. Other myosins such MYL6 and MYL12A were also found affected.

Conclusions: For the first time we identified alterations in MYH9 interacting proteins and networks within MYH9-RD platelets as compared to MYH9-WT ones. Despite the small number of patients these results contribute to amplify the understanding of MYH9 functioning and provide molecular data on the des-organization of MYH9-RD platelets.

PB 562 | Platelets in Severe Aortic Stenosis Have an Altered Protein Profile and Are More Vulnerable upon Activation Compared to Coronary Artery Disease

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Background: Patients with severe aortic stenosis (AS) have impaired von Willebrand factor and the altered coagulation might contribute to a bleeding tendency. Platelets are sensitive to high shear stress.

Aims: The aim was to evaluate the platelet proteome in severe AS and platelet response to activation compared to patients with coronary artery disease (CAD).

Methods: Platelets were purified from whole blood from AS (n=10) and CAD (n=10) upon admission before surgery. After preparation the proteins were analyzed by mass spectrometer. Whole blood was

TABLE 1 Platelet proteins in aortic stenosis compared to coronary artery disease

Protein name	LOG2 ratio AS/CAD	P-value	Protein name	LOG2 ratio AS/CAD	p-value
Protein CVD3 homolog	-0.31	0.003	Platelet endothelial aggregation receptor 1	-0.34	0.044
Dematin	-0.44	0.006	Microtubule-associated protein RP/EB family member 1	0.38	0.010
Septin-6	-0.36	0.007	Rho GDP-dissociation inhibitor 2	0.31	0.010
Bifunctional ATP-dependent dihydroxacetone kinase/FAD-AMP lyase	-0.55	0.016	Ras-related protein Rab-1B and 1C	0.36	0.014
ATP synthase subunit d. mitochondrial	-0.35	0.017	Tubulin alpha-8 chain	0.29	0.015
Hematopoetic lineage cell-specific prrotein	-0.26	0.010	HLA class I histocompatibility antigen	0.43	0.035
Filamin-A	-0.28	0.033	Integrin alpha-6	0.36	0.036
cGMP-dependent protein kinase	-0.68	0.039	Ras-related protein protein Rab-27B	0.35	0.043
Protein G6b	-0.50	0.042	Glycine-tRNA ligase	0.45	0.047

activated ex vivo by CRP-XL and ADP and the expression of fibrinogen, CD62P, and CD63 on platelets were evaluated.

Results: A total of 1567 protein groups were included in the quantitative analysis and 16 proteins were downregulated in the AS compared to CAD groups and 12 proteins were upregulated.

Upon activation with CRP-XL the fibrinogen expression tended to be higher in the AS group (p=0.062) and similar results were found after stimulation with ADP. CD62P expression but not CD63 were increased after ADP activation in the AS group (p=0.047).

Conclusions: Several of the platelet proteins which are different in AS compared to CAD are involved in the cytoskeleton function and microtubule formation which could contribute to the increase of platelet aggregation and release of alpha granule found in AS. The vulnerable platelets might function as a compensatory mechanism in an altered primary hemostasis in aortic stenosis.

PB 563 | Characterization of Large and Small Platelets

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Background: The mean platelet volume (MPV) is a composite of large and small platelets in the circulation. The MPV increases in situations of increased platelet turnover. Little is known, whether small and large platelets from the same individual differ in their functional characteristics.

Aims: To characterize functional and proteome differences in platelets with different MPV.

Methods: Platelet fractions with high and low MPV were separated from healthy blood donors by differential centrifugation. Platelet function was determined by flow cytometry (CD62P expression after stimulation with TRAP). Platelet adhesion and spreading was measured on collagen functionalized micro patterned arrays by live imaging and quantitative fluorescence microscopy. Cytosolic proteins were isolated and identified using a shotgun LC-MS/MS approach.

Results: Large and small platelet fractions significantly differed in MPV: 12.03 fl ± 0.88 vs. 7.76 fl ± 0.53, $p< 0.0001$. Large platelets showed higher CD62P-expression after stimulation with TRAP compared to small platelets: 29.81 MFI ± 8.575 vs. 21.22 MFI ± 6.996; p< 0.0039.Single large platelets covered a 1.4 fold larger area on a collagen surface: 63.76 μm^2 ± 21.13 in vs. 45.69 μm^2 ± 17.9 $p < 0.0001$. Out of 894 proteins identified in the cytosolic fraction, 19 proteins showed significantly different abundance in large and small platelets. Four proteins were more abundant in large platelets (ADP-ribosylation factor 1/3, Voltage-dependent anion-selective channel protein 3, Guanylate cyclase soluble subunit alpha-3, GTP-binding protein SAR1a) and 15 proteins were more abundant in small platelets (e.g. Apolipoprotein A-II, Alpha-1-antitrypsin).

Conclusions: We provide a method to separate large and small platelets for functional analyses. Platelets with high and low MPV of healthy volunteers differ in their proteome, and large platelets show enhanced functional capacities compared to small platelets.

PB 564 | TREM-like Transcript-1: A More Sensitive Marker of Platelet Activation than P-selectin Detectable in the Core and Shell of Thrombi

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Background: Recent findings suggest thrombi can be divided into a core of tightly packed, highly activated P-selectin-positive platelets, and a shell of loosely packed, P-selectin negative platelets. TREM-like transcript-1 (TLT-1) is an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor highly expressed in α-granules of platelets, which gets rapidly up-regulated to the surface upon activation. TLT-1 has been proposed to bind fibrinogen and facilitate platelet activation. However, the function of TLT-1 in thrombus formation and stability remains undefined.

Aims: To determine the expression of TLT-1 in activated platelets relative to P-selectin during platelet activation and thrombus formation.

Methods: TLT-1 and P-selectin surface expression were analysed in thrombin-activated human and mouse platelets *in vitro* by flow cytometry, and during thrombus formation following laser injury of cremaster arterioles in mice.

Results: Activation of platelets with 0.1 U/ml thrombin results in a more rapid and robust up-regulation of TLT-1 surface expression compared with P-selectin. This was seen at all concentrations of thrombin tested between 0.01-1 U/ml. TLT-1 was also found to more rapidly translocate to the surface of activated platelets compared with P-selectin during thrombus formation following laser injury of cremaster arterioles in mice. TLT-1 expression was detected throughout the entire thrombi compared with P-selectin, which was only observed in a highly localized region within the core of the thrombus, directly adjacent to the site of injury.

Conclusions: Findings from this study demonstrate more rapid up-regulation and peak surface expression of TLT-1 compared to P-selectin, in thrombin-activated human and mouse platelets *in vitro*, and during thrombus formation in mice following laser injury of arterioles. Moreover, they suggest platelet activation in the shell region of growing thrombi occurs more rapidly and to a greater extent than previously thought.

PB 565 | WASH-complex Subunit Strumpellin Selectively Regulates Integrin αIIbβ3 Expression

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Background: Endocytosis in megakaryocytes (MKs) and platelets is crucial for loading of granule cargos, such as the uptake of fibrinogen from plasma by αIIbβ3 integrin, and thus platelet effector function. However, the underlying mechanisms of cargo sorting and receptor trafficking are poorly understood, and key regulatory proteins remain to be identified. Strumpellin is a component of the Wiskott-Aldrich syndrome protein and Scar homologue (WASH) complex, the major endosomal actin polymerization-promoting complex. In other cell types, the WASH-complex is recruited to subdomains of early endosomes and generates an actin network thereby coordinating endosomal protein sorting. The function of WASH complex in MKs and platelets is unknown.

Aims: The aim of this study was to investigate the role of Strumpellin in platelet biology.

Methods: Mice lacking Strumpellin in MKs and platelets were analyzed using flow cytometric, histologic and immunoblot analyses as well as confocal and transmission electron microscopy.

Results: Strumpellin deficient mice had normal platelet count and size. Flow cytometric analysis revealed a 20% decrease in integrin αIIbβ3 surface expression on resting platelets. By contrast, the expression of other receptors (e.g. GPV, GPIb, α2 and β1 integrins) was unaltered compared to wild-type controls. P-selectin exposure and integrin αIIbβ3 activation after platelet stimulation was moderately reduced. Furthermore, a reduced number of αIIbβ3 integrins could be mobilized to the surface upon activation indicating a decreased internal pool of αIIbβ3 in knockout platelets. However, these platelets showed unaltered integrin αIIbβ3-dependent spreading on fibrinogen. Mutant bone marrow MKs displayed normal ploidy and morphology, but integrin αIIbβ3 surface expression was reduced suggesting a MK intrinsic defect.

Conclusions: These data point to a distinct role of Strumpellin in maintaining integrin αIIbβ3 expression in MKs and platelets, and provide new insights into regulatory mechanisms of platelet integrins.

PB 566 | Mechanisms of Platelet Adhesive-receptor Shedding in Platelet Populations in Thrombus Formation

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Background: The platelet adhesive receptors, glycoproteins (GP)Ibα and GPVI, are cleaved by members of the family of A Disintegrin And Metalloproteases, ADAM10 and ADAM17. The signaling mechanisms and functional consequences of receptor shedding are poorly understood.

Aims: We hypothesized involvement of four distinct activation mechanisms in adhesive receptor shedding: elevated Ca^{2+}, protein kinase C (PKC), phospholipid scrambling and caspase activity. The contribution of these mechanisms was assessed in agonist-stimulated platelets and in thrombi formed under flow.

Methods: Shedding of both GPVI and GPIbα (GPIX as control) was measured in activated platelets by multi-color flow cytometry or in thrombi formed under arterial flow conditions by confocal microscopy.

Results: Agents raising Ca^{2+} caused complete cleavage of GPIbα and GPVI, with similar kinetics, in a distinct population of phosphatidylserine (PS)-exposing platelets. Shedding was abolished by inhibition of ADAM10/17, but not of calpain or caspases. Surprisingly, shedding occurred independently of PS exposure, as it was unchanged in Scott syndrome platelets (no PS exposure). PKC stimulation caused incomplete cleavage of only GPIbα, which was annulled by kinase inhibition and overruled by Ca^{2+} elevation. Shedding of both glycoproteins with the apoptotic agent ABT-737 was mostly confined to a PS-positive platelet population and relied on caspase activity and (partially) Ca^{2+} elevation. Shedding induced by mitochondrial uncoupling was also Ca^{2+}-dependent. In thrombi formed on collagen, ADAM-dependent shedding of GPIbα and GPVI relied on high-Ca^{2+} and coincided with PS exposure. Platelets with shed receptors showed an increased ratio of prothrombin/VWF binding and detached normally.

Conclusions: Glycoprotein shedding is primarily mediated by Ca^{2+} elevation and caspase activity, along with but not via PS exposure; and enhances the platelet procoagulant activity.

PB 567 | The GYPSIE (GlYcoProtein Six in Stroke) Study: GPVI-dimer Level is Increased in Ischaemic Stroke Patients, But Total GPVI Is Not

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Background: Platelet glycoprotein VI (GPVI) binds to subendothelial collagen exposed by vessel injury or plaque rupture, initiating signalling culminating in thrombus formation. The collagen-binding form of

GPVI is a dimer of two GPVI-molecules (GPVI-dimer), which is constitutively present on resting platelets. This suggests that GPVI-dimer levels, not total GPVI, may be related to the incidence of ischaemic stroke.

Aims: To determine if GPVI-dimer levels, compared to total GPVI, are elevated in ischaemic stroke patients.

Methods: Blood samples were obtained from stroke patients at admission with informed consent and used for all tests within 2 h. GPVI was measured by flow cytometry in triplicate (as MFI) using the non-inhibitory, GPVI-dimer-specific antibody 204-11 Fab and pan GPVI antibody HY101; appropriate control antibodies and fluorescently labelled secondary antibodies were used. P-selectin expression was also measured by flow cytometry.

Results: Ischaemic stroke patients showed significantly higher GPVI-dimer level (P< 0.0001) on day of admission (n=70) and at the 90-day follow-up (n=27) compared to healthy controls (n=90). In contrast, levels of total GPVI did not differ between the patients (0- and 90-day) and the healthy controls. GPVI-levels tended to be elevated in strokes classified as large artery strokes (LAS), cardioembolic strokes (CES), and small vessel occlusions (SVO). Compared to healthy controls, resting platelets of stroke patients showed increased P-selection expression compared to healthy controls, suggesting the presence of activated platelets. This is an ongoing study, with further patient data to be added by the time of presentation.

Conclusions: GPVI-dimer levels, not total GPVI, are elevated in ischaemic stroke. This suggests that GPVI-dimer could serve as a future biomarker and pharmacological target in patients with ischaemic stroke.

PB 568 | Gradient-dependent Modulation of Platelet Stimulatory G-protein Coupled Receptors (GPCR) Signaling

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Background: The hemostatic activity of platelets is usually confined to the vicinity of an injured vessel. Factors or intracellular mechanisms responsible for this spatiotemporal regulation of platelet activation are not well understood.

Aims: To verify and characterize the agonist gradient dependent desensitization of platelets receptors as a mechanism for adaptive modulation of platelet GPCR signaling.

Methods: We assessed the effect of agonist gradients on different aspects of platelets activation such as aggregation, alpha-granule release and calcium mobilization by aggregometry, flowcytometry, and fluorimetry, respectively. Western blotting was used to assess the phosphorylation status of VASP in platelets treated with different gradients.

Results: We show that gradient-dependent desensitization is a common feature of stimulatory GPCRs, modulating the response to agonist stimulation by a mechanism involving phosphorylation of vasodilator-stimulated phosphoprotein (VASP) and protein kinase A. Different GPCRs display distinct patterns of gradient-dependent desensitization. Protease-activated receptor 1 (PAR1) and the ADP receptors desensitize rapidly and require higher temporal agonist concentration gradients than PAR4 and thromboxane receptors. In contrast, signaling from a collagen receptor, the non-GPCR glycoprotein VI, does not exhibit gradient dependence. We also show that platelets from surgical patients undergoing cardio-pulmonary bypass exhibit activation responses suggestive of desensitization of signaling from PAR1 but not PAR4, consistent with our observation *in vitro* that PAR1 is more susceptible to gradient-dependent desensitization.

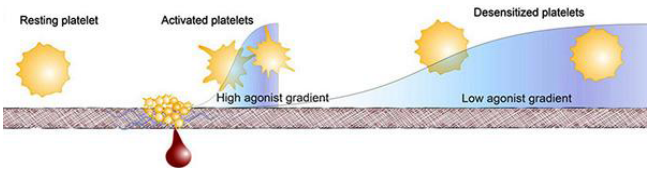

FIGURE 1 Illustration showing the platelet response to different concentration gradients

Conclusions: Platelet activation by stimulatory GPCRs is not dependent only on the concentration of the agonist but also on how rapidly the concentration changes (Fig. 1). Our findings suggest that gradient-dependent desensitization could represent a new clinically relevant mechanism for hemostatic regulation in platelets.

PB 569 | Activation of αIIbβ3 Integrin Involves the Cys608-Cys655 Disulfide Bond and the Electrostatic Network of the β-tail Domain

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Background: Integrin αIIbβ3 needs to be activated to mediate platelet aggregation. The β3 subunit of the integrin contains four EGF-like domains and a β-tail domain (βTD) which are rich in cysteine residues that form a network of disulfide bonds. Some of these bonds, such as the βTD 608-655 bond, were suggested to regulate integrin activation through disulfide bond exchange. Expression studies showed that the C655S mutation leads to αIIbβ3 activation, while the C608S mutation keeps the integrin inactive.

Aims: To examine the role of the 608-655 disulfide bond in αIIbβ3 activation.

Methods: Molecular dynamics simulations were conducted using β3 fragments comprising the βTD and the EGF-4 and EGF-3 domains, for either wild-type (WT) or one of the mutations disrupting the 608-655 bond, sometimes combined with the nearby Asp606 residue (C655S, C608S, C655S/D606A and C608S/D606A). Each of the WT and the

mutated β3 subunits were expressed in cells together with WT αIIb. The activity state of WT and mutated integrins was measured by flow cytometry.

Results: In the C655S simulation, the free thiol of Cys608 distanced from Ser655 and approached Asp606, causing a separation between the βTD α-helix 1 and β-strand 4, which further leaded to an opening of the angle between the βTD and the EGF-4 domain. In the C608S simulation, the thiol of Cys655 became very close to Asp606 enabling it to make a strong polar bond which kept α-helix 1 and β-strand 4 in close proximity, not allowing further conformational rearrangements. In the C655S/D606A and C608S/D606A double mutants simulations no strong polar bonds between α-helix 1 and β-strand 4 were shown. When expressed in cells, only the WT and the C608S integrins were non active while the other mutants were constitutively active.

Conclusions: Electrostatic interactions in the βTD are important for stabilization of the inactive αIIbβ3 conformer and interruption of this electrostatic network by disrupting the 608-655 bond is probably involved in normal activation of the integrin.

PB 570 | Clathrin Mediated αIIbβ3 Endocytosis Plays an Important Role in Platelet Activation

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Background: Dynamic endocytic and exocytic trafficking of integrins is an important mechanism for cell migration, invasion and cytokinesis. Endocytosis of integrin can be classified as clathrin dependent and clathrin independent manners. And rapid delivery of endocytic integrins back to the plasma membrane is key intracellular signals and is indispensable for cell movement. Integrin αIIbβ3 plays a critical role in thrombosis and haemostasis. Although previous studies have demonstrated that internalization of fibrinogen-bound αIIbβ3 may regulate platelet activation, the roles and mechanisms of integrin αIIbβ3 endocytosis in platelet activation are unclear.

Aims: To reveal the roles and mechanisms of integrin αIIbβ3 endocytosis in platelet activation.

Methods: A novel selective clathrin terminal domain inhibitor pitstop2 was used to study the role of clathrin-mediated endocytosis in platelet activation. Platelet activation were assessed by flow-cytometry, aggregometry and spreading on immobilized fibrinogen *in vitro*. The dynamic location of αIIbβ3 was traced by Alexa488-Fg uptake or fluorescence labeled αIIbβ3 internalization and distribution.

Results: We found that pitstop2 inhibited human platelet aggregation and spreading on immobilized fibrinogen. Pitstop2 significantly extended the occlusion time in vivo arterial thrombosis model. Mechanism studies revealed that pitstop2 did not block the endocytosis of αIIbβ3, but inhibit the recycling of αIIbβ3 to plasma membrane during platelet or CHO cells bearing αIIbβ3 spreading on immobilized fibrinogen. And pitstop2 enhanced the association of αIIbβ3 with clathrin and AP2 indicate that pitstop2 inhibit platelet activation is probably due to disturb the dynamic dissociation of αIIbβ3 from clathrin and AP2. Further study demonstrated that Src/PLC/PKC was the key pathway to trigger the endocytosis of αIIbβ3 during platelet activation.

Conclusions: Our findings suggest integrin αIIbβ3 endocytosis is clathrin-dependent and plays a critical role on platelet activation.

PB 571 | An Inhibitory Mutant of Snake Venom Rhodocytin Blocks CLEC-2/Podoplanin Interaction-dependent Platelet Aggregation and Experimental Lung Metastasis

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Background: The snake venom rhodocytin (also called aggretin), produced by the Malayan pit viper (*Calloselasma rhodostoma*), induces platelet aggregation through its binding to the platelet receptor CLEC-2. CLEC-2 is a physiological target protein, podoplanin expressed on some types of tumor cells, indicating that it is involved in podoplanin-induced platelet aggregation and tumor metastasis. Accumulating evidence suggests that CLEC-2 is a promising target for both anti-platelet and anti-metastasis drugs without increasing bleeding risk. We noticed that rhodocytin could be used not only as a research tool but also as a lead protein in order to develop anti-CLEC-2 drugs. In previous ISTH 2015 Congresses, we have reported for the first time generation of functional recombinant rhodocytin (rRhodo).

Aims: The aim of this study is to validate mechanism of platelet activation through CLEC-2 using mutagenesis analysis on rRhodo.

Methods: We constructed mutant α or β subunit of rhodocytin using site-directed mutagenesis. These purified mutant rRhodo were analyzed for the formation of multimer with Blue-native PAGE, the induction/inhibition of platelet aggregation with aggregometer, and the binding to CLEC-2 with FACS. In addition, we investigated whether mutant rRhodo could suppress podoplanin-induced metastasis in an experimental lung metastasis model in mice.

Results: Blue-native PAGE showed that wild-type rRhodo and native rhodocytin formed heterooctamer. Mutagenesis analysis showed that D4 in α subunit was one of critical sites for platelet aggregation through CLEC-2. Moreover, mutant rRhodo αWTβR53A/K56A formed heterotetramer. This mutant bound to CLEC-2 without platelet aggregation, and inhibited CLEC-2/PDPN interaction-dependent platelet aggregation and experimental lung metastasis.

Conclusions: We revealed that the formation of octamer and critical sites for platelet activation on rhodocytin. These findings provide strong support that mutant rRhodo can be a potential source for therapeutic agents that target CLEC-2.

PB 572 | Human and Murine Neonatal Platelets Display Reduced CLEC-2 and GPVI Expression and Function Profiles Compared with Adult Platelets

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Background: Newborn platelets (NP), *vs*. adult platelets (AP), are hyporreactive by unclear and likely multifactorial mechanisms. The role of ITAM/ITIM-containing receptors, i.e GPVI, FcγRIIA and CLEC-2/G6bB and PECAM-1, in NP hyporreactivity is unknown.

Aims: To compare the expression and the function of ITAM/ITIM-containing receptors of NP and AP in human and in mice.

Methods: Human adult and cord blood and murine blood samples, at distinct pre- and postnatal ages, were assessed for:

1) Levels of GPs Ibα, αIIbβ3, αIIβ1 and ITAM-ITIM receptors by flow cytometry;

2) Functional status of GPCR and ITAM receptors, by measuring P-selectin expression and fibrinogen (Fg) binding upon platelet stimulation with agonists (PAR-1, PAR-4, ADP, CRP, CD9 and rhodocytin in human; PAR-4, CRP and rhodocytin in mice;

3) GPVI and CLEC-2 signaling in human washed NP and AP activated with CRP or rhodocytin, by immunobloting of phosphorylated PLCγ2 and Syk in platelet lysates.

Results: Human NP showed lower levels of GPVI, CLEC-2, PECAM-1 and αIIbβ3 than AP. Changes in these receptors were also observed in murine embryos and neonates compared to adults.

In both species, NP had impaired response to ITAM and GPCR stimulation, this hyporesponsiveness being more marked for GPVI and CLEC-2 agonists.

In human NP Fg-binding was reduced in response to all agonists, while severe impairment in P-selectin secretion was restricted to CRP and rhodocytin suggesting a defect in the first steps of GPVI and CLEC-2 signaling. Concordantly, human NP showed reduced CRP- and rhodocytin- induced phosphorylation of PLCγ2 and Syk.

Conclusions: Our study shows changes in platelet receptors during development both in human and mice, including reduced GPVI and CLEC-2 levels. This, in addition to impaired ITAM receptor signaling,

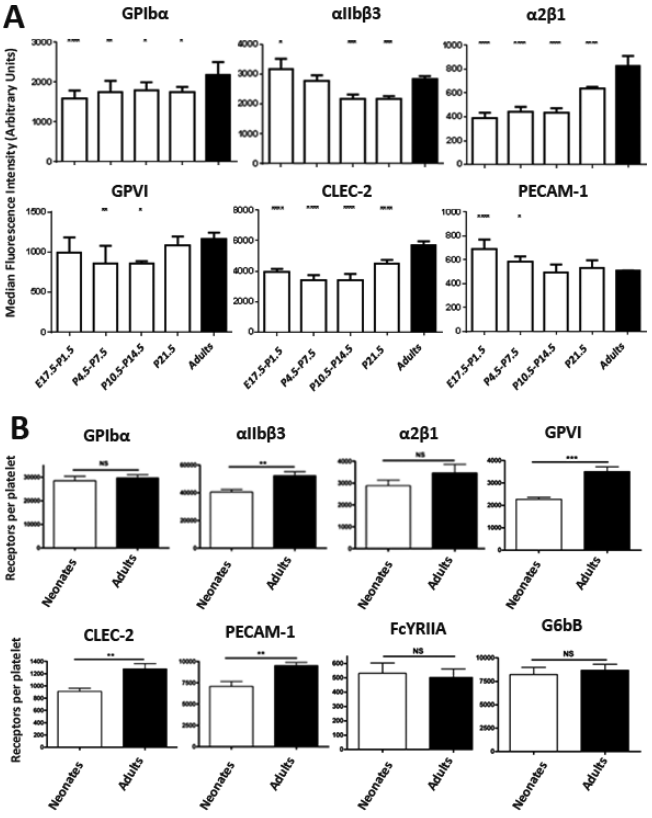

FIGURE 1 Receptor expression in adult and neonate platelets from mice (a) (n≥3), and humans (b) (n≥8)

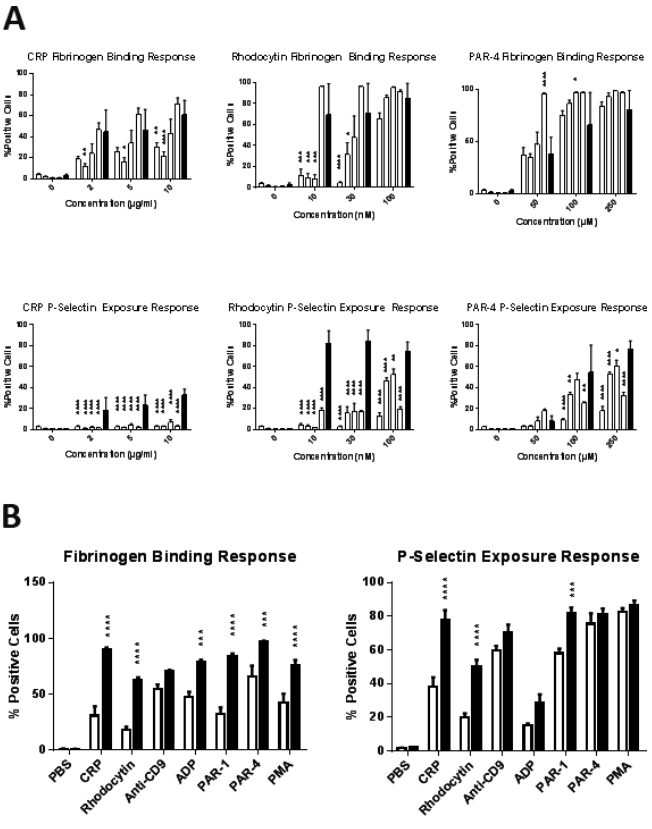

FIGURE 2 Fibrinogen binding and P-Selectin exposure response of platelets from mice (a) (n≥3) and human (b) (n≥8) neonates and adults.

contributes to defective reactivity of NP to GPVI and CLEC-2 agonists. A GPVI defect seems to play a major role in the well-known neonatal platelet hyporreactivity to collagen. *ISCIII&Feder (PI14/01956).*

PB 573 | DCBLD2 but Not GARP Has A Minor Role in Thrombus Formation in Mice which is Not in Line with the Findings in Zebrafish

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Background: Functional genomics in zebrafish identified a role for GARP (or LRRC32) in the promotion and DCBLD2 in the inhibition of thrombus formation (Blood 2009;113:4754-62).

Aims: To unravel the role of GARP and DCBLD2 in murine thrombus formation.

Methods: Platelet and endothelial specific *GARP*$^{-/-}$ mice (generated using Cre-loxP recombination) and full *Dcbld2*$^{-/-}$ mice were used. Surface expression of GPIb, GPVI and integrin αIIb and the function of platelets without GARP or DCBLD2 was measured by flow cytometry and aggregometry after activation with PAR4-activating peptide, collagen related peptide or adenosine diphosphate. *In vivo* tail bleeding time, as well as occlusion time of the mesenteric and carotid artery after $FeCl_3$-induced thrombosis, were determined in *GARP*$^{-/-}$ and *Dcbld2*$^{-/-}$ mice and their wildtype littermates.

Results: Platelets lacking DCBLD2 or GARP expression had normal platelet numbers and size and normal surface expression of GPIb, GPVI and integrin αIIb. Platelets without GARP displayed normal agonist induced activation and aggregation responses. Platelets lacking DCBLD2 demonstrated a stronger activation and aggregation towards suboptimal agonist levels. Nevertheless, in platelet specific *GARP*$^{-/-}$ mice, endothelial specific *GARP*$^{-/-}$ mice as well as in *Dcbld2*$^{-/-}$ mice, neither tail bleeding time nor occlusion time in the carotid- and mesenteric artery after $FeCl_3$-induced thrombus formation were affected.

Conclusions: Evidence is provided that platelet and endothelial GARP expression is not important in hemostasis and thrombosis in mice. DCBLD2 has a minor role in downregulating platelet activation and aggregation, which however is not translated into significant effects *in vivo*. These results are not in line with the observations previously made in zebrafish. Thus, zebrafish are a useful *in vivo* model to perform a first screening on gene function in thrombosis, but mammalian models are needed to evaluate biological relevance.

PB 574 | The Association of PAR4 Polymorphism with Human Platelet Reactivity in Japanese

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Background: Thrombin is the most important agonist to activates human platelets via two thrombin receptors, PAR1 (protease activated receptor 1) and PAR4. However, the details of PAR system in human platelets are still obscure mainly due to the different receptor expression between human and mouse. Especially, in contrast to PAR1 which has been established as a major thrombin receptor, the role of PAR4 in human platelet activation is not clear.

Aims: To elucidate the role of PAR4 in human platelets, we analyzed inter-individual variation of platelet activation and signaling induced by PAR4 in Japanese subjects.

Methods: We analyzed detailed platelet aggregation, integrin αIIbβ3 activation, granule release, calcium mobilization, and protein phosphorylation induced by PAR4-AP (PAR4-activating peptide) using human platelets obtained from healthy volunteers. PAR4 sequence was analyzed and investigated the association with platelet reactivity. We further analyzed the role of PAR4 in 293T cells transfected PAR4 expression vector.

Results: Sequencing analysis revealed PAR4 polymorphism p.Ala120Thr (c.533G>A) and the frequencies of 120A/A, A/T, and T/T in 202 Japanese were 56.9, 37.1, and 5.9% respectively. The concentration of PAR4-AP inducing irreversible platelet aggregation was significantly lower in 120T/T than those in 120A/A. PAR4-AP induced αIIbβ3 activation, granule release, and calcium mobilization were induced at lower concentration of PAR4-AP in 120T/T than 120A/A. Western blot analysis revealed PAR4-AP induced ERK phosphorylation, but not MLC phosphorylation, was enhanced in 120T/T platelets. 293T cells transfected with PAR4-120T or -120A confirmed that PAR4-AP induced calcium mobilization is much greater in PAR4-120T than PAR4-120A 293T cells.

Conclusions: We demonstrate that PAR4-120T/T platelets are much more sensitive to PAR4-AP stimulation than PAR4-120A/A.

PB 575 | Engineering scFvs (Single Chain Variable Fragments) to Platelet Glycoprotein VI

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Background: The display of antibodies as Fabs or scFvs on filamentous phage was first described in 1990 (McCafferty et al.). It provides a powerful technique for selecting a specific antibody from a mixed population of antibodies together with the gene that codes for it.

Large and diverse libraries now in use are capable of generating large panels of diverse, high affinity (sub-nanomolar) antibodies to a given antigen. As phage display is an *in vitro* technique, it also allows a wide range of standard DNA manipulation procedures to be used. Another advantage of working with antibodies in the form of recombinant DNA is the flexibility it allows over choice of final format. An antibody can be expressed as a scFv or Fab fragments or as fusion proteins with other protein domains attached.

Aims: Selecting large panels of diverse, high affinity **single chain variable fragments scFv** to both monomeric and dimeric GPVI.

Methods: Our methods include scFv selection on monomeric GPVI (depleted on Fc fusion GPVI) and Fc fusion GPVI, i.e, dimeric (depleted on monomeric GPVI) using Cambridge University phage library, isolation, and cloning the sequence of eluted phages in expression vector, scFv expression, antigen screening, and platelet aggregation, flow cytometry and real-time adhesion.

Results: Our screening on monomeric and Fc fusion GPVI showed that some clones are dimer-specific, few are either monomer-specific or bind to both forms of GPVI. Most of the clones recognized platelet GPVI in real-time platelet adhesion using xCELLigence RTCA Roche. In addition, we found that some of the clones were activating (aggregate platelets) and others inhibit platelet binding to Collagen Related Peptide (CRP).

Conclusions: Using phage display technology we selected scFv specific to glycoprotein VI forms, and some of them are inhibitory and others are activating.

PB 576 | Activation of Human Blood Platelets through CLEC2 is Slackened due to the Diffusion of Receptors into the Signalosome Region, but Still Can Induce Cytosolic Calcium Spiking in Single Platelets

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Background: Platelet surface receptor CLEC2 has a significant role in maintaining blood vascular integrity during inflammation. It shares signaling pathways with GPVI receptor. However, CLEC2 activation is notably delayed comparing to GPVI and the nature of this delay remains obscure. Different concepts of CLEC2 signaling have been proposed, for example Hughes et al. (Blood, 2015) believe that activation of Syk kinase lies upstream of SFK and Btk, while Manne et al. (J. Biol. Chem. 2015) assume the opposite. And whether CLEC2 activation affects platelet calcium signaling remains questionable.

Aims: Systems biology analysis of intracellular signaling after ligation of CLEC2 in single platelets.

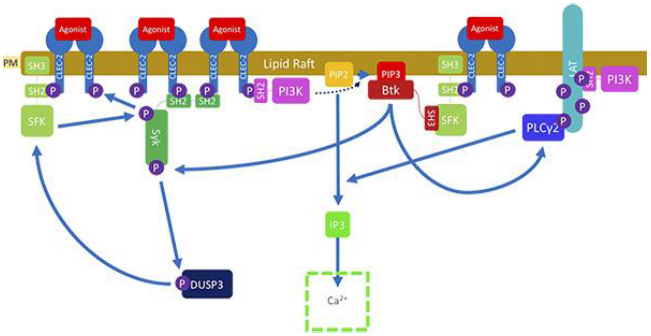

FIGURE 1 Model of CLEC-2 signalisation

Methods: A comprehensive 3D model of CLEC2 signaling was constructed based on experimental data and solved in VCell software (vcell.org). Fucoidan (Sigma 9072-19-9) was utilised as CLEC2 agonist in all experiments. For single platelet analysis Fura-2 loaded platelets were immobilised on fibrinogen and analised by TIRF microscopy. Alternatively, spreading of platelets on immobilized fucoidan was observed by the same technique. For model validation platelet activation by fucoidan was analised by aggregometry, flow cytometry and spectrofluorimetry.

Results: The model describes diffusion of CLEC2 molecules to the lipid raft and recruitment of downstream proteins to the signalosome. Signaling scheme is described in fig 1. Changing cholesterol saturation of cell membrane affected diffusion speed of the CLEC2 molecules, thus slowing down formation of the CLEC2 cluster. The model predicted cytosolic calcium spiking, which was confirmed in experiments with single platelets.

Conclusions: Slow activation of platelets by CLEC2 is due to the diffusion of it's oligomers to the signalosome, where a lipid raft prevents the cluster from the dissolving. CLEC2 activation leads to cytosolic calcium oscillations in single cells. Suggested scheme explains initial CLEC2 signaling stages and ties up representations of Hughes et al. and Manne et al.

PB 577 | Decompensated Heart Failure Patients Demonstrate Shedding of Platelet Receptors Glycoprotein (GP) Ibα and GPVI in Contrast to Stable Heart Failure Patients

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Background: Pathological shedding of platelet receptors glycoprotein (GP)-VI and GPIbα may contribute to platelet dysfunction and increased bleeding risk. Such changes have been demonstrated in patients with circulatory support devices associated with high shear

such as ventricular assist devices and extracorporeal membrane oxygenation. Surprisingly, we have also previously detected similar findings in patients with decompensated heart failure. Pathophysiologic mechanisms beyond altered shear forces may include inflammation or fibrin-mediated effects.

Aims: To investigate whether shedding of platelet receptors GPVI and GPIbα occurs in stable patients with compensated systolic heart failure, whether changes in von Willebrand factor (VWF) multimers were present, and how these patients compared to patients with decompensated heart failure and healthy donors.

Methods: 20 outpatients with compensated heart failure were recruited for measurement of platelet count, coagulation parameters, VWF multimers and assessment of platelet receptor status by flow cytometry or ELISA methods.

Results: Levels of shed GPVI (sGPVI), high molecular weight VWF multimers and platelet surface GPVI and GPIbα in stable heart failure patients were not significantly different to healthy controls. In contrast, patients with decompensated heart failure demonstrated significantly increased shedding of GPVI and GPIbα.

Conclusions: Stable heart failure patients appear to have similar platelet receptor expression profiles to healthy controls. However, heart failure patients developing clinical decompensation demonstrate increased shedding of platelet GPVI and GPIbα. The underlying mechanisms are not clear but may include alteration in shear stresses, differential expression of inflammatory mediators or effects of increased fibrin. Further research elucidating the pathobiology of these observations could potentially identify critical factors related to decompensation in heart failure.

PB 578 | Switching Integrin Alpha IIb-beta3 Conformations under Physiological and Stress Conditions

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Background: The heterodimeric transmembrane platelet receptor integrin alpha IIb-beta 3 (αIIbβ3) plays a crucial role in haemostasis and is involved in the autoimmune disease Immune Thrombocytopenia (ITP). ITP patients develop a higher bleeding risk due to autoantibody mediated platelet destruction. The immunogenicity (i.e. capacity of the immune system to induce an immune response) is potentially related to different conformations of αIIbβ3.

Aims: We aim to study the influence of mutations, external stress factors (e.g. pH and temperature) and binding partners on the αIIbβ3 conformation (e.g. closed, open and intermediate) in a membrane environment.

Methods: Integrin αIIbβ3 is incorporated into 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol (DMPG)/ 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) liposomes. Binding affinity and specificity of αIIbβ3 to binding partners (e.g. fibrinogen) is quantified under different conditions using surface plasmon resonance (SPR) and quartz crystal microbalance (QCM). Further the binding of specific antibodies to different αIIbβ3 conformations is investigated using enzyme-linked immunosorbent assay (ELISA).

Results: Different incorporation protocols are compared with regards to liposome- αIIbβ3 conjunction. We show using SPR and QCM that manganese ion concentration modifies the conformation of αIIbβ3. Our results indicate a higher binding affinity to interaction partners, e.g. fibrinogen, in presence of manganese ions.

Conclusions: Our results reveal that the biophysical methods SPR and QCM allow studying interactions between αIIbβ3 and ligands under native conditions. Future experiments could show the changing in binding properties of αIIbβ3 to ITP autoantibodies due to external factors or mutations.

PB 579 | C-Type Lectin-like Receptor 2 Promotes Hematogenous Tumor Metastasis and Prothrombotic State in Tumor-bearing Mice

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Background: C-type lectin-like receptor 2 (CLEC-2) is a platelet activation receptor of sialoglycoprotein podoplanin, which is expressed on the surface of certain types of tumor cells. CLEC-2-podoplanin interactions facilitate hematogenous tumor metastasis. However, direct evidence of the role of CLEC-2 in hematogenous metastasis and cancer progression is lacking.

Aims: The aim of this study is to examine the role of CLEC-2 in podoplanin-expressing cancer progression.

Methods: We generated platelet-specific CLEC-2-depleted mice using rat anti-mouse CLEC-2 monoclonal antibody 2A2B10. Podoplanin-expressing B16F10 mouse melanoma cells were used to evaluate hematogenous tumor metastasis, tumor growth, and overall survival.

Results: Hematogenous tumor metastasis was significantly inhibited in CLEC-2-depleted mice. B16F10 cells co-cultured with wild-type platelets, but not with CLEC-2-deficient platelets, showed increased proliferation. However, B16F10 cell proliferation was not inhibited in CLEC-2-depleted mice. Histological analysis showed that thrombus formation in tumor vessels was significantly inhibited and functional vessel density was significantly increased in CLEC-2-depleted mice. These data suggest that CLEC-2 deficiency may inhibit thrombus formation in tumor vessels and increase the density of functional vessels,

thus improving oxygen and nutrient supply to tumors, indirectly promoting tumor proliferation. Furthermore, the overall survival of CLEC-2-depleted mice was significantly prolonged, which may be due to the suppression of thrombus formation in the lungs and subsequent inhibition of systemic inflammation and cachexia.

Conclusions: Targeted inhibition of CLEC-2 as a new strategy for preventing hematogenous tumor metastasis and for inhibiting cancer-related thromboembolism. This study was published in *J Thromb Haemost* in 2016.

PB 580 | αIIb or Not IIb: Novel Insights into the Regulation of αIIb Expression and Localization in Activated Platelets

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Background: Integrin αIIbβ3 plays a crucial role in hemostasis and thrombosis, mediating platelet adhesion to extracellular matrix proteins, aggregation and thrombus formation; therefore appropriate regulation of integrin αIIbβ3 activity and quantity is essential to maintain platelet homeostasis. However, while much is known about the regulation of αIIbβ3 activation and derived signaling, comparatively little is known of the biochemical mechanisms that modulate its surface expression and localization in platelets.

Aims: To establish if the expression and localization of αIIb is modulated upon platelet activation.

Methods: Immunofluorescence (IF) microscopy was used to compare platelet αIIb spatial distribution on surfaces of fibrinogen following activation with thrombin and in the presence of various platelet pharmacological inhibitors. Western blot analysis was used to determine changes in platelet αIIb expression following activation with thrombin or ADP alone and in the presence of protein translation inhibitors.

Results: We show increased αIIb expression upon activation of platelets with thrombin or ADP. IF imaging revealed the presence of αIIb clusters in the center of thrombin-stimulated platelets adhering on fibrinogen. Inhibition of thrombin-activated platelets with PAR1 and PAR4 antagonists was not sufficient to prevent the formation of centralized αIIb clusters. Interestingly, inhibition of GPIb with the blocking antibody SZ2, but not AK2, abrogated αIIb centralization and clustering. Blockade of αIIb with integrilin had a mild inhibitory effect on αIIb relocalization, which was enhanced in the presence of apyrase, an ADP chelator.

Conclusions: Our results suggest that αIIb expression and localization can be regulated in platelets in a fashion dependent on GPIb, ADP and integrin signaling. Future efforts will define molecular routes of trafficking of αIIb in platelets.

PB 1258 | A Default in Acetyl-CoA Carboxylase Phosphorylation Increases Thrombus Growth *in vitro* and *in vivo*, and in a Collagen-dependent Manner

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Background: We have previously shown that AMPKα1 is activated in thrombin- or collagen-stimulated murine platelets. Accordingly, acetyl-CoA carboxylase (ACC), the bona-fide substrate of AMPK, is phosphorylated/inhibited upon platelet stimulation but its role in platelets has never been investigated. ACC is a central regulator of lipid metabolism. Given the primary roles of lipids in platelets, namely structural, energy storage and signaling, we hypothesized that acute ACC phosphorylation could affect platelet functions.

Aims: To determine the impact of acute ACC phosphorylation on platelet functions and investigate its contribution to haemostasis and thrombosis.

Methods: Platelets were isolated from a knock-in mouse model (KI) expressing a genetically modified ACC that can no longer be phosphorylated/inhibited by AMPK. *In vitro*, platelet adhesion and thrombus formation were measured using a flow chamber-based assay. Haemostasis was assessed via the measurement of bleeding time. Thrombosis was studied upon ferric chloride-induced carotid artery injury.

Results: ACC phosphorylation was unchanged in non-stimulated KI relative to control (WT) platelets. Thrombin or collagen stimulation led to a rapid and significant increase in ACC phosphorylation in WT, but not in KI platelets. This effect was accompanied by a prolonged presence of calcium in the cytosol of stimulated KI platelets. Most importantly, thrombus formation on collagen-coated surface was effectively augmented under flow, in the absence of ACC phosphorylation. Accordingly, KI mice had a subsequent shorter bleeding time. They also showed increased thrombus growth reflected by a higher increment in thrombus surface area over a 10-minute time interval, compared to WT mice ($7242\mu m^2 \pm 1265$ versus $2520\mu m^2 \pm 1219$ for WT mice).

Conclusions: Overall, our study defines a new and important role for platelet ACC phosphorylation in regulating thrombus formation *in vitro* and *in vivo*, and in a collagen-dependent manner.

PB 1260 | Fibrin Activation and Not GPCR Activation Induces Shedding of Platelet GPVI: Soluble GPVI is Elevated in Thermal Injury and ICU Patients

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Background: Upon activation of the major platelet collagen and fibrin receptor, glycoprotein VI (GPVI), the sheddase ADAM10 is activated and cleaves GPVI, releasing a soluble GPVI (sGPVI) fragment into plasma.

Aims: To determine whether sGPVI levels are elevated in patients with injury, assess sGPVI associations with clinical parameters, and whether fibrin induces GPVI shedding.

Methods: Plasma sGPVI levels were measured in thermal injury patients (n=99) and patients admitted to ICU (n=83) by ELISA. Washed platelets from healthy controls were stimulated with fibrin and G-protein coupled receptor (GPCR) agonists to induce GPVI shedding. Intact and cleaved GPVI was quantitated by western blot and densitometry.

Results: sGPVI levels at admission were associated with 28- and 90-day mortality of ICU patients (p=0.02 and 0.005). sGPVI was elevated day 14 post thermal injury (p< 0.005) and was higher in patients who developed sepsis (p=0.002). sGPVI positively correlated with D-dimer levels (fibrin degradation products) at day of admission for ICU patients (r=0.41) and at day 14 in thermal injury patients (r=0.46). Platelet activation by GPCR agonists, PAR-1, PAR-4, U46619 and ADP did not induce GPVI shedding. However, platelet activation by thrombin under conditions supporting fibrin formation induced metalloproteolytic GPVI shedding reducing intact GPVI to 33% of control GPVI levels (100%). Fibrin-mediated shedding was independent of integrin αIIbβ3 and ADAM10 but required fibrin polymerisation. GPVI signalling through Src and Syk, did not have a major role in fibrin-mediated GPVI shedding. Together this data suggests roles for multiple sheddases or alternative mechanisms including shear or Factor X activation.

Conclusions: Elevated sGPVI was predictive of mortality in injured patients, where minimal collagen exposure is assumed. Fibrin degradation strongly correlated with sGPVI in both patient cohorts. Fibrin induces GPVI shedding and may explain elevated sGPVI levels observed in patient plasma.

PB 1261 | A Humanized Monoclonal Antibody that Inhibits Platelet-surface ERp72 Reveals a Role for ERp72 in Platelet Function and Thrombus Formation

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Background: We have previously reported that multiple thiol isomerases are released by platelets and growing evidence supports the importance of PDI, ERp5 and ERp57 in the regulation of platelet activation. The role of ERp72, which is released and binds to the activated platelet surface, however has not been established.

Aims: To investigate if ERp72 inhibition using enzyme activity-blocking antibodies impacts on platelet functional responses and thrombus formation.

Methods: Humanized anti-ERp72 antibodies were generated using Human Combinatorial Antibody Library (HuCAL) phage-display technology. Eleven ERp72-selective antibodies were screened for their ability to inhibit both enzyme activity and platelet aggregation. One antibody (anti-ERp72) was selected for further study. Platelets were pre-incubated with anti-ERp72 or control immunoglobulin (Ig) prior to stimulation with collagen (1μg/mL) or CRP-XL (0.5μg/mL). Platelet aggregation and granule secretion were measured using lumi-aggregometry and flow cytometry. Platelet adhesion was measured by ELISA and thrombus formation in mice examined using intravital microscopy.

Results: Anti-ERp72 (10-25μg) caused a concentration-dependent reduction in platelet aggregation compared to control Ig (25μg). Anti-ERp72 also diminished platelet adhesion on collagen and fibrinogen and decreased dense and α-granule secretion. Fibrinogen and PAC-1 binding were substantially inhibited suggesting that ERp72 may regulate integrin αIIbβ3 function or outside-in signaling through this receptor. Anti-ERp72 significantly delayed thrombus formation in mice.

Conclusions: In this study we generated humanized antibodies that blocked ERp72 enzyme activity, inhibited platelet responses and delayed thrombus formation in mice. These data demonstrate that like PDI, ERp5 and ERp57, ERp72 is an additional thiol isomerase of importance for haemostasis and thrombosis and may represent an important target for antithrombotic therapies.

PB 1262 | Patient Derived HLA Monoclonal Antibodies Induce Fcγriia Dependent Platelet Activation

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Background: Pregnancy or transfusion can lead to the development of alloantibodies against MHC class I (HLA) molecules. As yet it is difficult to predict the clinical significance of HLA antibodies; in the majority of patients the presence of HLA antibodies does not result in a decline in platelet numbers or function after transfusion. In a subset of patients HLA antibodies coincide with refractoriness following platelet transfusion.

Aims: Evaluate the effect of HLA antibodies on platelet activation and clearance.

Methods: Human HLA IgG monoclonal antibodies (mAbs) with specificities for different HLA subtypes were incubated with platelets obtained from healthy donors with known HLA typing. Platelet activation (CD62P surface exposure, von Willebrand factor (VWF) and SPARC release) and platelet aggregation were measured. Binding of HLA mAbs to the platelets and HLA expression levels on platelets were determined.

Results: While all HLA mAbs bound to HLA matched platelets, 3 out of 11 induced platelet activation. CD62P membrane exposure was upregulated upon incubation with WIM8E5 (directed against A1/A10/A11/A9/A29/A30/A31/A33/A28), SN607D8 (A2/A28) and GV5D1 (A1/A9) with platelets expressing these MHC class I alleles. Also release of SPARC and VWF was observed upon incubation with WIM8E5, SN607D8 and GV5D1. These mAbs also induced platelet aggregation although at a rate that was 10 times lower than observed for collagen. Upon incubation with IV.3 (a blocking antibody directed towards FcγRIIa) WIM8E5, SN607D8 and GV5D1 induced aggregation and α-granule release was completely blocked. Blocking of downstream signaling from FcγRIIa employing Syk inhibitor IV also interfered with mAb induced α-granule release.

Conclusions: We provide evidence for FcγRIIa dependent activation of platelets by patient derived HLA mAbs. We suggest that these HLA antibodies contribute to enhanced clearance of transfused platelets from the circulation.

PB 1263 | Platelet PN-1 Regulates Clot Structure and Retraction

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Background: Serpin E2 or Protease Nexin-1 (PN-1), a cellular serpin, is a strong inhibitor of serine proteases. We recently established platelet PN-1 as a negative regulator of thrombin generation and thrombosis. The mechanical properties of clot are dependent of thrombin-induced platelet activation and fibrin formation. Altered clot architecture and retraction are known to be associated with abnormal thrombin generation. We hypothesize that platelet PN-1 can regulate clot architecture.

Aims: To evaluate the role of platelet PN-1 on clot structure and retraction.

Methods: Clot retraction was assessed after recalcification in glass tubes of human platelet-rich plasma (PRP) incubated or not with a PN-1-blocking IgG, or of PRP obtained from wild-type (WT) or PN-1 knock-out (PN-$1^{-/-}$) mice. Clot architecture was evaluated with confocal microscopy of human or mice platelet-rich clots (PRC) supplemented with Alexa647-fibrinogen and Alexa488 anti-CD41, a platelet marker. Clot viscoelasticity properties were analysed with rotational thromboelastometry test (ROTEM).

Results: Surprisingly, clot weight was increased by 66% in human PRC incubated with a blocking anti-PN-1 IgG and by 40% in PN-$1^{-/-}$ PRC compared to their respective controls, indicating a positive effect of PN-1 on clot retraction. Confocal microscopy images showed that the fibrin network structure was more porous in PRC from PN-$1^{-/-}$ mice or in human PRC incubated with a blocking anti-PN-1 IgG. TEMograms showed that the blocking anti-PN-1 IgG induced a 26% decrease of the maximum clot elasticity, a clot strength parameter. In our experimental conditions, no difference was observed in PRC incubated with tranexamic acid and in platelet-poor clots indicating a direct role of PN-1 in platelet contractile force.

Conclusions: Despite its anti-thrombin effect, platelet PN-1 accounts for the formation of a tight structure of PRC. We thus identify a critical positive role of PN-1 on platelet-driven clot retraction.

PB 1264 | Statins Inhibit Platelet Function and Attenuate Clot Structure

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Background: Inhibition of the mevalonate pathway by statins impairs prenylation of proteins by depleting cells of lipid geranylgeranyl diphosphate (GGPP). One such protein is Rab27b, a GTPase involved the secretion of platelet dense granules.

Aims: To investigate the impact of statins on prenylation of Rab27b in platelets and downstream effects platelet function.

Methods: Whole blood pre-treated with atorvastatin (ATV) was analysed by thromboelastography. ADP was quantified as a marker of dense granule secretion in thrombin-stimulated ATV treated platelets. P-selectin exposure and fibrinogen-binding to thrombin-stimulated platelets ± ATV was analysed by flow cytometry. Fibrin clot formation in platelet-rich plasma (PRP) ± ATV was examined by confocal microscopy. Unprenylated Rab27b was detected in the aqueous fraction of platelet lysates by Western blot. All samples were treated with increasing concentrations of ATV (0, 10, 20 and 40 μM) for 24 h at 37°C.

Results: ATV significantly increased clot formation time at 20 μM ($p< 0.005$). Clot firmness was attenuated by ATV at 20 μM (39.6 mm) ($p< 0.005$) compared to untreated (51.8 mm). Incubation of PRP with ATV (20 μM) significantly ($p< 0.001$) attenuated clot retraction. Fibrinogen-binding and P-selectin exposure were significantly lower ($p< 0.05$) in stimulated platelets treated with 40 μM ATV. Similarly, confocal microscopy of PRP clots revealed a reduction in fibrin binding to platelet aggregates following ATV treatment. ATV induced a dose-dependent accumulation of unprenylated Rab27b and inhibition of ADP release from activated platelets. Exogenous GGPP partially rescued the ADP release defect in ATV treated platelets suggesting these changes arise from attenuated prenylation of Rab27b.

Conclusions: Statins attenuate fibrinogen-binding to activated platelets, clot retraction, a-granule and dense granule secretion. Statins modulate fibrin binding to platelet aggregates, suggesting a potential alternative cardiovascular protective mechanism for these drugs *in vivo*.

PB 1265 | Cyclophilin D Restricts the Lytic Susceptibility of Fibrin via Modulation of Platelet Functions

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Background: In growing thrombi platelets are exposed to a variety of stimuli, resulting in morphologically and functionally distinct platelet subpopulations. Platelet activation by ‚strong' stimuli results in opening of the mitochondrial permeability transition pore (mPTP). Cyclophilin D (CypD), an enhancer of mPTP has been previously suggested as a suitable target to influence thrombosis. However, its role in ‚mild' platelet activation as well as in the platelet-driven stabilization of fibrin is unknown.

Aims: To compare the effects of CypD in platelet activation by ‚strong' (thrombin+collagen-analogue convulxin) and ‚mild' (ADP) stimuli, and to define the role of CypD in the lytic susceptibility of fibrin.

Methods: CypD function was blocked using pharmacologic inhibition (cyclosporin A, CsA) in human and genetic ablation in murine platelets. Platelet morphology was assessed with transmission- (TEM) and scanning electron microscopy (SEM). Adhesion and spreading were followed using an impedance-based assay. Platelet-platelet interactions were studied with optical aggregometry. Fibrinolysis was evaluated in a turbidimetric assay.

Results: TEM and SEM evidenced that ‚strong' stimulus-induced organelle-swelling (5-fold increase in the normalized organelle area index of human platelets) and membrane fragmentation were reverted by CsA. The rate of ADP-induced platelet spreading in $CypD^{-/-}$ and CsA-treated platelets was doubled compared to their respective controls. CsA but not the genetic ablation of CypD increased both the rate (+92%) and maximal values (+28%) of ADP-induced platelet aggregation. The fibrin-stabilizing effect of wild type platelets was abolished in clots containing $CypD^{-/-}$ platelets according to our fibrinolysis assays.

Conclusions: Any anti-thrombotic strategy that targets CypD function should consider the opposing consequences of CypD inhibition that depend on the stimulus and stage of platelet activation.

PB 1266 | Biomechanical Mechanisms of Platelet-fibrin Interactions as a Basis of Blood Clot Contraction

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Background: Contraction is a final stage of blood clot maturation that strengthens hemostatic clots, enhances wound healing and restores blood flow past otherwise obstructive thrombi. Despite biological and clinical importance, the physical mechanisms of clot contraction are largely unknown.

Aims: To unveil and quantify the structural biomechanics of clot contraction at various spatial scales from a single cell/single fiber level up to the network and macroscopic levels.

Methods: We used confocal microscopy and rheometry to perform concurrent 3D dynamic measurements of the platelet-fibrin meshwork over the course of clot contraction.

Results: We found that activated platelets bend and shorten individual fibrin fibers via cellular filopodia that undergo sequential extension and retraction, pulling hand-over-hand. Platelets also induce compaction of fibrin fibers into platelet-attached agglomerates. By pulling on multiple, closely-set fibrin fibers, platelets move toward each other and form secondary clusters. Contracting platelets remodel a fibrin network by increasing its density causing enhanced clot stiffness. Kinetic analysis revealed at least three distinct phases that differ in duration and rate constants. All the observed changes were reduced or abrogated in the presence of inhibitors of non-muscle myosin IIA (blebbistatin) and the integrin $\alpha_{IIb}\beta$ (abciximab), indicating that cell contractility and platelet-fibrin interactions are crucial for contraction of blood clots. Finally, blood clot contraction was found to be a spatially non-uniform process with faster compression of the clot edge and slower deformation of the clot interior.

Conclusions: We discovered structural mechanisms by which local platelet-fibrin interactions result in dramatic modifications of the whole clot architecture. The mechanisms of blood clot contraction that were revealed demonstrate an important new biological application of cell motility principles.

PB 1267 | Non-genomic Effects of the Pregnane X Receptor: Inhibition of Platelet Functions

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Background: Platelets, despite being enucleated, express nuclear receptors that are capable of regulating platelet activity. The pregnane X receptor (PXR) is a nuclear receptor, involved in the detoxification of xenobiotic compounds. Recently, its presence was reported in the human vasculature and its ligands were proposed to exhibit anti-atherosclerotic effects.

Aims: To explore the presence of PXR in human platelets and evaluate the role of its ligands in regulating platelet function.

Methods: Western blotting and immunoprecipitation were performed to examine the expression of PXR. Platelet aggregation and dense granule secretion were studied on washed platelets with and without treatment with PXR ligands (rifampicin or SR12813). The extent of fibrinogen binding and α-granule secretion was analysed using flow cytometry. Calcium mobilisation was measured by spectrofluorimetry in FURA-2AM loaded platelets and integrin $\alpha_{IIb}\beta_3$ outside-in signalling was studied by measuring clot retraction. The effects of PXR ligands on thrombus formation *in vitro* (whole blood) were tested in $DiOC_6$ loaded platelets.

Results: The expression of PXR in human platelets was confirmed using western blotting and immunoprecipitation. Platelets treated with rifampicin or SR12813 (10,20,50 & 100μM) reduced collagen or thrombin-mediated platelet aggregation and dense granule secretion. PXR ligands attenuated CRP-XL or thrombin-stimulated fibrinogen binding and P-selectin exposure, indicating a reduction in integrin $\alpha_{IIb}\beta_3$ up-regulation and α-granule secretion, respectively. Calcium mobilisation and clot retraction were also inhibited upon treatment with either of the ligands. Lastly, significant reduction in thrombus formation under arterial flow conditions *in vitro* was observed, demonstrating the potential role of the ligands in regulating thrombosis.

Conclusions: PXR exist in human platelets and exposure to its ligands inhibits platelet function. Future work will determine the specificity and mechanisms by which PXR ligands function in platelets.

PB 1268 | A Potential Role of Platelet Activation in the Progression of Atrial Fibrillation

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Background: Atrial fibrillation (AF) progresses in time and is associated with microvascular destabilization, tissue fibrosis and an increased risk for heart failure. AF is associated with low grade inflammation, a hypercoagulable state and activated platelets.

Aims: Our working hypothesis links AF progression to microvascular destabilization via hypercoagulability. To elucidate how this affects the integrity of the microvasculature, tissue factor (TF)-mediated coagulation on endothelial cells (ECs) was studied in the absence or presence of platelets (PLTs).

Methods: Primary ECs were treated with 1 ng/ml tumor necrosis factor α (TNFα). TF-induced coagulation was determined using a FXa generation assay. The role of PLTs was studied by adding PLTs to an EC-monolayer or to isolated extracellular matrix (ECM), which becomes exposed when the integrity of the monolayer is lost. TF protein was determined with an antibody (10H10) recognizing cryptic/proteolytically inactive TF (cTF). Expression of protein disulphide isomerase (PDI) was determined, which is involved in decryption/activation of TF.

Results: Without TNFα, ECs do not show TF-activity nor cTF protein expression (Figure A1 vs B1) and this is not altered when PLTs are added (A3 vs B3). PLT incubation on TNFα-stimulated ECs significantly increase TF-activity coinciding with a decrease in cTF protein (A4 vs B4), indicating decryption of TF. Interestingly, PLTs incubated on ECM±TNFα showed a similar protein pattern (B7-8 vs B3-4), but a synergistically potentiating effect on activity (A8 vs A4). Although PDI is constitutively expressed in ECs (B1-4), only the addition of PLTs resulted in significant FXa generation, suggesting that PLT-PDI is responsible for the decryption of TF. While resting PLTs express low amounts of PDI (B9), they increase PDI expression when encountering ECM (B7-8 vs B5-6).

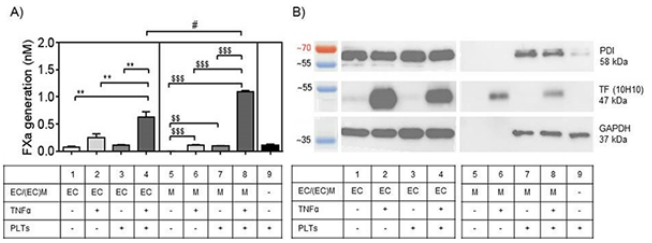

FIGURE 1 FXa generation (A) and immunoblots (B) on ECs and ECM from the same source. One-way ANOVA P<0,05, within EC (*) or ECM ($) and between EC and ECM (#)

Conclusions: We show that PLTs may aggravate the pro-coagulation state in AF. These data indicate that patients with AF may benefit from treatment with platelet inhibitors.

PB 1269 | Analysis of Microparticles Associated with Procoagulant Platelets by Imaging Flow Cytometry

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Background: A procoagulant platelet subpopulation, distinguished by phosphatidylserine (PS) surface membrane exposure, is formed upon activation. In a study of the physical characteristics of PS-exposing platelets, we made the novel observation that the majority of PS-positive (+) platelets had one or more associated microparticles (MPs).
Aims: To evaluate MPs associated with PS-exposing platelets using imaging flow cytometry.
Methods: Washed platelets were stimulated with 1U/mL thrombin and 10μg/mL collagen (T+C) or 3μM A23187, and labeled for the platelet marker glycoprotein (GP)IX with anti-CD42a-eFluor450, and for the platelet activation markers: activated αIIbβ3 with PAC1-FITC; P-selectin with anti-CD62P-PE; and PS exposure with annexin A5-Alexa Fluor647. At least 150,000 cells/sample were acquired on the Amnis ImageStream^X Mark II imaging flow cytometer and analyzed with IDEAS software. Values are mean±SEM, n=4.
Results: The majority of PS+ platelets produced by T+C stimulation (92.0±1.6%) or A23187 stimulation (97.1±0.7%) possessed at least one associated MP; % PS-negative platelets with associated MPs was negligible. The number of associated MPs per platelet ranged from 1-8, with the majority of PS+ platelets possessing 1 or 2 MPs (T+C: 80.2±1.0%; A23187: 72.8±2.5%), with diameters 20-25% of that of the parent platelet. Platelet-associated MPs were heterogeneous as indicated by the expression of GPIX and the activation markers in various combinations of up to all 4 markers. Those positive for GPIX, P-selectin, and PS, and not expressing activated αIIbβ3, were in the highest proportion: 23.1±5.2% (T+C) and 29.4±4.0% (A23187).
Conclusions: As demonstrated by imaging flow cytometry, almost all PS+ platelets have associated MPs that show heterogeneous marker expression. These MPs are not detectable by traditional flow cytometry. Whether the MPs are the result of blebbing of the platelet membrane, or have been released from platelets and subsequently re-associate with a platelet surface, has yet to be determined.

PB 1270 | Controlled Adhesion of Endothelial Cell and Platelets onto Patterned CoCr Alloy Surfaces by Direct Laser Interference Patterning Technique for Cardiovascular Applications

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Background: Stent restenosis and thrombosis are the main drawbacks of current carfiovascular bare-metal stents. Nano- and micro-scale modification of implant surfaces is a strategy to recover the functionality of the artery by stimulating and guiding molecular and biological processes at the implant/tissue interface.
Aims: To explore changes in the adhesion of endothelial cells (EC) and platelets on CoCr surfaces due to modifications via Direct Laser Interference Patterning (DLIP).
Methods: CoCr alloy surfaces were modified via DLIP to create a lineal patterning with different periodicities (≈ 3, 10, 20 and 32 μm) and depth (≈ 20 and 800 nm). Changes in surface topography, chemistry and wettability were characterized by scanning electron microscopy, X-ray photoelectron spectroscopy, and sessile drop method, respectively, before and after modification. The effect of the different DLIP patterns on both the growth of human umbilical vein EC and the adhesion of platelets on the CoCr surfaces were explored, by using immunofluorescence and perfusion techniques with flowing blood.
Results: Patterned surfaces with low depth presented a chemical rather than topographical lineal pattern, while high depth surfaces appeared chemically homogeneous. Adhesion and spreading properties of HUVECs were similar on all patterned and plain CoCr surfaces. However, high depth series induced EC elongation and alignment along the pattern lines. Platelet adhesion and aggregation of platelets, under flow conditions, decreased on all the patterned surfaces compared to the CoCr control, mainly due to changes in wettability.
Conclusions: These results provide evidence of the potential of DLIP topographies to modify the surface of metal stents to foster endothelialization without enhancing platelet adhesion.

PB 1271 | Authophagy of Microparticles by Platelets is Partially Mediated through TLR-4 and Enhances Thrombogenicity

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Background: Cardiovascular disease involves multiple inflammatory processes, including activation of innate immunity toll-like receptors (TLR). Circulating microparticles of platelet origin (PMP) are the most abundant (70-90%). PMPs contribute to the activation of inflammatory cells, accompanied by formation of heterotypic aggregates (HA) between platelets and leukocytes. Platelets play a key role in ischemic complications and can internalize procoagulant microvesicles, fostering their thrombogenicity.
Aims: We have investigated the association of PMP with platelets, and explored the involvement of TLR-4.
Methods: PMPs were separated by ultracentrifugation of expired platelets concentrates. We assessed: 1) Interactions of PMPs with platelets using confocal microscopy; 2) Association between platelet-PMP and formation of HA, by flow cytometry; and 3) Modifications in

thromboelastometry parameters of blood enriched with platelets exposed to PMPs, by ROTEM technology. Effects of a specific antibody to TLR-4 were also evaluated.

Results: Confocal microscopy studies demonstrated uptake of PMP by platelets. Flow cytometry revealed PMP-platelet associations (86.1±6.7% vs. 2% control; p< 0.01) and increased HA formation (34.4±7.0% vs. 29.7±6.7% control; p< 0.05). Incubation of platelets with anti-TLR4 prior to exposure to PMPs decreased both, PMP-platelet associations (57.1±7.9% vs. PMP without anti-TLR4; p< 0.05) and prevented HA formation. Addition of 30% platelets containing PMPs accelerated the clotting time (54.6±4.9 sec. vs. 62.1±5.2 sec. control; p< 0.05), and the clot formation time (79.4±5.8 sec. vs. 119.6±6.1 sec. control; p< 0.05) in the ROTEM system. The anti-TLR4 normalized alterations in the latter parameters.

Conclusions: We demonstrate autophagic activity of platelets to their procoagulant microparticles, a process partially mediated by innate immunity receptor TLR-4 and with implications in platelet thrombogenicity.

Grants: *RD12/0042/0016;FIS-PI13/00517;PIE15/00027;DTS16/00133*

PB 1272 | Analysis of Venous and Arterial Hemostatic Plugs Reveals Strikingly Similar Composition and a Gradient of Platelet Activation

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Background: Prior experiments in the mouse microvasculature have established that the architecture of hemostatic plugs is characterized by a gradient of platelet activation with heterogeneous packing density and platelet activation.

Aims: To determine and compare the architectural composition of hemostatic plugs in large arteries and veins.

Methods: We developed a new methodology to study hemostasis in large veins and arteries that combines scanning electron microscopy (SEM), confocal/two-photon microscopy, and histological sections. Hemostatic plugs in mouse jugular veins and carotid arteries were produced by needle injuries, then fixed and imaged in situ from either the intraluminal or extraluminal side.

Results: Our results revealed great similarity in platelet morphology and packing density in the jugular vein and carotid artery. Hemostatic plugs in both vessel types were composed almost entirely of platelets, which varied in activation state depending on spatial localization in the platelet mass. The extravascular portion in both cases was composed primarily of densely packed, highly activated, platelet-derived microvesicles and fibrin. In both the vein and artery, there was little fibrin observed on the intravascular side, suggesting thrombin activity is primarily restricted to the extravascular portion of the plug. A gradient of platelet activation extending from the injury site was observed on the intravascular side of both vein and artery hemostatic plugs. Though flow rates differ in the carotid and jugular, bleeding times were similar.

Conclusions: In conclusion, our results show that platelet rich hemostatic plugs in both large arteries and veins display a gradient of platelet activation, with a drastic difference between the appearance and composition of the internal and external surface of the plug in both vessel types. These novel findings suggest localized spatial distribution of thrombin and other platelet agonists at a site of vascular injury.

PB 1273 | Platelet Reactivity and Receptor Density in Platelets with Different MPV

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Background: It is often stated that larger platelets (plt) are more reactive than smaller ones. However, most studies only describe associations between a high mean platelet volume (MPV) and different clinical conditions. The fact that activated plt undergo substantial reorganisation which impact on plt size complicates studies to prove the hypothesis. Pre-analytical activation may also contribute to these problems.

Aims: To investigate if plt reactivity and receptor density varies between resting plt with different MPV.

Methods: Blood from healthy donors was centrifuged (150g, 15 min) and the fractions containing the largest and smallest plt were separated. MPV and numbers of PAR1, GPIb and GPVI receptors were determined. Plt reactivity was investigated by agonist stimulation, with surface exposure of activation markers P-selectin, active GPIIb/

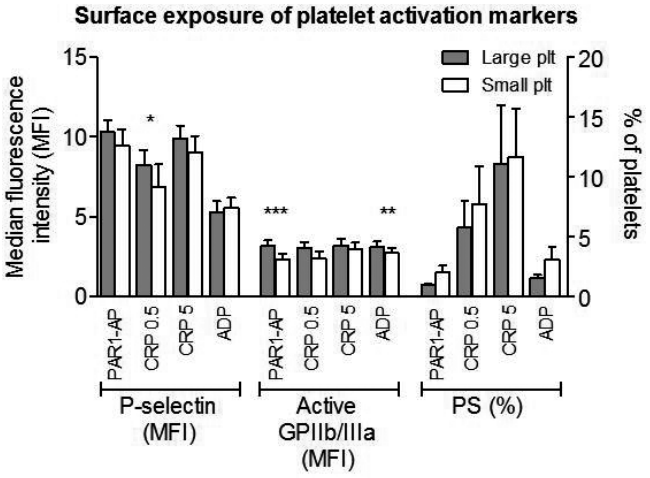

Figure 1. Plt were stimulated with PAR1-activating peptide (AP; 30μM), collagen-related peptide (CRP; 0.5 or 5μg/ml) or ADP (5μM). Mean ± standard error of the mean, n=6. * Indicate statistically significant differences between large and small plt.

FIGURE 1

IIIa and phosphatidylserine (PS) evaluated with flow cytometry. T-test was used to compare large and small plt.

Results: We successfully separated plt with different MPV. A low resting P-selectin exposure verified that pre-analytical plt activation was low and thus did not influence the results, table I. Receptor numbers were higher in large plt. However, if plt were assumed to be spherical, the resulting number of receptors/μm^2 were higher in small plt, table I. Only small differences in plt reactivity was found, with higher exposure of P-selectin and active GPIIb/IIIa in large plt at some conditions. Exposure of PS did not differ between large and small plt. These results, although non-significant, rather indicate a higher PS exposure in small plt, figure 1.

Conclusions: Centrifugation allowed successful separation of plt with different MPV, while maintaining a resting state. Large plt were slightly more reactive than small plt regarding P-selectin and active GPIIb/IIIa, but not PS. It can however be questioned whether such minute differences are physiologically relevant and can explain the reported associations between platelet size and different clinical conditions.

TABLE I Features of resting platelets.

	Large platelets	Small platelets
MPV (fL)	9.2 ± 0.5	7.8 ± 0.5 **
P-selectin (%, resting plt)	8.1 ± 1.0	7.6 ± 1.0
GPIb (receptors/plt)	28283 ± 1346	25338 ± 1013 *
PAR1 (receptors/plt)	1792 ± 166	1430 ± 184 **
GPVI (receptors/plt)	6204 ± 517	3986 ± 368 **
GPIb (receptors/μm2)	14.22 ± 0.59	15.95 ± 0.66 **
PAR1 (receptors/ μm2)	0.84 ± 0.03	0.95 ± 0.04 **
GPVI (receptors/ μm2)	4.00 ± 0.17	4.49 ± 0.19 **

Mean ± standard error of the mean, n=6. * Indicates statistically significant differences between large and small plt.

PB 1274 | Connexin 62 Hemichannels and Gap Junctions Regulate Platelet Function

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Background: Connexin (Cx) is a large family of proteins, expressed in numerous mammalian cells. Cxs oligomerise into hexameric structures [hemichannels, (HCs)] in the plasma membrane. These HCs on adjacent cells can dock together, forming gap junctions (GJs) that regulate trafficking of molecules between cells. Previously, we reported the presence of Cx37 and Cx40 in human platelets and that selective inhibition, or deletion, attenuates platelet functions. Notable levels of Cx62 transcripts were also reported in megakaryocytes.

Aims: The objective of this study was to determine the role of Cx62 in human platelets.

Methods: The presence of Cx62 in human/mouse platelets was examined by immunoblotting. A ^{62}Gap27 targeting Cx62 and scrambled control were designed. Platelet aggregation and dense granule secretion was studied in washed platelets, and the degree of fibrinogen binding and α-granule secretion measured through flow cytometry. Calcium mobilisation was measured by spectrofluorimetry in FURA-2AM loaded platelets. Integrin $\alpha_{IIb}\beta_3$ outside-in signalling was analysed by clot retraction. In vitro thrombus formation was examined in DiOC6 labelled platelets.

Results: Western blotting confirmed the expression of Cx62 in human/mouse platelets. ^{62}Gap27 but not scrambled control inhibited CRP-XL or thrombin-stimulated platelet aggregation and dense granule secretion. CRP-XL or thrombin-stimulated fibrinogen binding and P-selectin exposure was also reduced by ^{62}Gap27. Platelets treated with ^{62}Gap27 displayed reduced calcium mobilisation and clot retraction. Thrombus formation *in vitro* (arterial flow conditions) was inhibited substantially by ^{62}Gap27 suggesting probable role of Cx62 in regulating haemostasis and thrombosis.

Conclusions: Cx62 is present in human/mouse platelets and exposure to ^{62}Gap27 dampens platelet function. Work is ongoing to identify the nature of the molecules trafficking through Cx62 HCs (isolated platelets) or GJs (within thrombus) along with the mechanisms through which their conductance is regulated.

PB 1275 | Chloride Intracellular Channel 1 Cooperates with Integrins to Promote Thrombus Formation and Angiogenesis

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Background: Chloride intracellular channel 1 (CLIC1) has been shown to be involved in thrombus formation as well as angiogenesis but its role in these processes is largely unknown.

Aims: To determine if CLIC1 supports cell adhesive processes that are relevant for endothelial and platelet function.

Methods: Human umbilical venous endothelial cells (HUVEC) were probed for cell proliferation on plastic and cell invasion/survival after embedding in fibrin after transfection with siCLIC1. The subcellular localization of CLIC1 in HUVEC as well as platelets was analyzed with fluorescence microscopy following treatment with the synthetic CLIC1 inhibitor IAA94, which was also used to assess the effect of CLIC1 on integrin activation and platelet aggregation. The role of CLIC1 on thrombus formation *in vivo* was assessed by intravital fluorescence microscopy in a mouse dorsal skin fold chamber model.

Results: Knocking down endothelial CLIC1 with siRNA caused a defect in cell spreading that was associated with decreased cell proliferation, invasion and survival. The effects of CLIC1 inhibition could be traced back to diminished CLIC1 cell membrane expression, which, in turn, resulted in disorganized lamellipodia formation. Paralleling these results, we detected integrin-dependent CLIC1 membrane relocation in platelets. Treatment of platelets with the synthetic CLIC1 antagonist

IAA94 targeted membrane CLIC1 and, in the process, reduced integrin αIIbβ3 activation. As a consequence, inhibition of CLIC1 impaired platelet aggregation *in vitro* and vaso-occlusion in a mouse model of photo-chemical thrombus formation *in vivo*.

Conclusions: CLIC1 cooperates with integrins during cell adhesion and as such mediates functions related to thrombus formation, endothelial homeostasis and angiogenesis.

PB 1276 | A Novel Role for the Membrane Protein G6f in Platelet Activation Induced by Weak Stimulation

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Background: The lymphocyte antigen 6 complex locus protein (G6f) is a type I transmembrane protein of the immunoglobulin super family that is expressed on the surface of human platelets. The intracellular tail of G6f contains a single tyrosine in a single YXXI motif, which undergoes phosphorylation in response to GPVI and αIIbβ3-mediated platelet activation. This leads to the binding of the adaptor protein Grb2 to G6f. G6f is currently considered to be platelet-specific, but its function remains unknown.

Aims: In this study, we investigated if antibodies against this membrane protein can influence platelet activation and aggregation caused by various agonists.

Methods: Washed platelets were stimulated with low doses of agonists: thrombin (0.03-0.05 U/ml), ADP (10 μM), IgG immune complexes (IC, 40-100 nM) and collagen related peptide (CRP, 0.25-1 μg/ml) with or without anti-G6f antibodies. Platelet aggregation and dense granule release were assessed by aggregometry and the serotonin release assay, respectively.

Results: Platelet aggregation and granule release induced by low dose thrombin, ADP (aggregation only) and IC were strongly inhibited (>80%) by the anti-G6f antibodies whereas CRP-induced platelet activation was inhibited by 40%. These effects were not observed with higher agonist concentrations.

Conclusions: Our results suggest a novel role for G6f in platelet activation caused by a broad range of agonists. The results also indicate that anti-G6f antibody promotes an inhibitory effect against agonist-induced platelet activation and aggregation. G6f may therefore represent a possible anti-thrombotic target. However, additional mechanistic studies are required to address this.

PB 1277 | Biomimetic Surfaces for Studying Neutrophil-platelet Interactions

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Background: Neutrophils play a central role in cardiovascular disease and thrombosis. They interact with platelets through receptor-ligand interactions such as neutrophil L-selectin and PSGL-1 as well as integrin Mac-1 and platelet glycoprotein Ibα (GPIbα). The presentation of L-selectin as well as GPIbα varies under different physiological and pathophysiological conditions. However, the biological consequences of these dynamic changes remain poorly understood and hard to assess. I*n vivo* models are often too complex for the isolated observation of single receptor-ligand interactions while classical *in vitro* systems do not allow precise tuning of local receptor density and, simultaneously, hydrodynamic shear stress.

Aims: Therefore, our aim was to develop a nanotechnology-based platform for studying ligand-receptor interactions under realistic biophysical conditions.

Methods: A nanopatterning/site-directed biofunctionalization approach allowed the presentation of surface receptors in their natural orientation as well as precise adjustment of the local and global surface density of these receptors. Additionally, these biofunctionalized surfaces can be integrated into a microfluidic system, enabling the imitation of physiological flow conditions. We employed this method to mimic interactions between neutrophil Mac-1 and surface-bound GPIbα as well as between L-selectin and PSGL-1.

Results: Under physiological flow conditions, neutrophils required minimum spacings of GPIbα to successfully adhere. Under low shear stress, adhesion to GPIbα was similar at all tested densities, however receptor spacings differentially regulated neutrophil spreading kinetics and locomotion. For the interaction between L-selectin and PSGL-1, attachment of cells increased with higher ligand densities.

Conclusions: We were able to show that initial attachment and rolling of neutrophils increases at higher ligand densities. For complex cellular behaviors such as spreading and migration there is an optimum density at medium ligand concentrations.

PB 1278 | Heparin Decreases Thrombin Induced Procoagulant Platelet Formation via a Direct Platelet Effect

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Background: Lack of procoagulant platelets is associated with bleeding while excess is associated with atherothrombosis. Heparins are known to bind to exosite II on thrombin and a direct inhibition thrombin induced platelet activation has been postulated.

Aims: Determine if heparin has a direct effect on procoagulant platelet formation.

Methods: We adapted a procoagulant platelet flow cytometry assay based on novel cell death agent, GSAO (Hua et al,Blood, 2015) to measure procoagulant platelet formation ex vivo. Whole blood from healthy volunteers was pre-incubated with varying doses of unfractionated heparin (UFH) or vehicle prior to thrombin stimulation. In some experiments, washed human platelets were stimulated without plasma, or resuspended in anti-thrombin III deficient plasma or autologous plasma prior to preincubation with UFH. Comparison was made with preincubating with anticoagulants with structural similarity to heparin but variable binding to thrombin: enoxaparin, the heparinoid danaparoid (which acts on FXa); and structurally dissimilar anticoagulants: fondaparinux, rivaroxaban and apixaban. Procoagulant platelets were defined as being GSAO+/CD62P+ by FACS.

Results: UFH demonstrated a dose dependent inhibition of procoagulant platelets within the therapeutic range, and abrogated response at high doses (13.3±8vs1.81±0.5,p< 0.001). Inhibition was reproduced with washed platelets and platelets resupended in anti-thrombin III deficient plasma indicating a direct effect on platelets (fold change 0.1±0.05, p< 0.001). Heparin based anticoagulants enoxaparin, and the heparinoid indirect FXa inhibitor, danaparoid, reproduced these findings (fold change 0.46±0.1,p< 0.01 and 0.61±0.11,p< 0.05), while the structurally dissimilar pentasaccharide, fondaparinux and direct FXa inhibitors did not.

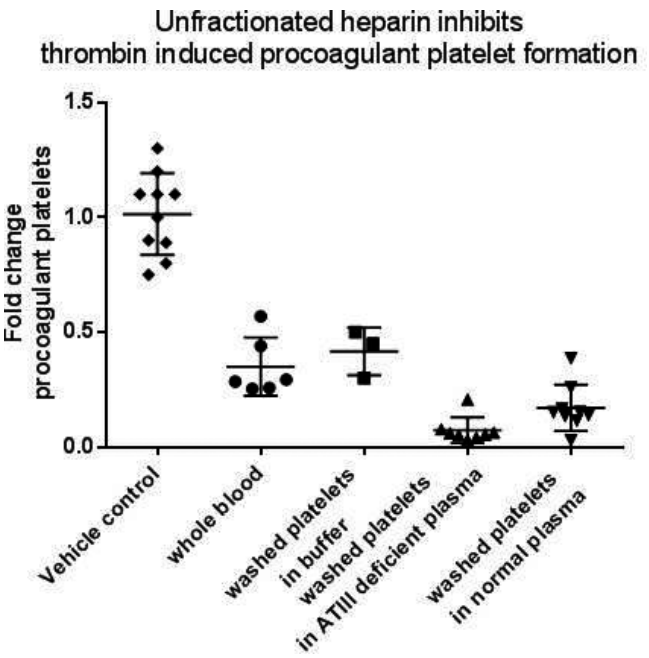

FIGURE 1 UFH or vehicle control was preincubated prior to thrombin stimulation and measurement of procoagulant platelets

Conclusions: Anticoagulants with structural similarity to heparin inhibit procoagulant platelet formation independent of the anticoagulant activity. Exploration of the mechanism could give insight into new therapeutic targets.

PB 1279 | Changes in Platelet Surface Receptor Expression upon Activation Are Mainly Contributed by Large Platelets

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Background: Upon activation, platelets undergo a shape change, including filopodia protrusion. Thus more plasma membrane, along with receptors, may be exposed on the surface.

Aims: We determined if surface receptor expression changes upon platelet activation and if larger platelets show a greater change.

Methods: Receptors in resting, CRP, Cvx, or thrombin activated platelets were measured by an Accuri C6 flow cytometer, as MFI, using specific primary Abs and Alexa fluor 488-anti-mouse Fab. In the analyses, the total platelet population (P1) was divided in half: smaller ones (P3) and larger ones (P2).

Results: In activated platelets, P1 showed increased integrin $\alpha 2\beta 1$ and $\alpha IIb\beta 3$, no change in GPVI, and decreased GPIb (Ib). P2/P1, the ratio of the MFI value in P2 and P1, was calculated for each receptor in activated platelets. The ratios for $\alpha 2\beta 1$, $\alpha IIb\beta 3$, Ib, and GPVI were increased from about 130% to 190% in PRP and 130% to 160% in washed platelets. The ratio for CD62P and $\alpha IIb\beta 3$ increased to >300% after thrombin activation. P3 (smaller platelets) showed much lower changes in expression. Immunoblotting of the platelet supernatant and confocal imaging showed that decrease in GPIb expression was due to shedding, while decreased GPVI expression was caused by internalization.

Conclusions: More receptors are exposed on the surface of platelets when they become activated, corresponding to platelet shape change and filopodia extension. Changes in receptor expression are mainly contributed by large platelets (P2), while small ones (P3) show only slight changes. These results indicate it is mainly the population of large platelets that become activated, supporting the recently proposed hypothesis that large mean platelet volume (MPV) is a risk factor for cardiovascular incidents. Further, these results suggest that flow-cytometric measurement of P2 platelets for increased receptor expression would be a more sensitive method to detect activated platelets.

PB 1280 | Autophagy Inhibitors Reduce Platelet Function by Preventing Granule Exocytosis

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Background: A critical role for autophagy, the process by which cells eliminate excess or damaged content, in platelet activation and thrombosis has recently been shown using mice deficient in autophagy proteins. Given its role in regulating protein secretion, autophagy may regulate platelet function by regulating granule exocytosis. This

mechanism has been suggested to explain the platelet inhibitory effects of chloroquine (CQ), but experimental evidence in support of this mechanism is lacking.

Aims: Our aim was to elucidate by which mechanism inhibition of the autophagy machinery decreases platelet function.

Methods: Platelet-rich plasma (PRP) or whole blood from healthy volunteers was treated with different concentrations of CQ, bafilomycin A1 (BAF), 3-methyladenine (3MA), or vehicle. Lumiaggregometry was used to simultaneously measure release of ATP and platelet aggregation. P-selectin expression was measured with flow cytometry. Release of platelet factor 4 (PF4) and Platelet-derived Growth Factor (PDGF) were assessed. Platelet adhesion and aggregation to collagen, von Willebrand factor (VWF), and fibrinogen was studied using a flow chamber model, and were recorded in real-time using fluorescent microscopy.

Results: We observed a dose-dependent decrease in ADP and ADP/TRAP-induced P-selectin expression, PF4 -, and PDGF release in PRP treated with CQ or 3MA. CQ and 3MA also dose-dependently inhibited platelet aggregation and ATP release in suspension induced by different agonists. Platelet aggregation to collagen and VWF under flow was severely reduced in whole blood treated with CQ or 3MA. However, there was no difference in platelet adhesion to fibrinogen, which is independent of platelet secretion. We did not observe any effects on platelet function using BAF.

Conclusions: Disruption of the autophagy machinery using CQ or 3MA leads to impaired platelet function by decreasing the release of α- and δ-granule content. It seems that autophagy has however, no effect on secretion-independent functions of platelets.

PB 1281 | Kinetics of Prothrombin Interaction with Subpopulations of Activated Platelets

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Background: One of the major reactions of blood coagulation is membrane-dependent activation of prothrombin to thrombin by prothrombinase. There are at least two activated platelet subpopulations with dramatically different procoagulant properties. Systematic kinetic studies for the binding of a prothrombin to the membranes of activated platelet subpopulations are lacking.

Aims: We aimed to characterise the binding of a prothrombin to the membranes of activated platelet subpopulations.

Methods: Platelets were isolated from whole blood by washing and gel-filtration. Written informed consent was given by all participants, and the study was approved by the institutional ethical committee. They were stimulated with thrombin (100 nM), subpopulations were identified with Fura Red is dye for cytosolic calcium Flow cytometry and confocal microscopy were used to investigate interaction of fluorescein-labeled prothrombin with the membranes of activated platelets.

Results: In agreement with previous reports, prothrombin was predominantly bound to the phosphatidylserine-positive subpopulation of activated platelets. This interaction was high-affinity and calcium-dependent. Binding to the phosphatidylserine-negative platelets was several-fold less and calcium-independent binding. Prothrombin was bound to platelets of patients with Glanzmann´s thrombasthenia with the same parameters as for the platelets of healthy donors indicating that binding to phosphatidylserine-negative subpopulation did not depend on integrin $\alpha_{2b}\beta_3$. Apparent equilibrium binding of prothrombin to phosphatidylserine-positive platelets was specific, with 10,000±2,500 binding sites per platelet and apparent k_d of 1.5 ±0.4 microM (n=3). The association/dissociation data were fitted to an exponential decay model with rates of k_+= 1.8±0.3 microM^{-1}s^{-1} and k_- = 0.96 ±0.04 s^{-1}.

Conclusions: Here, we quantitatively characterized interaction of prothrombin with the two subpopulations of activated platelets from healthy donors and patients with Glanzmann´s thrombasthenia.

PB 1282 | Inhibition of MRP4 Mediated Transport Reduces Platelet Function in a cAMP-cGMP Independent Manner

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Background: Platelet Multidrug Resistance Protein4 (MRP4) overexpression has a role in reducing the pharmacodynamics effects of aspirin. In platelets obtained from aspirin treated patients, high on aspirin residual platelet reactivity (HARPR) positively correlated with MRP4 levels.

Aims: We studied the effects of an MRP4 mediated transport inhibitor, cilostazol, on collagen induced platelet activation.

Methods: All studies were performed in platelets obtained from healthy volunteers (HV; N=10) and patients under chronic aspirin treatment (N=45). Platelet aggregation (PA) was evaluated and the results are reported as percent of aggregation observed after 4 min stimulation (%PA) in response to collagen (threshold concentration). ATP release (luciferin-luciferase assay) was measured to evaluate platelet secretion. We evaluated the phosphorylation status of VASP on the Serine 239 through Western Blot. As cilostazol is also a cAMP elevating agent (PDE inhibitor), to separate the effect on MRP4 and PDE, platelets were treated at different time points.

Results: An inhibitory effect on PA and secretion was found when platelets were activated immediately (T0) after addition of cilostazol (6.4±2.4 vs 88.8±1.9 PA%; 2.4±1.6 vs 26.8±2.2 ATP-release %).

VASP phosphorylation was absent at this time (T0), indicating that inhibition is not cAMP-cGMP correlated. The effect of Cilostazol on PA is dependent on MRP4 inhibition, as similar PA reduction was obtained using an MRP4 selective inhibitor, Ceefourin1(6.4±5.8 vs 84.1±5.5 PA%).

Cilostazol effect was evident also in aspirin treated platelets. A reduction of PA and secretion were observed (38.8±16 vs 61.8±17 PA%; 14.9±8.2 vs 20.9±10.8 ATP-release %).

Inhibition of MRP4 reduces HARPR, in patients under chronic aspirin treatment.

Cilostazol treatment reduces collagen induced PA (17.5 ±7.7 vs 53.2±7.8 PA%) in patients with HARPR.

Conclusions: The inhibition of MRP4-mediated transport reduces residual platelet function in patients with HARPR in cAMP-cGMP independent manner.

PB 1283 | Single Platelet Screening Platform Based on Cytoskeletal Adhesion Morphology

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Background: For sealing injured blood vessels, platelets respond to biophysical and biochemical stimuli by remodeling the cytoskeleton. How this reorganization gives rise to a highly contractile phenotype that drives clot contraction remains poorly understood. In vitro, spreading platelets form unique self-organized cytoskeletal patterns, but whether these correlate with platelet function under pathophysiological settings is elusive.

Aims: We aimed at establishing a mid-throughput imaging platform to systematically classify single platelet morphologies and test how these respond to known effectors of platelet aggregation.

Methods: Washed platelets were cultured in the presence of ADP on fibrinogen-coated glass, fixed and stained for F-actin and vinculin. Morphological parameters of single cells were extracted from confocal images by advanced image analysis. Super-resolution fluorescence and SEM were used to obtain further structural insights.

Results: Platelets from healthy subjects predominantly adopted a polarized and highly aligned cytoskeletal organization with strong F-actin bundles anchored at peripheral adhesion sites. This morphology was exclusively observed on fibrinogen or fibronectin, but not on collagen I or laminin. The specific integrin $\alpha_{IIb}\beta_3$ inhibitors RUC-4 and Eptifibatide induced an isotropic cytoskeletal organization in a dose-dependent manner before affecting spreading area. Platelets from a patient with Glanzmann thrombasthenia formed actin rings and very slowly proceeded to a more polarized organization. Differences between morphologies were statistically highly significant with $p << 10^{-10}$.

Conclusions: Our results show that outside-in signaling of the platelet integrin $\alpha_{IIb}\beta_3$ determines the cytoskeletal organization on fibrinogen. Our assay sensitively distinguishes between different platelet phenotypes that are representative for platelet aggregation behavior. The developed platform will be further explored for a clinical screening of platelets.

PB 1284 | Systemic Alteration of Platelet Activity during Each Trimester of Normal Pregnancy

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Background: Markers of platelet activation are elevated during normal pregnancy. However, the clinical significance of platelet activation during pregnancy is not understood.

Aims: In this study we therefore aim to compare platelet function in healthy pregnant women with non-pregnant donors.

Methods: 20 healthy pregnant women were recruited with informed consent at their first-trimester hospital visit. A 12 ml blood sample was donated at each trimester (T1: 9-15 weeks, T2: 16-24 weeks, T3: 25-36 weeks gestation), and within 8 weeks of delivery.

Platelet aggregation was measured in response to a collagen related agonist (CRP), a thrombin-derived activator peptide (TRAP), and a thromboxane mimic (U46619). In parallel platelet ATP/ADP secretion assays were performed using dose-ranges of agonists to quantify changes in platelet sensitivity.

Results: The results of this study shows differential modulation of platelet activation during normal healthy pregnancy. Specifically platelet aggregation in response CRP is enhanced (>two- fold) compared to non-pregnant controls. In parallel the potency of CRP to induce a secretion response is significantly altered (EC_{50} 0.34+/-0 .05 ug/ml vs 8.72 +/-2.77 ug/ml) in T1 pregnancy vs control (Figure 1). Differences in potency persist in T2 and T3 and revert to normal levels in postpartum samples. These alterations in responsiveness may reflect the physiological importance of collagen in critical stages of pregnancy. In parallel, responsiveness to other soluble platelet agonists shows

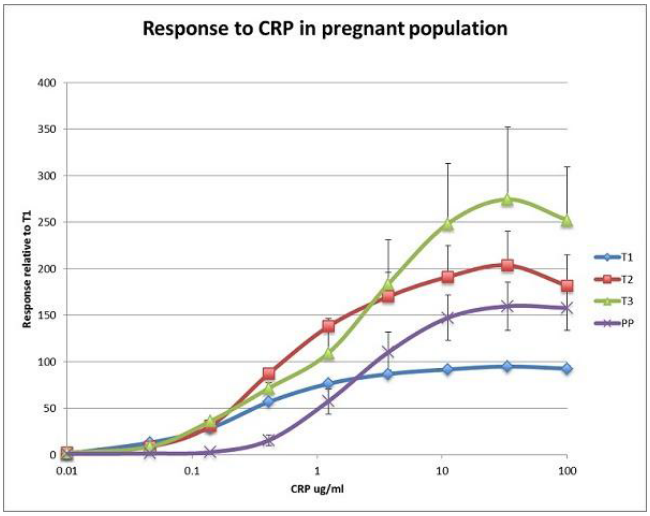

FIGURE 1 Platelet ATP secretion was data from pregnant donors in response to doses of CRP. All data is normalized to data obtained from trimester 1

modulation throughout gestation. In contrast platelet aggregation and secretion in response to U46619 and TRAP was greater at T3 than T1.
Conclusions: From this study we hypothesize that this subtle regulation of platelet responsiveness during pregnancy reflects a nuanced regulation of platelet function that is necessary for a normal healthy pregnancy.

PB 1285 | The Inhibitory Effect of Protamine on Arterial Thrombosis and Platelets in Rats

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Background: Protamine-induced thrombocytopenia (PIT) is a new immuno-hematological and thrombotic disorder described in patients treated with unfractionated heparin (UFH) and protamine (PRT) during cardiopulmonary bypass (CPB). We previously found elevated anti-protamine IgG antibodies (PLoS One 2015) and erythrocyte-platelet aggregates in lungs of mice treated with UFH followed by PRT (UFH/PRT) (Front Pharmacol 2016).
Aims: We aimed to investigate the effect of PRT alone or in complex with UFH on arterial thrombosis and platelets in rats.
Methods: Wistar male rats were divided into 4 groups treated with vehicle, UFH, PRT and UFH/PRT. Platelet aggregation and platelet count were assessed one hour after single administration. In the chronic experiment, UFH/PRT administration was repeated 5 times, once weekly. Arterial thrombosis was electrically-induced in the carotid artery of rats after the last injection. We assessed: thrombus weight, platelet count and aggregation in whole blood, aPTT, PT, fibrinogen, prostacyclin metabolite and D-dimer concentrations in rat plasma.
Results: The platelet aggregation was inhibited, while no changes in platelet number occurred one hour from a single injection of PRT (Table 1). In the chronic experiment, PRT slightly inhibited thrombosis development (0.83±0.17 vs. 1.09±0.22 mg in the vehicle; p< 0.05), but it did not affect the platelet count and platelet aggregation in rats. There were no changes in other parameters.
Conclusions: PRT inhibits directly platelets activity, without decreasing the platelet count, while UFH slightly weakens this effect. We showed an antithrombotic rather than prothrombotic potential of PRT in a repeated-dose experiment in healthy rats. We suggest that PIT and thrombotic complications after PRT treatment could depend on the individual cardiovascular risk, interactions between UFH/PRT/CPB and other drugs, rather than PRT alone.
Funding: National Science Centre, 2016/23/N/NZ7/00442.

PB 1286 | The Role of Neuraminidases in Platelet Function

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Background: Platelets contain many glycans capped by sialic acid, and its cleavage has been implicated in clearance of senescent and cold-stored platelets as well as in immunothrombocytopaenia. The majority of glycans are attached to the platelet adhesion receptor glycoprotein (GP)Ibα. So far, 4 human sialidases (neuraminidase, NEU1-4) have been identified: NEU1 being lysosomal and NEU2 cytosolic.
Aims: To investigate the role of neuraminidases in platelet function.
Methods: Donors were consented to donate either whole-blood to prepare platelet rich plasma (PRP, n=6) or apheresis platelets (n=8). Platelets were stimulated with ristocetin (3mg/ml), ADP (20μM) and arachidonic acid (AA, 800μM). NEU1 and NEU2 surface expression was measured by flow cytometry, as were platelet-attached glycans using Ricinus Communis Agglutinin-1 (RCA-1; detecting galactose-residues) and Wheat Germ Agglutinin (WGA; detecting sialic acid and N-acetyl-D-glucosamine, GlcNAc-residues). GPIIb/IIIa-integrin and/or GPIbα mediated signalling was inhibited by RGDS, GlcNAc or O-sialo-glyco-endopeptidase respectively. Apheresis platelets were studied on collection day (D0) and day 1, 2, 5, 7, 9 post-collection.

TABLE 1 Platelet count and platelet aggregation after one hour experiment. Results are shown as mean ± SD, n = 6-7 * P<0.05 Mann-Whitney test

		VEHICLE	UFH	UFH+PRT	PRT
Platelet count (10^3/mm^3)	-5 min	609 ± 103	609 ± 103	609 ± 103	609 ± 103
	15 min	583 ± 138	549 ± 167	605 ± 159	622 ± 58
	30 min	595 ± 196	607 ± 213	605 ± 146	630 ± 162
	60 min	563 ± 196	639 ± 56	621 ± 82	643 ± 40
Platelet aggregation	Maximal extension	8.2 ± 1.8	9.2 ± 1.6	7.0 ± 3.3	5.4 ± 2.1*
	Slope of platelet aggregation	3.8 ± 0.6	4.4 ± 0.7	3.5 ± 1.4	3.0 ± 0.7*
	Lag phase	146.0 ± 38.0	141.0± 36.0	191.0 ± 64.0	219.0 ± 48.0*
	Area under the curve	18.4 ± 6.2	21.9 ± 6.4	14.0 ± 12.2	8.2 ± 5.7*

Results: Ristocetin stimulation induced a 3-fold increase in RCA-1 binding ($p < 0.05$), and reduced WGA binding ($p < 0.05$), ADP and AA had no effect. Interestingly, basal platelet membrane expression of both NEU1 and 2 was observed, which was increased by 5- and 3-fold respectively following ristocetin stimulation ($p < 0.05$). RGDS inhibited NEU1 expression by 40%; however, GPIbα inhibition decreased both NEU1 and NEU2 by 80%. In apheresis platelets, binding to all lectins, without or with ristocetin stimulation was highly variable between donors.
Conclusions: These results show a potential novel role for NEU1 and NEU2 in platelet activation, which is highly dependent on GPIbα-mediated signalling. The significance of the variation in apheresis platelet products requires further investigation.

PB 1287 | Platelet Procoagulant Activity Development and Thrombin Generation in Recalcified ADP-supplemented Platelet-rich Plasma

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Background: Only upon strong CRP- or thrombin-induced stimulation, phosphatidylserine(PS)-positive platelet subpopulation is formed. However, ADP can regulate the number of procoagulant platelets, modulate action of other agonists and potentiate thrombin generation in recalcified platelet-rich plasma.
Aims: The objective of this study was to investigate possible formation of the PS-positive platelets in recalcified plasma following ADP-induced platelet activation and contribution of ADP-stimulation and thrombin generation in this process.
Methods: Whole blood was collected from healthy donors into sodium citrate. We used flow cytometry to investigate the formation of PS-positive and PS-negative platelet subpopulations.
Results: In accordance with previously published data, ADP-induced stimulation of washed platelets caused no formation of PS-positive platelets. The same ADP-induced stimulation of platelets in plasma caused formation of PS-positive platelet subpopulation as detected by annexin V binding. The formation of PS-positive platelets in plasma upon ADP-induced platelet activation was only due to spontaneous thrombin generation, because this effect was completely inhibited by irreversible thrombin inhibitor D-Phe-Pro-Arg-chloromethylketone (PPACK).
Conclusions: Our data indicate that ADP-induced activation of platelets in plasma can lead to formation of PS-positive activated platelets only due to indirectly generated thrombin.

PB 1288 | Role of HSP47 in Platelet Collagen Interaction

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Background: Heat shock protein 47 (HSP47) belongs to the heat shock protein superfamily, which is involved in chaperoning activities within secretory system of cells to maintain protein folding and configuration. HSP47 is unusual in that its chaperoning activities are confined to collagen and procollagen monomers. We reported presence of HSP47 within and on the surface of platelets for the first time in proteomic analysis of platelet peripheral membrane proteins. Through the use of agents that disrupt interactions between HSP47 and collagen we demonstrated its importance in the control of platelet function.
Aims: To understand the mechanism by which HSP47 modulates platelet-collagen adhesion.
Methods: Immunofluorescence and sucrose density gradient subcellular platelet fractionation were used to determine HSP47 location in human platelets. Quantification of HSP47 was achieved using quantitative western blotting. The actin polymerisation inhibitor (latrunculin-A) was used to test the dependency of HSP47 mobilisation on actin polymerisation. Release of HSP47 was tested using immunoblotting and flow cytometry.
Results: The presence of HSP47 in platelets was confirmed in immunofluorescence confocal microscopy where it was found to colocalise with the dense tubular system (DTS) within platelets. Consistent DTS association, HSP47 was also seen in low-density sucrose gradient fractions. HSP47 was expressed at around 10,000 copies per platelet and its mobilisation to the cell surface was shown to be dependent on actin polymerisation following platelet activation. Immunoblotting of platelet releasate confirmed the release of HSP47 from human platelets in a soluble form and on platelet-derived microvesicles.
Conclusions: HSP47, located in the DTS is mobilised to the platelet surface in a manner that is dependent of cytoskeletal reorganisation. Our future priority will be to determine to what is HSP47 binds on platelet surface, and its ability to influence collagen-stimulated cell signalling.

PB 1289 | Assessment of Platelet Procoagulant State - Significance of Different Methods

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Background: Platelets are key players in hemostasis. During primary hemostasis platelets adhere to injured endothelium and to exposed subendothelial matrix proteins. In consecutive steps, coagulation factors are bound to phosphatidylserine rich sites in the platelet membrane thereby facilitating the progression of the clotting cascade. Platelet microvesicles (MV) shedded in the course of activation also play an intensifying and spreading role.

Aims: The objective of our study was to determine platelet coagulant status by use of different methods and to evaluate conditions that could induce the release of MV.

Methods: Platelets were either prepared from citrated whole blood obtained from healthy donors or from pooled platelet concentrates obtained from a blood bank at day 5 of preparation. Flow cytometry was applied to measure binding of fluorescently-labeled Annexin V (AV). Thrombin generation assay (TGA) triggered with tissue factor, and thrombelastometry (TEM) were performed. As exogenous challenge, different shear rates were applied by passage through a flow chamber.

Results: Annexin V binding was found on platelets and MV. Shear forces reduced the AV binding on platelets and increased AV binding in the MV fraction. In TGA the whole platelet samples clearly enhanced thrombin generation. A major part could be attributed to the effect of MV. Application of shear forces was particularly suitable to generate activated states in conjunction with MV release.

Conclusions: TGA provides a sensitive and valuable quantitative method for the determination of the platelet procoagulant status. Complementary data can be obtained by flow cytometry which allows the direct comparison of AV binding to platelets and MP. Together with the application of shear forces a measure of the platelet procoagulant status and of platelet integrity can be acquired.

PB 1290 | Exploration of additional parameters of multiple electrode aggregometry is encouraged

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Background: Platelet function evaluated by multiple electrode aggregometry is increasingly employed. Area under the curve (AUC) of the platelet aggregation signal is at present the only reported parameter in the literature. However, other parameters of platelet aggregation are readily available and may provide further insight on platelet function.

Aims: To provide reference intervals of maximum aggregation amplitude and aggregation velocity in order to facilitate future exploration of these parameters.

Methods: 121 healthy individuals were included. Multiple electrode aggregometry was performed in hirudin whole blood using ADP, arachidonic acid, collagen and ristocetin (RISTOlow and RISTOhigh). Day-to-day variation was investigated, and the associations of the aggregation parameters with platelet count, white and red blood cell counts were explored.

Results: Reference intervals were established for maximum amplitude and aggregation velocity, and will be presented at the congress. Day-to-day variation was ≤11% for both parameters except when using RISTOlow (30 and 18% respectively). In contrast to the AUC and velocity, the maximum amplitude was not associated with the platelet count inside the reference interval in all assays (p-values ≥ 0.4) except RISTOHigh (p=0.001). Similar to the AUC, a positive association with white blood cell count was found using all agonists (p-values ≤ 0.02) except RISTOhigh. No associations with red blood cell count or haematocrit were observed (p-values > 0.08).

Conclusions: Reference intervals for maximum aggregation amplitude and aggregation velocity were established. The day-to-day variation was acceptable. The presented data facilitates future exploration of these parameters that may provide further insight on platelet function and therefore should be encouraged in research settings.

PB 1293 | Nicotine Suppresses the Collagen-induced Platelet Aggregation

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Background: Nicotine is reported to have various physiological activities, but little is known about its effect on platelets.

Aims: We investigate the effect of continuous nicotine loading on hemostatic processes, especially on platelet activation.

Methods: Male ICR mice of 6 months old were administered with nicotine (100 μg/mL) by supplementation in drinking water for 4 weeks. (1) Loading dose was calculated from amount of water intake. (2) Systolic blood pressure and heart rate were measured by tail cuff method. (3) Citrated blood was collected from inferior vena cava, cells number was counted using cell counter. (4) The whole blood sample was centrifuged at 200 *g* for 10 min to obtain platelet-rich plasma (PRP), and platelet poor plasma (PPP) was obtained by further centrifugation (1,500 *g*, 10min). PRP was adjusted to 250,000 platelets per mL with PPP, and stimulated with collagen (4~10 μg/mL) or ADP (1~10 μM) to evaluate platelet aggregation by light transmission (MCM HEMA TRACER 712, MC Medical). (5) Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured using CA-104 (Sysmex).

Results:

(1) Nicotine intake of each mouse was calculated to be 12.2±0.2 mg/kg body weight/day.

(2) Nicotine loading did not affect systolic blood pressure and heart rate,

(3) nor blood cells counting.

(4) Nicotine loading inhibited platelet aggregation induced by collagen (5 μg/mL) from 77.1±3.5% of vehicle control (0.2% saccharin containing d.w.) to 46.4±9.4% (n=12), while ADP-induced one showed no significant suppression.

(5) Nicotine loading did not affect PT and aPTT.

Conclusions: These results indicate that nicotine supplementation suppresses platelet activation, especially when stimulated by collagen. Our interest is focusing toward the intracellular mechanism of nicotine on the platelet activation.

PB 1294 | Platelets Enhance Freshly Isolated Lymphocytes Adhesion to Fibrin

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Background: The processes of primary hemostasis, which are caused by adhesion, aggregation and subsequent retraction of the platelet plug can't be viewed in isolation from coagulation. The damaged part of the vascular wall is always expressed tissue factor, which triggers the extrinsic pathway of coagulation and by PF4 initiated an internal pathway of prothrombinase and fibrin clot formation. In a previous study we demonstrated that CD4+lymphocytes interacted with platelets and formed heterotypic lymphocyte-platelet aggregates (LPA).

Aims: The aim was to determine the role of fibrin in the interaction of lymphocytes and platelets isolated from blood of healthy donors with the adhesive surface in static conditions.

Methods: We used the Millipore filters coated with fibrin. Filters impregnated with Na CL 0,9% served as 1-st control and autologous platelet poor plasma 2-nd control. At the bottom of the wells plastic plate placed filters and added to a previously prepared lymphocyte-platelet suspension was extracted from the blood of 12 healthy individuals on Ficoll gradient. After incubation in static conditions in the wells and subsequent washing, the cell pool resuspended and counted in a light microscope. The numbers of lymphocyte-platelet aggregates remaining in the cell suspension were counted.

Results: We observed that after incubation of platelets and lymphocytes to the filter surface with fibrin their number in the suspension was reduced to nearly 2.5 times as compared with filters impregnated NaCL 0,9% or platelet poor plasma. This reduces the average number of platelets per one lymphocyte and total lymphocyte count ($p < 0.05$). Reduction of the number of LPA in a suspension showed the ability of lymphocytes in aggregates with platelets adheres to surface coated by fibrin.

Conclusions: Thus, lymphocytes are able to contact with fibrin, while they forming coagregates with platelets. Moreover, we proved a leading role of fibrin, but not blood plasma in this process.

PB 1295 | Synergistic Inhibitory Effect of Capsaicin and Dihydrocapsaicin on the Arachidonic Acid Pathway of Platelet Aggregation

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Background: Capsaicinoids, including capsaicin (CAP) and dihydrocapsaicin (DHC), the pungent principles of chilli peppers, individually inhibit *in vitro* platelet aggregation. However, their effects when present in the relative proportions that they are found in different fruits, i.e., 60:40 CAP:DHC, are not known.

Aims: To determine the effects of CAP and DHC, alone and together, on platelet aggregation, platelet count, and thromboxane B2 (TXB2) formation.

Methods: The individual (12.5 μM) and combined (CAP+DHC, 3.75+2.5, 7.5+5 μM) effects of CAP and DHC were determined on arachidonic acid (AA; 300μg/mL), ADP (5 μM), and collagen (4μg/mL) induced aggregation (AggRAM aggregometer; %AUC normalised to aggregation in the absence of capsaicinoid, mean±SEM), platelet count (Sysmex 1000i analyser) and TXB2 release (ELISA) (n=4 healthy donors). Data were analysed using ANOVA/linear regression.

Results: Compared to vehicle, CAP and DHC (12.5 μM) each inhibited AA-induced aggregation (by 23.2 and 25.3%, respectively; both $p< 0.01$). In combination, CAP+DHC inhibited AA-induced aggregation (CAP+DHC, 3.75+2.5 μM by 36.5%, $p=0.01$; CAP+DHC, 7.5+5 μM by 57.5%, $p< 0.001$), compared to vehicle (Fig 1). In contrast to AA-induced aggregation, neither CAP nor DHC individually, or in combination, significantly inhibited ADP- or collagen-induced aggregation. Incubation of platelets with CAP or DHC (12.5 μM), and in combination, for up to 2 hours did not significantly affect the platelet count. The 60:40 CAP+DHC (7.5+5 μM) combination significantly inhibited TXB2 formation ($p< 0.001$), compared to the individual capsaicinoids.

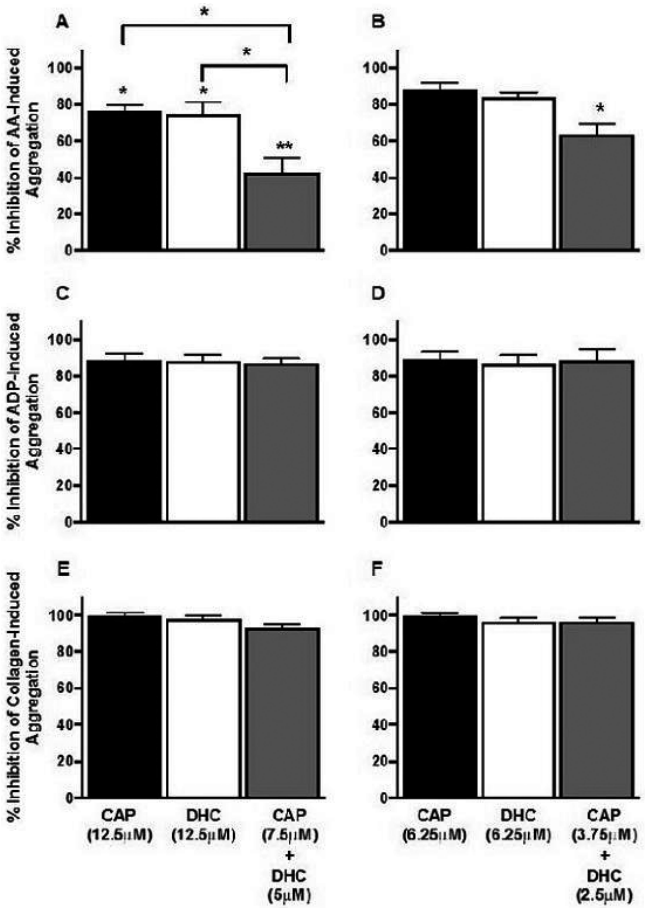

FIGURE 1 Effect of CAP, DHC, and their combination, on AA- (A & B), ADP- (C & D) and collagen- (E &F) induced aggregation. * $p<0.05$, ** $p<0.001$

Conclusions: The combination of CAP+DHC in the proportions they are present in chilli peppers, produces a significantly greater inhibitory effect against AA-induced platelet aggregation and TXB2 formation, compared to the individual capsaicinoids. Further investigations are warranted to determine whether these capsaicinoids may be exploited for therapeutic benefit by dampening platelet activity via the AA-pathway.

PB 1296 | Crosstalk between PI3K Pathway and MAPK Pathway Regulates Platelet Activation and Protein Synthesis via Phosphoinositide-dependent Kinase-1

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Background: Phosphoinositide-dependent protein kinase 1 (PDK1) is known to regulate PAR4 induced platelet activation and thrombus formation through GSK3b. However, whether PDK1 signaling also involves the ADP receptor and, if so, downstream functional consequences are unknown.

Aims: To evaluate the role of PDK1 in ADP-induced platelet activation and protein synthesis.

Methods: We employed both pharmacologic (e.g. the selective PDK1 inhibitor, BX795) and genetic (platelet specific deletion of PDK1) approaches to dissect the role of PDK1 in ADP-induced platelet activation and protein synthesis.

Results: Inhibition of PDK1 with BX795 reduced 2MeSADP-induced platelet aggregation by abolishing thromboxane generation. Similar results were observed in PDK1-/- mice (Fig A). Inhibition of PDK1 protected mice from collagen and epinephrine-induced pulmonary embolism (Fig B). PDK1 was also necessary for the phosphorylation of MEK1/2, Erk1/2 and cPLA2, indicating that PDK1 regulates an upstream kinase in MAPK pathway. We next identified that this upstream kinase is Raf1 (necessary for the phosphorylation of MEK1/2), as pharmacologic inhibition and genetic ablation of PDK1 was sufficient to prevent Raf1 phosphorylation (Fig C). Pharmacologic inhibition and genetic ablation of PDK1 blocked MAPK- and mTORC1-dependent protein synthesis in platelets through a mechanism requiring the phosphorylation of S6K and eIF4E. Concordantly, PDK1 is necessary for signal-dependent synthesis of the protein bcl3, which is under mTORC1-dependent control (Fig C).

Conclusions: Taken together, our findings show for the first time that PDK1, a master kinase in the PI3K pathway, directly governs thromboxane generation, thrombosis, and protein synthesis in platelets through regulating MAPK and mTORC1 pathways.

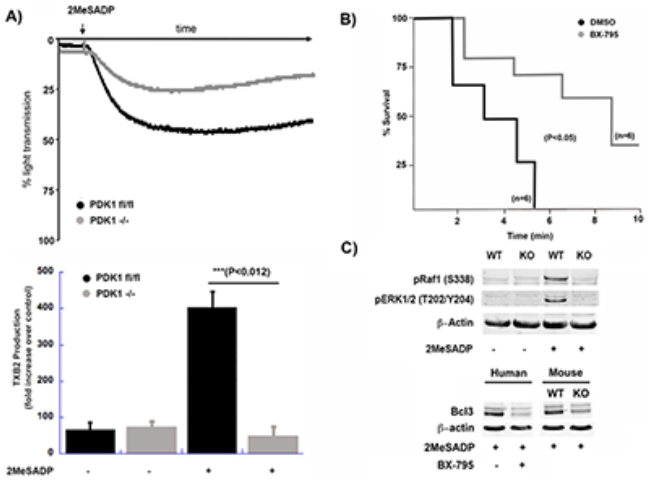

FIGURE 1 PDK1 regulates platelet aggregation, TxB2 generation, and thrombosis through the MAPK kinase pathway

PB 1298 | Diacylglycerol Kinase Zeta is a Negative Regulator for GPVI-Mediated Platelet Activation

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Background: Diacylglycerol kinases (DGK) are a family of enzymes that catalyze the conversion of diacylglycerol (DAG) into phosphatidic acid (PA). Removal of DAG, a potent activator of protein kinase C, from the metabolic pool has the effect of dampening platelet activation. Human platelets contain mRNA transcripts encoding many of the isoforms of DGK, and an inhibitor of DGKα, β and γ, R59949, has been shown to amplify platelet activation induced by collagen, but not thrombin. These data support a role for DGKs is suppressing platelet activation, however the specific identity of the DGK isoforms that function in platelets is unknown.

Aims: The aim of this study is to determine which DGK isoforms play a role in platelet production and function.

Methods: Platelet count and function from mice lacking the ζ isoform of DGK was determined using standard biochemical and cell biological techniques.

Results: DGKζ-KO mice are born in normal Mendelian ratio, and show normal platelet counts and volume. Flow cytometric analysis revealed a 50% increase in surface expression of GPVI, whereas expression of integrins α2β1 and αIIbβ3 were normal. Platelet activation responses to the GPVI agonists, collagen and collagen-related peptide (CRP) was potentiated in DGKζ-/- platelets; and the onset of platelet activation was earlier. Consistent with this, signaling events downstream of GPVI activation, including phosphorylation of PLCγ2 and ERK, was significantly increased in DGKζ-/- platelets. Interestingly, in contrast to GPVI-mediated platelet activation, thrombin-mediated activation was reduced.

Conclusions: Taken together these data support the notion DGKζ is a selective negative regulator of GPVI-, but not thrombin-, mediated platelet activation. Pharmacologic suppression or genetic deletion of this DGK isozyme might prove useful in producing platelets that exhibit improved hemostatic effectiveness without inducing overt pathological thrombosis.

PB 1299 | ELMO1 Negatively Regulates Glycoprotein VI-mediated Signaling by RhoG in Platelets

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Background: PI3-kinase is an important signaling molecule activated upon platelet activation leading to generation of Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) in turn recruiting pleckstrin homology (PH) domain containing proteins to the membrane. We screened for proteins that interacted with PIP_3 and among these proteins we found engulfment and cell motility-1 (ELMO1). ELMO1 is a scaffold protein with no catalytic activity and is well known to regulate actin cytoskeleton involved in cell motility and cell spreading in nucleated cells. ELMO1 is expressed in platelets and interacts with active RhoG however the function of ELMO1 is not known.

Aims: The focus of this study is to determine the function of ELMO1 in platelets.

Methods: We utilized $ELMO1^{-/-}$ mice to characterize the function of ELMO1 in platelets using *ex vivo* and *in vivo* methods.

Results: Platelet aggregation and dense granule secretion was enhanced in $ELMO1^{-/-}$ platelets in response to GPVI agonists, CRP (Figure 1A & B) and collagen, but unaltered using PAR4 agonist (AYPGKF). Surface expression of GPVI and Glycoprotein IIb/IIIa (GPIIb/IIIa) were normal, suggesting that ELMO1 plays a specific role downstream of GPVI. $ELMO1^{-/-}$ platelets exhibited enhanced clot retraction and spreading indicating its role in GPIIb/IIIa-mediated outside-in signaling. Whole blood from $ELMO1^{-/-}$ mice perfused over collagen under arterial shear conditions exhibited enhanced thrombus formation. $ELMO1^{-/-}$ mice showed reduced survival compared to control following pulmonary embolism. $ELMO1^{-/-}$ mice also exhibited shorter time to occlusion using the ferric-chloride injury model and shorter bleeding times compared to control. This indicates that ELMO1 plays an important role in hemostasis and thrombus formation *in vivo*. At the molecular level, RhoG activity was enhanced within 1 minute in $ELMO1^{-/-}$ murine platelets (Figure 1C) compared to control in response to CRP.

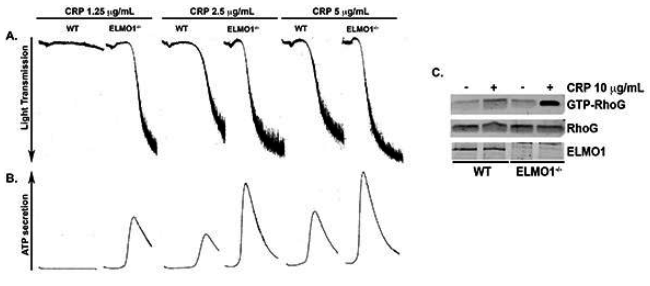

FIGURE 1 Representative figure of (A) platelet aggregation, (B) dense granule secretion, and (C) active RhoG pull-down by GST-ELMO1 from platelets.

Conclusions: ELMO1 negatively regulates GPVI-mediated thrombus formation possibly by RhoG.

PB 1300 | All Roads Lead to Filamin A: Ligand Binding and Shear Force Signal to Calpain-mediated Cleavage of Filamin A upon Platelet Activation

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Background: Responding to mechanical stimuli is a critical function of living cells. Filamin A (FlnA) is a 280 kD highly conserved protein. It has been implicated in cytoskeletal integrity and mechanotransduction. As an essential scaffolding protein that physically links membrane-bound glycoproteins (GP) to the actin cytoskeleton, it has been implicated in platelet aggregation under conditions of shear as both integrin alphaIIbbeta3 and GPIb-V-IX receptors have been shown to interact with FlnA through its C-terminal region.Nonetheless, many aspects of FlnA's signaling mechanisms and functions remain unknown.

Aims: Study signaling mechanism leading to Filamin A cleavage.

Methods: Use of human platelets and monoclonal antibodies to GPIb (6D1), and alpha2beta1 (6F1), as well as the small molecule inhibitor of alphaIIbbeta3 RUC-4.

Results: When platelets are activated by thrombin-receptor activating peptide (T6), ADP, or convulxin (Cnx), FlnA is cleaved downstream of ligand binding to integrin alphaIIbbeta3 in a PKC dependent fashion. On the other hand, inducing von Willebrand factor binding to GpIb with ristocetin or collagen binding to integrin alpha2beta1 results in FlnA cleavage without a PKC requirement. Cleavage generates a 180 kD N-terminal fragment, while the 100 kD C-terminal is further cleaved into 90 and 10kD fragments. We also demonstrate that FlnA cleavage requires not only receptor activation but ligand binding since RUC-4 inhibits cleavage in platelets treated with the alphaIIbbeta3 activating antibody PT25 or DTT in the presence of fibrinogen. Furthermore, platelet activation and Ca^{++} mobilization *per se* are not sufficient to induce FlnA cleavage as treating platelets with PMA in combination with RUC-4 inhibits it. Finally, we demonstrate that FlnA cleavage occurs only under aggregating conditions since stirring is required in combination with activation by T6, ADP, collagen, Cnx or ristocetin.

Conclusions: FlnA cleavage is an essential outcome of ligand binding and platelet aggregation under shear force.

PB 1301 | Kindlin-3 identified as a key negative regulator of the small Rho GTPase, Cdc42 during platelet spreading

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Background: Integrin bidirectional signaling allows the fine regulation of platelet functions in hemostasis and thrombosis. Upon stimulation, integrin ligation and clustering trigger outside-in signaling, which is critical for platelet spreading and retraction. These processes depend on Rho family small GTPases (RhoA, Cdc42 and Rac1) that regulate actin-binding proteins and actin dynamics. Kindlin-3 (K3) is described as an essential regulator of platelet integrin inside-out signaling and recent studies suggest roles for kindlins in outside-in signaling. Previously, we identified a patient with a complete K3 deficiency suffering LAD-III, characterized by strong defect in platelet inside-out $\alpha_{IIb}\beta_3$ signaling.
Aims: Taking advantage of the K3 deficiency in platelets of the LAD-III patient, we studied its role in $\alpha_{IIb}\beta_3$ integrin outside-in signaling and in cytoskeleton changes during spreading.
Methods: Platelet adhesion and spreading, confocal and reflection interference contrast fluorescence microscopy, Cdc42 activation assay.
Results: We found that K3-deficient platelets normally elongate filipodia and perform early steps of spreading but cannot extend lamellipodia and form stress fibers. Platelet treatment with a combination of ADP and Mn^{2+} restores soluble fibrinogen binding and partial aggregation. However, impairment of spreading and clot retraction persists. Conversely to healthy platelets, K3-deficient ones are stuck at the level of actin nodule formation. These nodules are abnormal since they lack $\alpha_{IIb}\beta_3$ integrin, Cdc42 and Rac1 clusters and show impaired protein phosphorylation (Ser-Thr and Tyr). Interestingly, increased and prolonged Cdc42 GTP-binding is noticed in K3-deficient platelets and full lamellipodia extension is restored by CASIN, a selective Cdc42 inhibitor.
Conclusions: This study points out K3 as key negative regulator of Cdc42 activity and actin cytoskeleton organization during $\alpha_{IIb}\beta_3$ integrin outside-in signaling in human platelets.

PB 1302 | RGS-insensitive G Proteins as a Model to Study G Protein-dependent Signaling

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Background: We first reported that the duration of G protein signaling in platelets is limited by RGS proteins. A mutation (G184S) in G_{i2} renders the α subunit resistant to accelerated turn-off by all RGS proteins, and therefore confers a gain of function for platelet activation. Using the alternative gene knockout approach, knocking out either RGS10 or RGS18 in mice results in a gain of platelet function.
Aims: The gain of function in RGS-insensitive G_{i2}(G184S) appears to be confined to G_{i2} signaling in platelets. Here we examine for the first time RGS-insensitive G_q as an in vivo probe of G protein regulation.
Methods: Using CRISPR-Cas9 genome-editing, we have recently generated a transgenic mouse line in which a substitution (G188S) in G_q subunit renders it resistant to accelerated turn-off by all RGS proteins. Platelet function was examined by flow cytometry.
Results: G_q(+/G188S) mice are grossly normal in appearance and have a normal platelet count. Western blot analysis show that there is no difference in G_q expression levels among G_q(G188S/G188S), G_q(+/G188S) and WT control. Flow cytometry using Jon/A to detect $\alpha_{IIb}\beta_3$ activation show that the dose/response curve for the PAR4 agonist peptide, AYPGKF, was shifted to higher concentrations in the G_q(+/G188S) platelets. The platelet response to U46619 or ADP was also impaired in the G_q(+/G188S) platelets. In contrast, responses to collagen-related peptide, which is not a G protein coupled receptor agonist, were unaffected. Similarly, α-granule secretion in the G_q(+/G188S) platelets was attenuated in response to AYPGKF, but not collagen.
Conclusions: In contrast to enhanced G_{i2} signaling in G_{i2}(G184S) mutant platelets, we observed decreased platelet activation in G_q(+/G188S) mutant platelets, indicating that regulation of G_q signaling is more complex than that of G_i signaling. This new G_q(G188S) mutant mouse may provide novel insight into how G_q signaling is regulated differently from G_i signaling.

PB 1303 | Arp2/3 is Critical for Platelet Homeostasis but Dispensable for Hemostatic Plug Formation

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Background: Actin reorganization regulates key processes in platelet activation including shape change, spreading, aggregation and granule secretion. The contribution of actin filament branching to platelet biology, beyond spreading, is poorly understood.
Aims: Here we examined the role of the Actin Related Protein 2/3 complex (Arp2/3), an essential component in actin branching, in platelet function and homeostasis.

Methods: Deletion of the Arp2/3 complex in mice results in early embryonic lethality; therefore we employed the Cre/Lox gene targeting strategy to generate mice with a megakaryocyte/platelet-specific deletion of the *Arpc2* gene, which encodes the p34 subunit of the Arp2/3 complex (*Arpc2*$^{fl/fl}$*PF4-Cre*). Loss of p34 subunit expression results in disruption of the entire Arp2/3 complex.

Results: Deletion of the Arp2/3 complex resulted marked thrombocytopenia (~60% reduction in the peripheral platelet count) and a significant decrease in platelet size, reminiscent of the micro-thrombocytopenia documented for Wiskott-Aldrich syndrome patients. Microthrombocytopenia in *Arpc2*$^{fl/fl}$*PF4-Cre* mice was a consequence of premature platelet release into the bone marrow compartment and impaired platelet survival in circulation. *Arpc2*$^{fl/fl}$*PF4-Cre* platelets exhibited alterations in their actin cytoskeleton and their peripheral microtubule coil, resulting in altered morphology of resting platelets. Thrombocytopenia was alleviated following clodronate liposome-induced macrophage depletion in *Arpc2*$^{fl/fl}$*PF4-Cre* mice. *Arpc2*$^{fl/fl}$*PF4-Cre* platelets failed to spread on a fibrinogen matrix and showed a mild defect in integrin activation, alpha-granule secretion and aggregation. However, no significant differences in hemostasis or thrombosis were observed between *Arpc2*$^{fl/fl}$*PF4-Cre* and control mice.

Conclusions: Our studies support a critical role for Arp2/3 in platelet homeostasis but, unexpectedly, not vascular hemostasis or thrombosis.

PB 1304 | Cold-induced Binding of von Willebrand Factor Facilitates Fast Clearance of Refrigerated Platelets

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Background: Refrigerated platelets are rapidly cleared after transfusion, but the underlying mechanism is not entirely clear. Enzymatic removal of the N-terminal domain of GPIbα on refrigerated murine platelets abrogated fast removal of these platelets mediated by Ashwell-Morell receptors, but the N-terminal domain of murine GPIbα contains no N-glycosylation sequence (Asn-X-Ser/Thr). Recently we demonstrated that botrocetin-mediated binding of VWF to GPIbα under shear induces unfolding of the mechanosensory domain (MSD) in GPIbα, leading to platelet clearance. It was reported previously that VWF binding to platelets was increased after refrigeration.

Aims: To investigate the role of VWF in the fast clearance of refrigerated platelets.

Methods: Freshly obtained murine and human platelet-rich plasmas were stored in gas-permeable bags at 4°C for 24 hours. VWF binding, MSD unfolding, GPIb-IX signaling events, and post-transfusion recovery and survival of platelets were measured by flow cytometry as previously reported.

Results: After cold storage binding of VWF to both human and murine platelets was significantly increased. Uniform shear treatment of cold-stored murine and human platelets resulted in increased MSD unfolding, β-galactose exposure and phosphatidylserine exposure on the platelet. Furthermore, post-transfusion recovery and survival of cold-stored VWF$^{-/-}$ platelets was significantly increased than that of cold-stored WT platelets.

Conclusions: These results suggest that cold-induced VWF binding to GPIbα critically induces GPIb-IX signaling and fast clearance of cool-stored platelets. Inhibition of the VWF/GPIbα interaction during cold storage may potentially enable cold storage of platelets.

PB 1305 | RGS Proteins Shape the Hemostatic Response by Regulating the Platelet Signaling Networks

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Background: Regulator of G protein Signaling (RGS) proteins limit the duration of G protein signaling by accelerating the hydrolysis of GTP on Gα. Platelets express RGS10 and RGS18. In resting platelets, RGS proteins are sequestered by scaffold protein, spinophilin. RGS18 deletion in platelets leads to gain of function.

Aims: To define the role of RGS10 in the hemostatic response to injury.

Methods: RGS10$^{-/-}$ mice were generated and evaluated for platelet function *in vitro* and *in vivo*. Free RGS10 levels in platelets were measured using novel assay.

Results: An increase in free RGS10 levels was observed in platelets upon activation by thrombin or rendered resistant to activation by PGI_2. Integrin $a_{IIb}b_3$ activation and α-granule secretion in the absence of secondary wave mediators revealed a marked gain of function by RGS10$^{-/-}$ platelets in response to G protein coupled receptors (GPCR) signaling agonists: PAR4 activating peptide, ADP and TxA_2 (U46619). Notably, while the dose/response curve for the PAR4 agonist was shifted to lower concentrations in the RGS10$^{-/-}$ platelets with no change in the maximum response, the dose/response curve for U46619 and ADP showed a substantial increase in maximal response. Intracellular calcium mobilization was also enhanced in response to GPCR agonists. Hemostatic thrombi formed in the RGS10$^{-/-}$ mice revealed a smaller core of fully activated platelets and a larger shell of partially activated loosely packed platelets. The decreased platelet packing density was confirmed in intra thrombus transport studies using fluorescent albumin. In line with augmented GPCR signaling, phosphorylation of serine/threonine kinase Akt and myosin light chain kinase was also enhanced in RGS10$^{-/-}$ platelets.

Conclusions: RGS10 is key regulator by limiting platelet activation downstream of GPCR signaling. RGS10 affects response sensitivity

and amplitude and is agonist selective. RGS10 regulates both Gi- and Gq-dependent signaling in platelets and, appears to have a role that is independent of RGS18.

PB 1306 | A Spatial Systems Approach to Identifying Roles for the Rho-specific Guanine Nucleotide Dissociation Inhibitor (RhoGDI) Ly-GDI in Platelet Function

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Background: Platelets undergo specific morphological alterations critical to hemostasis via actin cytoskeletal reorganizations driven by the Rho GTPases Rac1, Cdc42 and RhoA. While these Rho GTPases are known to be critically sequestered and regulated by Rho-specific guanine nucleotide dissociation inhibitor proteins (RhoGDIs) in other cell types, roles for RhoGDIs in platelet activation and hemostatic function remain uncharacterized.

Aims: Here we aim to determine how and whether RhoGDI proteins regulate Rho GTPase-driven platelet functions downstream of platelet integrin and glycoprotein receptors.

Methods: Through an approach combining pharmacology, cell biology and systems biology tools, we investigate roles for RhoGDI proteins in platelet function.

Results: We find that platelets express two RhoGDI family members, RhoGDI and Ly-GDI. Functional interference and platelet spreading experiments suggest a specific role for Ly-GDI in platelet function. Intracellular staining assays demonstrate that Ly-GDI displays an asymmetric, polarized pattern that largely colocalizes with Rac1 and Cdc42 as well as microtubules and protein kinase C (PKC) in platelets adherent to fibrinogen. Signaling studies rooted in quantitative phosphoproteomics and pathways analyses also support a regulatory role for Ly-GDI in platelets, as Ly-GDI is phosphorylated at PKC substrate motifs in a PKC-dependent manner in response to the platelet collagen receptor glycoprotein (GP)VI-specific agonist collagen-related peptide. Notably, inhibition of PKC diffuses the polarized organization of Ly-GDI in spread platelets relative to its colocalization with Rac1 and Cdc42.

Conclusions: Together, our results support roles for Ly-GDI as a localized regulator of Rho GTPases in platelets and link PKC and Rho GTPase signaling systems in the orchestration of the platelet activation program.

PB 1307 | RhoGAP6 and RhoGEF2 Are Novel Regulators of RhoA in Platelets

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Background: RhoA, a Rho family small GTPase, is the bridge between extracellular signals and cytoskeletal restructuring of platelets during activation. Vascular endothelium releases prostacyclin (PGI_2) and nitric oxide (NO) leading to platelet inhibition. PGI_2 and NO upregulate cAMP and cGMP which activate cAMP/cGMP-dependent protein kinases (PKA and PKG). PKA and PKG then phosphorylate unknown substrates causing RhoA inhibition and restricting platelet function.

Aims: To identify Rho GTPase activating proteins (RhoGAPs - terminate RhoA signalling) and Rho guanine nucleotide exchange factors (RhoGEFs - activate RhoA) as substrates of PKA and PKG that convert PGI_2/NO signals into RhoA inhibition.

Methods: Platelet phosphoproteome screening (Beck et al Blood 2014 and 2017) presented RhoGAP6 and RhoGEF2 as candidate substrates for PKA and PKG. Protein analysis includes quantitative mass spectrometry, mutagenesis studies, pulldown assays, PhosTag and SDS-PAGE/Western blotting in human platelets and transfected cells.

Results: We show that RhoGAP6 and RhoGEF2 proteins are expressed in human platelets and regulate RhoA activity. Both proteins are phosphorylated by PKA and PKG on serine/threonine residues. We give evidence of an interaction of RhoGAP6 with the phosphoserine/threonine binding protein 14-3-3. RhoGEF2 phosphorylation on serine 886 stimulates binding of RhoGEF2 to 14-3-3. Further interaction partners of RhoGAP6 and RhoGEF2 are being investigated while RhoGAP6 and RhoGEF2 protein complex regulation by PKA and PKG is being characterized.

Conclusions: This data shows two new RhoA regulatory proteins in platelets, RhoGAP6 and RhoGEF2 that are novel targets for PKA and PKG phosphorylation. Investigation of the implications of phosphorylation of these two proteins will provide a better understanding of endogenous platelet regulation by PGI_2 and NO.

PB 1308 | A Role of TNF Receptor-associated Factor 3 in Platelets A and Thrombosis

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Background: Platelets express CD40 and its ligand (CD40L) and are a major source of soluble CD40L. CD40L has been shown to potentiate platelet activation and thrombus formation, involving both

CD40-dependent and -independent mechanism. CD40L-mediated platelet activation requires the family of member of TNF receptor-associated factor 2 (TRAF2). However, the mechanism of platelet activation by CD40L has not been fully understood.

Aims: Identifying the expression and function of a novel member of the TNF receptor-associated factor family, the TRAF3, in platelets.

Methods: Western blot was used to verify the expression of TRAF3 in platelets. The function of TRAF3 in platelets was evaluated by various in vitro platelet function assays, tail-bleeding time assay, and FeCl3-induced carotid artery thrombosis assay.

Results: Here we show that platelet also express TRAF3, which plays a negative role in regulating platelet activation. Thrombin- or collagen-induced platelet aggregation and secretion are increased in TRAF3 knockout mice. The expression of integrin aIIbb3, GPVI, and PAR4 was similar between TRAF3 knockout platelets and wild type controls, suggesting that increased platelet activation in the TRAF3 knockout mice is not due to increased expression of platelet receptors. TRAF2 expression was not affect by deletion of TRAF3 either. Using the $FeCl_3$-induced arterial thrombosis model, we found that time to formation of thrombi in was significantly shortened in TRAF3 knockout mice.

Conclusions: TRAF3 plays a negative role in platelet activation and in thrombus formation in vivo.

PB 1309 | Annexin A7 (ANX7) Regulates Collagen-dependent Platelet Ca2+ Increase and Activation

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Background: Activation of platelets by subendothelial collagen results in an increase of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and is followed by platelet secretion, aggregation and thrombus formation with consecutive vascular occlusion.

Aims: This study aimed to determine the role of ANX7 in collagen-dependent platelet Ca^{2+} signalling and function in platelets from ANX7 knockout (*anx7*$^{-/-}$) mice.

Methods: Fura-2-AM spectrofluorometric Ca^{2+} measurements, *in vitro* flow chamber approaches, light transmission aggregometry and PGE_2 ELISA were performed using *anx7*$^{-/-}$ and *anx7*$^{+/+}$ platelets.

Results: Stimulation of the collagen receptor glycoprotein VI (GPVI) by collagen or collagen-related peptide (CRP) leads to platelet activation due to increased intracellular calcium concentration ($[Ca^{2+}]_i$). Fura-2-AM spectrofluoriometric Ca^{2+} measurements revealed that ANX7 deficiency strongly blunted Ca^{2+} mobilisation from intracellular stores as well as extracellular Ca^{2+} influx following activation with CRP or collagen. As platelet activation is linked with integrin $\alpha_{IIb}\beta_3$ activation and subsequent aggregation, light transmission aggregometry was performed uncovering a significant impaired platelet aggregation in *anx7*$^{-/-}$ platelets compared to *anx7*$^{+/+}$ platelets after stimulation with CRP or collagen. Furthermore, platelet dense granule secretion (reflected by ATP release) in response to CRP or collagen as well as *in vitro* thrombus formation under high arterial shear rates (1700 s^{-1}) on collagen-coated surfaces were significantly diminished in *anx7*$^{-/-}$ platelets as compared to *anx7*$^{+/+}$ platelets. In addition, *anx7*$^{-/-}$ platelets exhibit increased PGE_2 levels under resting conditions as well as upon activation with CRP and collagen.

Conclusions: In conclusion, ANX7 plays an important role upon GPVI-triggered platelet activation and thrombus formation due to impaired activation-dependent increase of $[Ca^{2+}]_i$. Impaired Ca^{2+}-dependent platelet activation results, at least in part, from increased PGE_2 levels in *anx7*$^{-/-}$ platelets.

PB 1310 | Four-receptor Platelet Signaling Computational Model Reveals a New Role of Protein Kinase A in the Control of Platelet Response

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Background: Under physiological conditions, platelets are stimulated by several agonists simultaneously. Moreover, receptor network can be important even when not all of them are occupied by ligands.

Aims: We developed a computational systems biology model of platelet signaling encompassing receptors PAR1, PAR4, $P2Y_{12}$, $P2Y_1$ and pathways to integrin activation and procoagulant activity in order to investigate the interplay of G_q- and G_i-dependent signaling.

Methods: Kinetics of fibrinogen and annexin V binding to Fura Red-loaded platelets were investigated by continuous flow cytometry, confocal and total internal reflection fluorescence microscopy. The model comprised set of ordinary differential equations integrated in COPASI software (www.copasi.org).

Results: The model included about one hundred reactions governing signal transduction after ligation of G-protein coupled receptors and leading to cytosolic calcium increase and protein kinase A (PKA) inactivation. PKA and calcium then influenced CalDAGGEFI activation and subsequent Rap1b-GTP formation and integrin activation.

The validation of the model was conducted using experiments on cytosolic calcium, fibrinogen and annexin V binding dynamics after platelet stimulation with a combination of ADP and thrombin or PAR-activating peptides. The model predicted that ADP would increase the frequency of calcium oscillations, the levels of fibrinogen binding and procoagulant activity, and $P2Y_{12}$ antagonists would decrease them even when platelets are stimulated via PAR1 only. The predictions were confirmed experimentally.

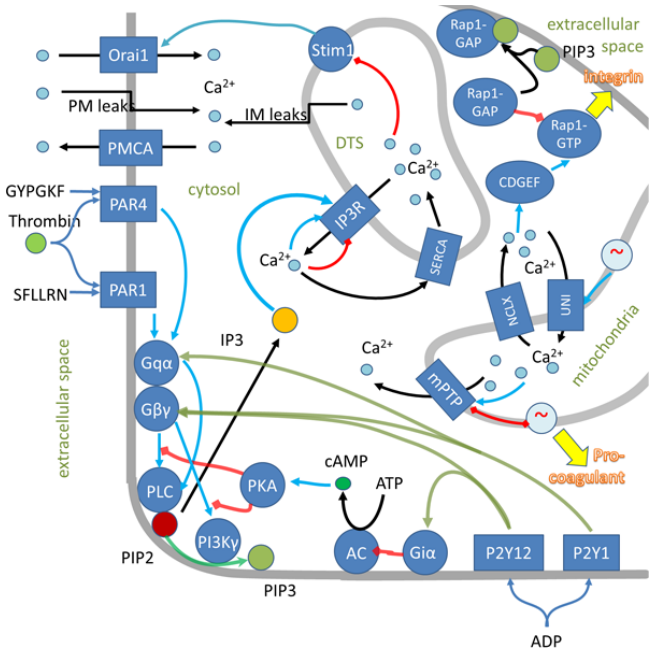

FIGURE 1 Scheme of the 4-receptor platelet signaling model]

Conclusions: Here we presented the first comprehensive model describing platelet intracellular signaling after simultaneous activation by ADP and thrombin. PKA is a crucial controlling element in platelet response even when stimulating signal comes though PAR receptors and does not contain ADP.

PB 1311 | Disabled-2 Controls Platelet Activation Through a Regulatory Circuit Involving Multiple Interacting Proteins

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Background: Platelet Disabled-2 (Dab2) is a key haemostatic regulator involving in thrombin signaling, integrin activation, and fibrinogen uptake. The underlying mechanism of Dab2 in platelet function has not yet been completely elucidated.

Aims: Dab2-interacting proteins (Dab2-IPs) in the resting and thrombin-stimulated human platelets were unveiled with an aim to elucidate Dab2 function in human platelet activation.

Methods: Dab2 was immunoprecipitated from the resting and thrombin-stimulated platelet lysates followed by differential labeling of $^{12}C_2{}^{1}H_6$ and $^{13}C_2{}^{1}H_2{}^{2}D_4$, respectively. A liquid chromatography system coupled with an Orbitrap Elite hybrid mass spectrometry was used for identification of Dab2-IPs. The biological processes regulated by Dab2-IPs were defined by GO enrichment analysis.

Results: There were a total of 10 Dab2-IPs exclusively present in the resting but not in thrombin-stimulated platelets. Additional 32 Dab2-IPs were present in both resting and thrombin-stimulated platelets. Among them, Dab2 had an increased interaction with one Dab2-IPs, a decreased interaction with 16 Dab2-IPs, and no alteration for the interaction with 15 Dab2-IPs when platelets were stimulated with thrombin. GO enrichment analysis of the 32 Dab2-IPs revealed the top 3 GO terms were vesicle mediated transport (n = 18), endocytosis (n = 13) and complement activation (n = 8). Notably, thrombin stimulation resulted in a decrease in Dab2-clathrin interaction which was known to regulate fibrinogen uptake. These findings were confirmed by immunoprecipitation of Dab2 followed by Western blotting of clathrin and consistent with the notion that Dab2 acts as a clathrin sponge and sequesters clathrin from its interaction with membrane receptor.

Conclusions: Dab2 controls platelet activation through a regulatory circuit involving multiple Dab2-IPs. Thrombin-stimulated dissociation of Dab2-clathrin protein complex provides a new function of Dab2 in the regulation of human platelet activation.

PB 1312 | Sustained Inhibition of Acetyl-CoA Carboxylase Decreases Platelet Dense Granules Secretion and Aggregation

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Background: Acetyl-CoA carboxylase (ACC) regulates fatty acids synthesis and oxidation. Our results show that ACC is phosphorylated in platelets of patients with coronary artery disease. ACC phosphorylation results in its inhibition. Given the primary roles of lipids in platelets structure, metabolism and signaling, we hypothesize that a sustained inhibition of ACC could affect platelets bioenergetics and functions.

Aims: To investigate the effect of a sustained ACC inhibition on platelets functions, signaling and metabolism.

Methods: Platelets were treated with TOFA, an ACC inhibitor, for 2 hours before thrombin stimulation. We measured lipogenesis via ^{14}C-acetate incorporation into fatty acids. Platelet functions were assessed by aggregometry and flow cytometry. Platelet signaling was investigated via western blot analysis, and metabolism, via the measurement of oxygen consumption rate.

Results: We show that TOFA significantly decreased lipogenesis. This reduction was associated with an inhibition of thrombin-induced dense granules secretion and platelet aggregation whereas αIIbβ3 activation and α-granules secretion were not affected, suggesting that the default in aggregation resulted from a lower autocrine effect of ADP. Mechanisms involved in the modulation of dense granules secretion implied PKC, in particular PKC-δ, and their

substrates, cytohesin-2 and protein kinase D (PKD). Platelet metabolism was also affected by a sustained ACC inhibition, as shown by the TOFA-induced decrease in reserve capacity and ATP-linked respiration.

Conclusions: A sustained inhibition of platelet ACC decreases lipogenesis and affects dense granule secretion and aggregation through (i) a PKC/PKC-δ dependent mechanism, and (ii) an alteration of platelets bioenergetics. We believe that it could affect thrombus stability in atherosclerotic patients.

PB 1313 | The G12/13 Signaling Pathway Contributes to the Racial Difference in PAR4 Signaling

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Background: Atherothrombotic disorders disproportionally burden blacks relative to whites. One of the independent risk factors for atherothrombosis is enhanced platelet reactivity. Compared to platelets from white donors, platelets from black donors were hyper-responsive to activation of the thrombin receptor Protease-Activated Receptor-4 (PAR4), which was at least partially due to an increase in Gαq signaling. It remains unknown if the difference in PAR4 signaling by race was due solely to differences in Gαq signaling or whether Gα12/13 also contributed to the racial difference in PAR4-mediated platelet activation.

Aims: Determine if the activation of both signaling pathways known to be regulated by PAR4, Gαq and Gα12/13, are enhanced in platelets from blacks compared to whites.

Methods: To determine if there was a racial difference in the Gα12/13 pathway in PAR4-stimulated platelets, RhoA activation, actin polymerization, shape change, and spreading were measured in washed human platelets from white and black donors. Moreover, PAR4-mediated Gq and G12/13 protein activation was directly measured in prepared membranes expressing recombinant human PAR4 with mutations known to be differentially expressed by race.

Results: PAR4 receptor with the Thr120 variant, a variant more commonly expressed in black donors, enhanced the activation kinetics of Gq and G12/13 compared to the Ala120 (white) variant. Similarly, RhoA activation, shape change, and spreading were enhanced in PAR4 stimulated platelets from blacks compared to whites.

Conclusions: The increased signaling through PAR4 observed by race, which is controlled in part by genotype differences, was not due to biased signaling toward Gaq. Rather an overall increased activity of the receptor leads to increased kinetic activation of both the Gαq and Gα12/13 signaling pathways in platelets.

PB 1314 | Changes in Gene Expression Levels in Immune Thrombocytopenia Patients Treated with Eltrombopag

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Background: Eltrombopag (ETP) is small non-peptide molecule, which interacts with the transmembrane domain of thrombopoietin receptor, initiating a JAK/STAT signaling pathway inducing the proliferation and differentiation of the megakaryocytes to increase platelets production.

Aims: To assess the gene expression profile (GEP) and the underlying signaling pathways modified before and during the ETP treatment in chronic immune thrombocytopenia (ITPc) patients.

Methods: ITPc patients (n=14) treated with ETP were evaluated (Table 1). Complete response (CR) was defined as a platelet count of ≥100x10^3/mm^3. RNA was isolated from mononucleated cells pre/post ETP treatment. Thus, 28-paired samples were hybridized in Affymetrix´s. The robust microarray analysis algorithm was used for normalization and signal expression was calculated by significance analysis of microarray to provide a robust statistical inference. The pathways and upstream regulators related with the most differentially expressed genes were analyzed by *in silico* analysis tools: IPathwayGuide and DAVID.

Results: Baseline patients' characteristics (Table 1). Median platelet counts and white blood cells increased after treated by ETP. All but two patients achieved CR (Table 1).

TABLE 1 Baseline characteristics of ITPc treatments included in the study

Age: median (range)	77,5 (35-87) years
Treatments before start Eltrombopag:	One: 5 (35,7%) / ≥2: 9 (64,3%) / Splenectomy: 3 (21,4%)
Blood cells count (BCC) basal: median (range). Platelets (P) and WBC in (x10^3/mm^3); Hb in g/dL	P: 14,45 (1,9-73,8) / WBC: 6,85 (2,2-19,3) / Hb: 13,85 (8,7-16,2)
BBC at day 14: median (range). P and WBC in (x10^3/mm^3); Hb in g/dL	P: 119,5 (4,7-230) / WBC: 7,58 (1,9-14,4) / Hb: 12,85 (8,6-16,3)
BBC at day 28: median (range). P and WBC in (x10^3/mm^3); Hb in g/dL	P: 132 (1,9-173,8) / WBC: 9,1 (2,7-14,1) / Hb: 13,35 (8,1-15,4)
Treatment response (n,%):	Complete: 12 (85,7%) / Failure: 2 (14,3%)
Doses response (n,%)	50 mg: 9 (64,3%) / 75mg: 3 (21,4%) / Failure: 2 (14,3%)
Day of response (n,%):	+14 day: 10 (71,4%) / +28 day: 4 (28,6%)

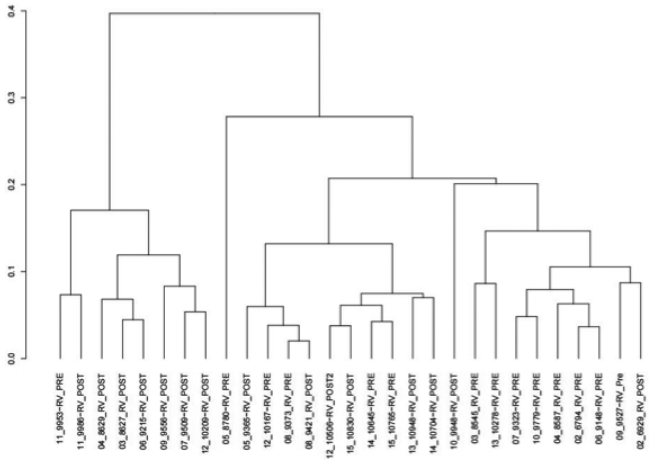

FIGURE 1 Samples distribution provide robust statistical inference of the most significant genes:

In silico analysis revealed that ETP treatment modified the expression of 147 genes, all of them were overexpressed after treatment. These genes cluster (2 groups), samples pre/post ETP treatment are shown in Figure 1. Mainly, genes (38) were involved in the maintenance of hemostasis and almost all of them were related to platelet activation (*PTGS1*, *GP1BA* or *GP6*). Interestingly, the paired GEP pointed out *E2F1 and GFI1B* as possible leaders of the increase of the megakaryopoiesis. The main signaling pathways overexpressed by ETP are downstream routes of PI3K/Akt (*GFI1B*) and platelet activation.

Conclusions: In ITPc patients, ETP not only induced overexpression of many genes related with platelet activation and megakaryopoiesis but also signaling pathways such as JAK/STAT and PI3K/Akt.

PB 1315 | Calcium Homeostasis in Platelets in Pregnancy Women with Preeclampsia

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Background: Platelet function is abnormal in preeclampsia. Changes of platelet functional activity associated with adhesion and aggregation, leading to increased bleeding and thrombus formation or enhance the development of pathological changes in blood flow. Ca^{2+} is required in platelets for many functions such as shape change, secretion and aggregation.

Aims: The aim of the study was to investigate changes in calcium homeostasis in platelets of pregnant women with preeclampsia.

Methods: Venous blood samples of obtained from 21 pregnant women with preeclampsia and 23 of healthy pregnant women, for platelets study. Informed consent was obtained from every study subject. No participants had taken any antiplatelet drugs in the previous 2 week. Intracellular Ca^{2+} concentration in the platelets was determined using the fura 2-AM.

Results: The resting platelet Ca^{2+} in a group of women with preeclampsia was higher (82,1±4,2) nM than in the control group (60,6±3,5) nM. Different Ca^{2+} transport mechanisms can to be altered in platelets from patients with preeclampsia, including the sarcoendoplasmic and plasma membrane Ca^{2+}-ATPases, plasma membrane Ca^{2+} channels, or the Na^{+}/Ca^{2+} exchanger. This study confirmed that in pregnant women with preeclampsia, platelets have increased basal Ca^{2+} and larger agonist-stimulated Ca^{2+} response compared with those in normal subjects. Ca^{2+} leak from the dense tubular system or the acidic stores, induced by a low concentration of thapsigargin or 2,5-di-(t-butyl)-1,4-hydroquinone (TBHQ), respectively, was clearly greater in pregnant women with preeclampsia than in control. Furthermore, it was found that in preeclampsia, the direction and activity of the platelet Na^{+}/Ca^{2+} exchanger was altered.

Conclusions: Platelets from patients with preeclampsia show abnormalities in intracellular Ca^{2+} homeostasis that are involved in platelet hyperaggregability and the development of thrombotic complications.

PB 1316 | PDK1 Regulates Platelet Activation through Phosphorylating of S6K on Thr229

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Background: Phosphoinositide-dependent kinase 1 (PDK1) regulates platelet activation through Akt-Thr308/Gsk3β-Ser9 signaling pathway. It is still unknown whether PDK1 could activate other substrates during the process of platelet activation.

Aims: The Aims of this study were to find out whether PDK1 regulated platelet activation through other substrates, to identify the role of S6K Thr229 in platelet activation.

Methods: Platelet aggregation and secretion test; platelet spreading; clot retraction; immublotting; Statistical analysis.

Results: Here, we reported that PDK1 played inhibitory roles in collagen and ADP induced platelet aggregation and secretion while Akt/Gsk3β signaling was blocked by the pharmacologic Gsk3β inhibitors. Moreover, PDK1 regulated collagen induced platelet aggregation partially through suppressing the secretion of ADP from dense granules. Further results demonstrated that the p70 ribosomal S6 kinases (S6K) was phosphorylated by PDK1 on Thr229 in the resting platelets and dephosphorylated in response to agonists stimulation. S6K inhibitor promoted platelet aggregation and secretion induced by collagen. S6K Thr229 phosphorylation might function as a regulator keeping platelets from activation. Thus, the diminished platelet aggregation in response to collagen stimulation caused by PDK1 was partially due to the phosphorylation of S6K on Thr229. Compared to thrombin, collagen induced the decrease of S6K Thr229 phosphorylation was restored by the Gsk3β inhibitor, which explained the different roles of Gsk3β in thrombin and collagen induced platelet aggregation.

Conclusions: Together, these results indicate that PDK1 negatively regulates collagen induced platelet activation through affecting phosphorylation of S6K on Thr229.

PB 1317 | Reverse Phosphorylation of the Protein Phosphatase 2A Inhibitor α-endosulfine (ENSA) at Two Distinct Sites (S67, S109) in Activated and Inhibited Human Platelets

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Background: α-endosulfine (ENSA; ARPP19-e) belongs to the cAMP-regulated phosphoprotein (ARPP) family. In *Xenopus*, PKA evokes ENSA S109 phosphorylation which keeps the oocyte in prophase whereas S67 phosphorylation is necessary for M-phase entry (Vigneron et al. Int J Dev Biol. 2016). In *Drosophila* and *Xenopus*, Greatwall kinase (MASTL in mammals) phosphorylates ENSA at S67 and converts it to a potent protein phosphatase 2A (PP2A) inhibitor resembling okadaic acid. Little is known about ENSA in platelets.

Aims: To elucidate the regulation and function of ENSA S67/S109 phosphorylation in human platelets.

Methods: Platelet proteomic studies were carried out as published (Burkhart et al. Blood. 2012; Beck et al. Blood. 2017). ENSA cloned from human platelets was expressed in HEK293 cells and *E.coli* BL21. ENSA phosphorylation was studied with intact human platelets, platelet extracts and recombinant proteins and mutants.

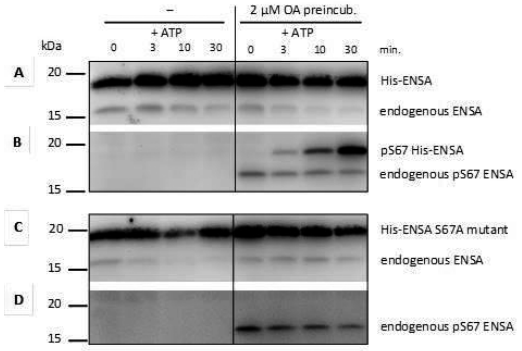

Figure 1: Demonstration of specific ENSA S67 protein-kinase activity in soluble extracts of human platelets. Washed human platelets were pre-treated without (-) or with 2 µM okadaic acid (OA) for 15 minutes at 30°C. Then, platelets were lysed by an EDTA/EGTA containing NP40 buffer at 0°C and recombinant His-tagged ENSA protein (wildtype and the S67A mutant as control) was added to the soluble platelet extracts. The phosphorylation reaction of endogenous protein kinase(s) was started by the addition of $MgCl_2$/ATP (0 min) and incubated at 30°C. Aliquots were removed from the reaction mixture before ATP addition (0 min) and after 3, 10 and 30 minutes of incubation. Western blots were probed for the presence of endogenous (~ 17 kDa) and recombinant His-tagged (~ 19 kDa) ENSA with a general antibody against human ENSA (A and C; cell signalling) and for the presence of phosphorylated endogenous and His-tagged pS67-ENSA with a phosphospecific antibody (B and D; cell signalling).

FIGURE 1 Demonstration of specific ENSA S67 protein-kinase activity in soluble extracts of human platelets

Results: ENSA and PP2A, but not MASTL, were detected in human platelets at significant protein levels. Quantitative phosphoproteomics showed strong ENSA S109 phosphorylation in response to cAMP- (Iloprost) and cGMP-elevating (NO-donors, Riociguat) platelet inhibitors whereas ADP and selective GPIb-stimulation decreased S109 phosphorylation. ENSA S67 phosphorylation was elevated upon phosphatase PP1/PP2A inhibition while decreased by cyclic nucleotide-elevating platelet inhibitors. Using recombinant ENSA wild type and mutant proteins a strong kinase activity towards S67 was observed in human platelet extracts (see Fig). Okadaic acid, a powerful pharmacological PP2A inhibitor, strongly inhibited various agonist responses in human platelets.

Conclusions: Our data demonstrate that in human platelets ENSA S67 is phosphorylated by a kinase other than MASTL. With its regulated phosphorylation sites S67 (essential for PP2A inhibition) and S109 (both PKA and PKG) ENSA represents an important signaling node in human platelets.

PB 1318 | Integration of Platelet Agonist Signaling during the Hemostatic Response in vivo

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Background: The local microenvironment within an evolving hemostatic plug shapes the distribution of soluble platelet agonists, resulting in a gradient of platelet activation emanating from the site of injury. While thrombin and ADP mediated platelet activation are clearly critical for establishment of this gradient, it remains unclear to what extent there is overlap of these and other platelet signaling pathways in time and space.

Aims: We sought to determine whether single agonists dominate the platelet activation state within specific regions of a hemostatic plug, or whether multiple agonist pathways are integrated to produce the platelet activation gradient observed. We also examined the relationships among different agonists in the face of anti-platelet or anti-coagulant therapy.

Methods: A combination of genetic and pharmacologic approaches was used in the mouse cremaster laser injury model to examine how thrombin, thromboxane A_2 (TxA_2), $P2Y_{12}$ and epinephrine signaling are coordinated in time and space to regulate the development of platelet activation gradients in vivo.

Results: We found that 1) inhibition of either TxA_2 or $P2Y_{12}$ signaling specifically attenuated accumulation of minimally activated platelets in the outer shell region of hemostatic plugs; 2) dual anti-platelet therapy was similar to inhibition of either TxA_2 or $P2Y_{12}$ signaling alone; 3) epinephrine signaling was completely dispensable for hemostatic plug formation; and 4) thrombin mediated, robust platelet activation does not require positive feedback via $P2Y_{12}$.

Conclusions: In conclusion, accumulation of minimally activated platelets requires integration of both TxA_2 and $P2Y_{12}$ signaling pathways, while maximal platelet activation requires thrombin mediated signaling. These data shed new light on the way multiple platelet signaling pathways are integrated during the hemostatic response, and predict the outcome of therapeutically targeting specific platelet signaling pathways alone and in combination on hemostatic plug organization.

PB 1319 | The Identification and Characterization of a Novel, Selective Platelet-surface ERp57 Inhibitor that Modulates Platelet Function Independently of Outside-in Signaling through Integrin αIIbβ3

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Background: Inhibition of platelet-surface ERp57 using function-blocking antibodies results in the inhibition of platelet function and *in vivo*, ERp57 controls thrombus growth. Despite ERp57 being an important antithrombotic target, to date, a selective ERp57 small molecule inhibitor is not available.

Aims: To use newly identified selective inhibitors of ERp57 to explore the role of ERp57 in platelet signaling through the integrin αIIbβ3.

Methods: 3641 compounds were screened for inhibition of ERp57 by insulin turbidity assay, the most potent that was selective for ERp57, CGP13501 (CGP) was selected for further investigation. Human platelets were incubated with CGP (7.5-300μM) or vehicle for 5 minutes prior to stimulation with CRP-XL (1μg/mL), U46619 (1μM) or thrombin (0.1U/mL). Platelet aggregation and granule secretion was measured using lumi-aggregometry and by flow cytometry. Calcium mobilisation from intracellular stores was measured by fluorescence-based assay, platelet spreading and thrombus formation were measured using confocal microscopy.

Results: CGP inhibited ERp57 enzyme activity but not the activity of PDI, ERp5, ERp72 or thioredoxin. CGP (7.5-300μM) concentration-dependently inhibited platelet aggregation in response to CRP-XL, U46619 and thrombin. Calcium mobilisation, dense-and alpha-granule secretion and platelet spreading on collagen and fibrinogen were similarly affected. CGP decreased substantially the size of thrombi formed under arterial flow conditions using human and murine blood and in blood from transgenic (diYF) mice that display defective integrin αIIbβ3 outside-in signaling.

Conclusions: CGP is a potent inhibitor of ERp57 that inhibits thrombus formation through a mechanism that is not dependent on outside-in signaling by integrin αIIbβ3. Small molecule inhibitors of ERp57 such as CGP provide additional means to explore the role of ERp57 in platelet function and may prove useful for the development of future antithrombotic therapies.

PB 1320 | Platelet Proteomic Profiling in a Patient with Severe Platelet Dysfunction Associated to an Anti-αIIbβ3 Autoantibody Triggering Outside-in Signaling

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Background: Acquired platelet dysfunction may be caused by anti-$\alpha_{IIb}\beta_3$ autoantibodies.

Aims: We report the case of a patient who has developed an atypical severe platelet disorder associated with an IgM antibody that induces integrin $\alpha_{IIb}\beta_3$ outside-in signaling.

Methods: We performed several functional assays (e.g. PAC-1 binding) to investigate the mechanism leading to platelet dysfunction. We also investigated the proteome of platelets (Label-Free quantitative analysis LC-MS/MS method), from the patient (n=3) and three unrelated controls. Functional analysis was performed using IPA software.

Results: The patient is a 53-year-old man with chronic refractory immune thrombocytopenia. Platelet function testing showed much reduced aggregation using all agonists, but normal agglutination with ristocetin. Expression of $\alpha_{IIb}\beta_3$ was reduced and there was nearly no activation of GPIbα or P-selectin. Surprisingly, an IgM antibody was strongly detected in permeabilized platelets, suggesting that this antibody was internalized. Patient´s platelet poor plasma activated $\alpha_{IIb}\beta_3$ of control platelets as evidenced by increased PAC-1 binding. We also demonstrated that constitutive activation of $\alpha_{IIb}\beta_3$ triggers outside-in signaling, as shown by the phosphorylation of β_3 Tyr773 and the autophosphorylation of FAK. In the same way, PRP clot retraction was conserved in our patient. Using a proteomic approach, 932 proteins were identified in our samples from which 306 were differentially expressed. Most of them are involved in platelet signaling or protein ubiquitination pathways. The signaling activation pathway appeared diminished, while proteins in the ubiquitination pathway were augmented.

Conclusions: We present the case of a patient with a severe platelet disorder associated with the presence of an IgM anti-$\alpha_{IIb}\beta_3$ autoantibody triggering outside-in signaling.

Paradoxically, others signaling activation networks were diminished, probably due to a proteolysis phenomenon linked to the proteasome system.

PB 1321 | STAT3 Phosphorylation Is Regulated by Thromboxane A2 Receptor in Platelets

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Background: Thromboxane A2 (TXA2) synthesis is part of the amplification loop of platelet activation. This effect is mediated by the action of TXA2 on its receptor on platelet membrane. However, signal transduction mechanisms related to TXA2-R are largely unknown. STAT3 is present in platelets, and is phosporylated in response to thrombopoietin (TPO) and collagen.

Aims: To investigate the implication of STAT3 phosporylation in TXA2-induced platelet activation.

Methods: Washed human platelets were stimulated with different agonists (collagen, thrombin, TXA2 analogs U46619 or IBOP, ADP and r-TPO). STAT3 phosphorylation were performed by immunoblotting. TXA2-R, ADP receptor P2Y12 and GPIIbIIIa were blocked with SQ29,548, 2MeSAMP and RGDS, respectively. STAT3 was inhibited with STA21.

Results: Platelet aggregation induced by collagen, thrombin, U46619 and IBOP, but not ADP, dose and time-dependently induced STAT3 phosphorylation. In all cases this phosphorylation was lower than those induced by TPO. TXA2-R blocking suppressed STAT3 phosphorylation induced with all agonist, and only partially reduced TPO-induced phosphorylation. Similar results were obtained when TXA2 synthesis was blocked by aspirin, suggesting that TXA2-R plays an important role in STAT3 phosphorylation. U46619-induced STAT3 phosphorylation was independent of aggregation or ADP release as demonstrated by GPIIbIIIa or P2Y12 receptors blocking. Incubation with STA21 reduced U46619-induced STAT3 phosphorylation, although only reduced platelet aggregation at low concentrations of agonist. Finally, TXA2-R and STAT3 were co-immunoprecipitated both from resting and activated platelets.

Conclusions: STAT3 phosphorylation induced by different platelet agonists is mainly related to TXA2 receptor. These results described for the first time a new signal transduction mechanism linked to TXA2 receptor. Grants. FIS13/00016. ACIF/2016/465. RETICS networks INVICTUS (RD12/0014/0004) and INVICTUS+ (RD16/0019/0008) Instituto de Salud Carlos III.

PB 1322 | Guanosine Exerts Antiplatelet and Antithrombotic Properties through cAMP-PKA Signaling and Independently of Platelet Adenosine Receptors

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Background: Guanosine is a natural product and an endogenous nucleoside that has shown to increase during myocardial ischemia. Platelets are critically involved in ischemic coronary events. It remains unknown, however, whether guanosine may affect platelet activation and function.

Aims: We sought to investigate the potential antiplatelet and antithrombotic properties of guanosine and decipher the mechanisms behind.

Methods: We firstly assessed the effects of guanosine on platelet activation/aggregation upon stimulation with several platelet agonists includingadenosine diphosphate (ADP), collagen, arachidonic acid (AA), and TRAP-6. Guanosine antithrombotic potential was also evaluated both *in vitro* (Badimon perfusion chamber) and *in vivo* (murine model). In addition we assessed any potential effect on bleeding. At a mechanistic level we determined the release of thromboxane B2, intraplatelet cAMP levels, the binding affinity on platelet membrane, and the activation/phosphorylation of protein kinase A (PKA), phospholipase C (PLC) and PKC.

Results: Guanosine markedly inhibited platelet activation/aggregation -challenged by ADP and, although to a lesser extent, also reduced platelet aggregation challenged by collagen, AA and TRAP-6. Guanosine significantly reduced thrombus formation both *in vitro* and *in vivo* without significantly affect bleeding. Guanosine antiplatelet effects were associated with the activation of the cAMP/PKA signaling pathway, and a reduction in thromboxane B2 levels and PLC and PKC phosphorylation. The binding affinity assay revealed that guanosine effects on platelets were adenosine receptor independent.

Conclusions: Guanosine effectively reduces ADP-induced platelet aggregation and limits thrombotic risk. These antithrombotic properties are associated with the activation of the cAMP/PKA signaling pathway and are independent of adenosine receptors.

PB 1323 | Anti-thrombotics from Snake Venom as Selective Inhibitor of $\alpha_{IIb}\beta_3$ Outside-in Signaling

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Background: Current $\alpha_{IIb}\beta_3$ antagonists are efficacious anti-thrombotics but have significant bleeding risk.

Aims: To address the issue, we examined whether two purified antagonists, TMV-2 and TMV-7, from *Trimeresurus mucrosquamatus* snake venom, prevent thrombosis without causing bleeding via selectively inhibiting $\alpha_{IIb}\beta_3$ outside-in signaling.

Methods: We investigated the relationship between structure activity and the capacity of binding to $\alpha_{IIb}\beta_3$ on mutually exclusive binding of cytosolic protein talin and $G\alpha_{13}$ to β_3 cytoplasmic domain among TMV-2, TMV-7 and its derivative by immunoprecipitation and binding study.

Results: We reported the purified Arg-Gly-Asp (RGD)-containing disintegrin TMV-7 endows with a binding motif toward aIIb b-propeller

domain of αIIbβ3, different from those of another disintegrin TMV-2 and abciximab, with advantages in that TMV-7 selectively inhibits Gα13-binding without affecting talin-binding to β3 in human and mouse thrombin-activated platelets. Compared with TMV-2 and abciximab, TMV-7 had minor effect on clot retraction, processes of hemostasis driven by talin. TMV-7 potently inhibited FeCl3-induced occlusive thrombosis in vivo, but did not affect bleeding time measured by tail bleeding time model, compared to abciximab and TMV-2. To mimic hemorrhage during surgical procedures, we used ROTEM method of surgical hemorrhage. In contrast to abciximab and TMV-2, TMV-7 had no effect on human whole blood coagulation-indexes even at higher dose. Additionally, we mutated the RGD-domain of TMV-7 from ARGDNP to AKGDXX, and found that this AKGDXX mutant (XX) also acts as a selective inhibitor of Gα13-mediated outside-in signaling and exhibits a higher potency in inhibiting platelet aggregation and preventing thrombosis in vivo, and its safety index is raised to 70-time higher than TMV-7.

Conclusions: Taken together, TMV-7 and XX prevent thrombosis by a mechanism different from that of all other tested $\alpha_{IIb}\beta_3$ antagonists, acting as efficacious antithrombotic agents with minimal bleeding risk.

PB 1324 | The Involvement of $\alpha_{IIb}\beta_3$ Integrin on the Release of Extracellular Vesicles and the Chemokines CXCL4 and CCL5 by Platelets

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Background: Platelets release extracellular vesicles upon activation as well as spontaneously during prolonged storage. Platelets represent a major source of extracellular vesicles (EV) and have been recognized to be involved in inflammatory diseases. Stimulation of platelets results in activation of $\alpha_{IIb}\beta3$ integrin, which enables ligand binding and subsequent outside-in signaling. Previous studies have shown that $\alpha_{IIb}\beta3$ integrin is involved in platelet EV release. Besides the release of platelet EV, platelets are a rich source of chemokines i.e. platelet factor 4 (CXCL4) and RANTES (CCL5), which can be secreted through the α-granules and are involved in the inflammatory recruitment of leukocytes. However, the exact mechanism behind the involvement of $\alpha_{IIb}\beta3$ integrin in the release of platelet EV and chemokines is currently unknown.

Aims: To identify the underlying mechanisms of platelet EV- and chemokine CXCL4 - and CCL5 - release by activated platelets

Methods: Platelets were isolated from healthy volunteers and stimulated with convulxin and TRAP-6. The release of platelet EV and chemokines (CXCL4 and CCL5) was determined by a prothrombinase-based assay and by ELISA, respectively. The mechanism of extracellular release was studied with several inhibitors e.g. eptifibatide.

Results: Convulxin-activated platelets increased the release of platelet EV compared to non-activated platelets. Chemokine CXCL4 and CCL5 levels were increased upon convulxin and TRAP-6 platelet activation. Pre-treatment with the $\alpha_{IIb}\beta3$ inhibitor eptifibatide reduced platelet EV release triggered by convulxin and attenuated CCL5 release, but did not affect TRAP6- activated platelets. Interestingly, CXCL4 release was not affected by eptifibatide pre-treatment of platelets.

Conclusions: These data conclude that the $\alpha_{IIb}\beta3$ integrin is involved in the release of platelet EV and the chemokine CCL5. Interestingly, the release of CXCL4 appears not to be dependent on the action of $\alpha_{IIb}\beta3$ integrin.

PB 1325 | Neuromedin U Potentiates ADP and Epinephrine Activation of Human Platelets

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Background: Neuromedin U (NmU), a pleiotropic hypothalamic neuropeptide involved in the gut-brain axis, interacts mainly through NMUR1 and NMUR2 receptors coupled to $G\alpha_{q/11}$ and $G\alpha_{i,}$, respectively. Transcriptome analysis shows evidence for expression of NmU and its receptor in human platelets, but the functional effect of NmU on these cells is unclear.

Aims: To explore whether NmU plays a role in platelet activation and to detect the presence in platelets of NMUR1, the receptor preferentially involved in NmU activity on peripheral cells.

Methods: Platelet aggregation was studied in a PAP-8E (Bio/Data Corporation) aggregometer; platelet rich plasma was stimulated with human NmU (Abcam, Cambridge-UK) alone or combined with ADP, epinephrine, serotonin, with(out) platelet inhibitors. Human anti-NMUR1 antibody (H-45; 1:200) was used in western blots of platelets.

Results: NmU itself (to 10μM) did not induce any measurable platelet aggregation, but significantly and concentration-dependently (10-100nM) potentiated ($p< 0.003$) platelet aggregation by submaximal ADP-concentrations (0.625-2.5μM). This effect was more pronounced when NmU was added 1-2 min prior to ADP. Neither aspirin (200μM, 15 min), nor MRS2179 (50μM, 5 min), a $P2Y_1$ inhibitor, modified the NmU effect on ADP. NmU also increased (mean 37%; CI 95% 23.3-52.2; $p< 0.0002$) aggregation by low epinephrine concentrations (0.078-1.25μg/ml), but did not affect serotonin (20μM)-induced aggregation. YM-254890, a specific inhibitor of Gαq receptors (1μM), abrogated the NmU/epinephrine-induced platelet activation. NMUR1 was detected in platelets from six donors.

Conclusions: This is the first description of the potentiating effect of NmU on platelet activation by several agonists. This effect is COX-1 and $P2Y_1$ independent; NmU/epinephrine acts through NMUR1, coupled to $G\alpha_q$ in platelets. NmU released during vascular activation and/or injury may enhance platelet responses to classical agonists, *via* NMUR1-signaling.

PB 1326 | Real-time Flow Cytometry Can Identify Differences in the Kinetics of Platelet Activation in Response to Different Agonists

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Background: The kinetics of platelet activation may be important in determining the size and structure of a forming thrombus. Real-time flow cytometry accurately measures the dynamics of platelet activation over time and can identify variations in response to different agonists.

Aims: To identify differences in the rate of platelet activation in response to stimulation via different signalling pathways.

Methods: Platelet-rich plasma was used in real-time flow cytometry to detect binding of FITC-labelled fibrin(ogen) antibodies in response to cross-linked collagen related peptide (CRP-XL; 0.1mg/ml), thrombin (0.3U/ml), ADP (1mM) and epinephrine (0.5mM). Data was collected over 300 seconds and a loess curve of fluorescence intensity vs. time was plotted in R and used to calculate a range of metrics, including maximum fibrinogen binding, maximum rate and acceleration of fibrinogen binding, and the time points at which these maximums occurred.

Results: In 300s platelets bound greater levels of fibrinogen when stimulated with ADP (3.8FU; P< 0.05 vs. CRP-XL) and epinephrine (4.4FU; P< 0.001 vs. CRP-XL) compared to CRP-XL (2.9FU) or thrombin (3.6FU). In contrast, the maximum acceleration of fibrinogen binding revealed the highest rates in response to CRP-XL ($15.1x10^{-4}$ FU/min) and thrombin ($11.79\ x10^{-4}$ FU/min) when compared to ADP ($7.68\ x10^{-5}$ FU/min; P< 0.05 vs. CRP-XL) and epinephrine ($3.59\ x10^{-5}$ FU/min; P< 0.0001 vs. CRP-XL), but occurred at later time points. These data indicate that ADP and epinephrine induce rapid platelet activation at a steady rate leading to high maximal fibrinogen binding at 300s. Stimulation with CRP-XL begins at a slow rate initially, before accelerating to a high activation rate, leading to lower levels of fibrinogen binding at 300s.

Conclusions: The rate of platelet activation and time to reach full activation varies depending on the signalling pathway. These measurements could determine how efficiently platelets are recruited into forming thrombi and how this would affect thrombus architecture.

PB 1327 | Interplay between Calcium and Zinc: Implications for Platelet Activation

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Background: At sites of vascular injury, Zn^{2+} is released from damaged cells, activated platelets and inflammatory cells. At high concentrations, Zn^{2+} acts as a direct platelet agonist. At low concentrations, Zn^{2+} potentiates platelet activation. However, the underlying mechanism(s) of Zn^{2+}-induced platelet activation remain unclear.

Aims: The aim of this work was to determine the mechanisms by which Zn^{2+} gains access to the platelet cytosol, and to investigate the mechanism by which Zn^{2+} effects platelet aggregation.

Methods: Zn^{2+}-induced platelet activation was investigated in washed platelet suspensions using various channel blockers. Zn^{2+} entry into the platelet cytosol was assessed using the Zn^{2+}-sensitive dye FluoZin-3. Fluctuations of intracellular Ca^{2+}, activation of integrin $a_{IIb}b_3$ and expression of P-selectin, LAMP-3 and phosphatidylserine were assessed by flow cytometry.

Results: Blockade of the sodium/calcium exchanger (NCX) and transient receptor potential (TRP) family members, resulted in reduced aggregation in response to Zn^{2+}. Co-application of TRP and NCX blockers abrogated aggregation. Further experiments revealed reduced aggregation responses to CRP and thrombin in the presence of these drugs. Pre-incubation of platelets with sub-activatory Zn^{2+} reduced intracellular Ca^{2+} responses, likely by impairment of release of Ca^{2+} from the dense tubular system. However, CRP- and thrombin-induced detection of activation markers (i.e. P-selectin, LAMP-3 and intergrin activation) was not significantly altered in the presence of subactivatory Zn^{2+}.

Conclusions: TRP channels and NCX are implicated in coordinating platelet responses to Zn^{2+}. Low concentrations of Zn^{2+} reduce agonist-induced Ca^{2+} responses, likely via reduction of Ca^{2+} release from the intracellular store. Given that the expression of activation markers was not affected by Zn^{2+}, these data indicate a potentiatory role for Zn^{2+} during platelet activation.

PB 1329 | Btk Kinase Activity is Not Critical for Platelet Activation by GPVI

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Background: Ibrutinib and acalabrutinib, two inhibitors of the non-receptor protein tyrosine kinase Btk, are used to treat B cell malignancies. They bind irreversibly to Cys481 of Btk, preventing it from phosphorylating itself and other substrates. Patients treated with ibrutinib have increased bleeding events and this correlates with inhibition of platelet aggregation downstream of the collagen receptor GPVI. Patients treated with acalabrutinib do not bleed excessively.

Aims: We sought to investigate this discrepancy between the two Btk inhibitors.

Methods: Aggregometry, tyrosine phosphorylation and Ca^{2+} mobilisation assays were performed in washed platelets pre-treated with ibrutinib or vehicle. Aggregometry was also performed with platelet rich plasma (PRP) from patients taking ibrutinib 420mg daily, acalabrutinib 100mg twice daily or a control treatment.

Results: Ibrutinib (40nM) blocked GPVI-induced phosphorylation of Btk on Y223 and PLCg2 Y759 and 1217, but only caused a slight delay

in aggregation and had no effect on Ca^{2+} mobilisation. Complete inhibition of aggregation, Ca^{2+} mobilisation and total tyrosine phosphorylation occurred at concentrations greater than 1μM ibrutinib. PRP from patients taking ibrutinib did not aggregate in response to GPVI ligation whereas PRP from patients taking acalabrutinib aggregated normally.

Conclusions: Our results confirm that ibrutinib blocks GPVI-induced platelet aggregation *in vitro* and *ex vivo* but that, contrary to current understanding, this is due to off-target effects. We hypothesise that reducing the dose of ibrutinib given to patients would abolish its off-target effects and therefore reduce bleeding. This is supported by the fact that the more specific Btk inhibitor acalabrutinib, which does not have a bleeding side effect, does not block aggregation to GPVI *ex vivo*.

Acknowledgement: This work was supported by the British Heart Foundation.

PB 1330 | ERK5 Associates Casein Kinase II to Regulate GPIb-IX-mediated Platelet Activation via PI3K/Akt Pathway

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Background: The platelet adhesion receptor, the glycoprotein (GP) Ib-IX complex play essential roles in thrombosis and hemostasis. Although mitogen-activated protein kinases (MAPKs) p38 and ERK1/2 have been shown to be crucial in GPIb-IX-mediated platelet adhesion and aggregation, the function and mechanisms of ERK5 (extracellular signal-regulated kinase 5) in GPIb-IX-mediated platelet activation remain unknown.

Aims: To study the function and mechanisms of ERK5 in GPIb-IX-mediated platelet activation.

Methods: The effects of ERK5 inhibitor on Botrocetin/vWf or Ristocetin/vWf induced human platelet two-phase aggregation and on platelet adhesion to VWF under shear stress were assessed. The ERK5 associated proteins were identified by co-immunoprecipitation and mass spectrometry analysis from CHO cell expressing HA-ERK5 and were confirmed by immunoprecipitation and western blot in human platelet. The role of ERK5 associated proteins in GPIb-IX-mediated platelet activation were clarified by specific inhibitor.

Results: Phosphorylation levels of ERK5 were significantly enhanced in human platelets stimulated by Botrocetin/vWf or Ristocetin/vWf. ERK5 inhibitor XMD8-92 significantly suppressed the second phase of Botrocetin/vWf or Ristocetin/vWf induced human platelet aggregation, and inhibited human platelet accumulation on immobilized VWF under shear stress. Immunoprecipitation and mass spectrometry analysis revealed that ERK5 associates with CKII (casein kinase II) in CHO cells expressing HA-ERK5 and in human platelets. CKII inhibitor TBB, similar as ERK5 inhibitor XMD8-92, specifically restrained the phosphorylation of PTEN, therefore suppressed Akt phosphorylation in human platelet treated with Botrocetin/vWf or Ristocetin/vWf.

Conclusions: ERK5 associates CKII to play critical roles in GPIb-IX-mediated platelet activation via PI3K/Akt Pathway.

PB 1331 | Agonist-induced Protein Disulfide Isomerase Activity in Platelets: Different Effect of Different Agonists

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Background: Protein disulfide isomerase (PDI) resides mainly in the endoplasmic reticulum but is also displayed on the surfaces of various cells including platelets. PDI is released from platelets following activation and facilitates platelet aggregation and thrombus formation in mice thrombosis models.

Aims: To investigate the effect of different agonists on PDI activity in human platelets and explore whether this effect correlates with platelets activation.

Methods: Washed platelets were prepared from healthy controls´ blood samples. Enzymatic reductase activity in platelets that were either activated or not by various agonists was assayed by measuring the kinetics of eosin 5-isothiocyanate-coupled glutathione disulfide (Di-E-GSSG) reduction. The contribution of PDI to reductase activity was assessed by adding a specific PDI inhibitor, quercetin-3-rutinoside (rutin). Platelet activation by the various agonists was measured by flow cytometry (FACS) using antibodies against the platelets activation markers P-selectin and active αIIbβ3.

Results: Resting platelets displayed basal reductase activity that was significantly increased following incubation with thrombin, TRAP, convulxin, ionophore and U46619. In contrast, collagen and ADP at low and high concentrations did not increase platelets reductase activity and did not further increase reductase activity induced with TRAP and U46619. Addition of rutin, significantly decreased reductase activity induced by the different agonists and also of resting platelets. FACS analysis showed that most agonists profoundly increased both P-selectin expression and αIIbβ3 activation; however, collagen and ADP caused smaller increase of both markers.

Conclusions: Resting human platelets harbor active PDI on their surfaces and platelets strong activation by some agonists induces the release of additional active PDI. Other agonists that caused lesser activation of platelets did not induce PDI release from platelets, indicating that PDI release requires strong platelets activation.

PB 1332 | Dose-dependent Inhibitory and Stimulatory Effects of Sphingosine-1-phosphate on Platelet Adhesion to Endothelium

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Background: Sphingosine-1-phosphate (S1P) is a biologically active sphingolipid with important cardiovascular functions. Activated platelets release large amounts of S1P locally during thrombus formation, whereas S1P itself induces platelet aggregation. In endothelial cells, S1P leads to P-selectin activation and enhances P-selectin-dependent leucocyte rolling. However, the effects of S1P on platelet adhesion to endothelium under flow are unknown.

Aims: To investigate the effect of different S1P concentrations on platelet adhesion to endothelial cells under flow.

Methods: Human umbilical vein endothelial cells were grown in microfluidic channels (Bioflux 200) until a monolayer was formed. Cells were stimulated with S1P in different concentrations (0.1µM, 1µM, 10µM). Whole blood of healthy individuals was perfused over the monolayer using pulsatile flow for 10 min. Thrombus formation was assessed using area coverage in percent. Data are mean ± standard deviation.

Results: Stimulation with 1µM S1P significantly decreased platelet adhesion to endothelial cells (20.1±4.9% vs. 16.3±3.7%, p< 0.0001), while 0.1µM S1P had no effect (19.1±4.5% vs. 20.2±4.4%, p=0.64). In contrast, 10µM S1P significantly increased platelet adhesion to endothelial cells (17.8±3.6% vs. 25.4±6.7%, p= 0.007). The S1P1 receptor antagonist W146 abolished the decrease in platelet adhesion by 1µM S1P (Control: 28.7±6%, 1µM S1P: 23.7±7%, 1µM S1P+W146: 33.8±4%) but had no effect on the increase in platelet adhesion by 10µM S1P (Control: 27.3±7%, 10µM S1P: 40.7±9%, 1µM S1P+W146: 38.7±11%). The enhanced adhesion by 10µM S1P was abrogated by a P-selectin blocking antibody (Control: 19.4±5%, 10µM S1P: 27±8%, 10µM S1P+anti-CD62P: 21.3±5%).

Conclusions: S1P affects platelet adhesion to endothelial cells dose-dependently. Physiological S1P concentrations suppress platelet adhesion to the endothelium through S1P1, whereas high S1P concentrations enhance adhesion independently of S1P1 and by activating P-selectin.

PB 1333 | Calcium Signalling Patterns in Adherent Single Platelets during Activation with Collagen-related Peptide

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Background: Platelet activation with thrombin, SFLLRN, ADP induces cytoplasmic calcium oscillations, while that with fibrillar collagen results in calcium increase without perceptible oscillations. Calcium patterns in single platelets activated with CRP are not well-characterized.

Aims: To study dynamics and dose-dependence of platelet calcium signalling upon CRP stimulation.

Methods: Whole blood was collected into sodium citrate. Platelets were loaded with Fura Red, immobilized on a fibrinogen surface for 20 min, followed by activation and real-time confocal microscopy. Annexin V and tetramethylrhodamine were used for detection of PS and mitochondrial potential.

Results: Calcium response during activation with different concentrations of CRP was concentration-dependent, and two types of dynamics were observed: short (~100 sec) high increase in calcium with following decrease and transition to stationary level (higher than rest level) for high concentrations of agonist, and the same dynamics without short increase but with low-speed one to the same stationary calcium level for low concentrations of agonist. All platelets had calcium spikes after activation that could be halted after a while. Calcium increase was delayed for decades of seconds for low agonist concentrations and was immediate for high concentrations of CRP. Formation of procoagulant platelets was accompanied by their mitochondrial collapse.

Conclusions: Activation of platelets with CRP leads to emergence of calcium oscillations distinct from responses induced by either thrombin, ADP, or collagen.

Study was supported by RFBR grant 16-34-01342.

PB 1334 | Reelin Modulates PLCγ2 Phosphorylation upon Platelet Activation and Integrin Outside-in Signaling

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Background: Reelin is known as an extracellular matrix protein mediating cell migration in brain development. Recent studies provide evidence that Reelin is expressed in platelets as co-localized to F-Aktin. Moreover, Reelin binds to the receptors amyloid precursor protein (APP) and apolipoprotein E receptor 2 (ApoER2) and is important for thrombus formation under high shear conditions suggesting that Reelin modulates glycoprotein(GP)Ib signaling in platelets. Consequently, defective thrombus formation protects Reelin-deficient mice (Reeler) against arterial thrombosis.

Aims: To analyze the role and the signaling mechanisms of Reelin in platelet cytoskeletal reorganization, activation and aggregation.

Methods: In vitro and in vivo analysis of Reeler mice.

Results: Reelin activated small GTPases of the Rho family and was shown to be important for the phosphorylation of Rho target proteins such as PAK1/2 supporting lamellipodia formation upon spreading on fibrinogen. Furthermore the adhesion and cytoskeletal reorganization of Reeler platelets on a collagen-related peptide (CRP) matrix was found to be reduced. Likewise strongly reduced phosphorylation of PLCgamma2 and Syk in response to CRP was detected in reeler platelets. Furthermore, platelet adhesion to immobilized reelin was significantly reduced when the collagen receptor GPVI was blocked. For further analysis of outside-in signaling of integrin αIIbβ3 clot retraction experiments were performed showing strongly reduced retraction of the clot in Reeler platelet rich plasma (PRP). Taken together these data provide first evidence for Reelin to mediate signaling through GPVI and to modulate integrin outside-in signaling suggesting that Reelin is a modulator of PLCgamma2 phosphorylation in platelets.

Conclusions: Our study reveals an important role for Reelin in hemostasis and arterial thrombosis, thus being a promising therapeutic target for antithrombotic therapy.

PB 1335 | Calcium Monitoring in Platelets: Tool for Diagnosing Platelet Function Defects

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Background: Laboratory tests currently used to investigate a bleeding diathesis may fail to reveal the underlying hemostatic disorder. Flow cytometry analysis (FCA) improved our tools for exploring platelet (PLT) function and profiling defects associated with bleeding diathesis.

Aims: To hone standard FCA by extending investigations to intracellular signaling, here monitoring free intracellular Ca^{2+} upon PLT activation.

Methods: Platelet-rich plasma was obtained from buffered citrated whole blood. PLTs were activated with increasing concentrations of thromboxane analogue U-46619, ADP, thrombin (THR), TRAP6 (PAR-1 agonist), AYPGKF (PAR-4 agonist), convulxin (CVX, GPVI agonist), and ionophore. PLT activation end-points for FCA were P-selectin expression and PAC-1 binding. Intracellular free Ca^{2+} was detected by its indicator Fluo-3 AM. After measurement of a stable baseline, PLTs were activated with agonists and Fluo-3 fluorescence was acquired over time.

Results: Optimal dose-response concentrations were determined for each agonist, and Ca^{2+} monitoring following PLT activation was performed. Strong and sustained cytosolic Ca^{2+} increase was observed after PLT activation with ionophore and CVX. THR induced an initial strong Ca^{2+} response, which slowly declined. Both TRAP6 and AYPGKF demonstrated a strong intracellular Ca^{2+} increase with a rapid decline. Their combined action was not able to fully replicate THR effect. Weak Ca^{2+} mobilization was observed with U-46619 and ADP. With Ca^{2+}-supplemented buffer, we observed both an increased Ca^{2+} mobilization and a qualitatively different response for THR and CVX combined either together or with U-46619 and ADP. Preliminary results indicate an increased calcium mobilization after *in vivo* desmopressin treatment.

Conclusions: The present work highlights the use of continuous Ca^{2+} monitoring to complement FCA of PLT function. This technique sharpens the ability to investigate agonist synergies and PLT signaling defects in bleeding patients investigated for PLT function disorders.

PB 1336 | Modelling Intracellular Zinc Release in Platelets

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Background: Zinc ions (Zn^{2+}) are released into the cytosol from intracellular stores of a number of different cell types in response to external stimulation, in a similar manner to intracellular calcium (Ca^{2+}) release. Platelets are small blood cells that are involved in the haemostatic response to vascular injury.

A hallmark of platelet activation is increased intracellular Ca^{2+} levels as a result of store release and influx through membrane channels. The role of Zn^{2+} release in platelets has yet to be studied in detail. Ionophores which enable ion transfer across membranes are often used to mimic intracellular ion release. Here we used zinc-specific ionophores to examine the effect of increased Zn^{2+} concentration on platelets.

Aims: The primary objective of this project is to examine the role of increases in intracellular Zn^{2+} in platelet processes, and to determine the potential store and release of Zn^{2+} in platelets.

Methods: The effects on platelets of Zn^{2+}-specific ionophores, clioquinol and pyrithione were compared with that of the calcium ionophore, A23187.

Results: A23187 induced platelet shape change followed by full platelet aggregation. Clioquinol induced shape change followed by sub maximal aggregation, whilst pyrithione caused significant platelet shape change without aggregation. Pre-treatment of platelets with the Zn^{2+} chelator, TPEN reduced the effects of Zn^{2+} ionophores in the platelets. The mechanism of ionophore-induced shape changes was examined using Cytochalasin D (Cyt D), which inhibits actin polymerization. Cyt D significantly reduced the shape change induced by Zn^{2+} ionophores.

Conclusions: These data suggest that intracellular Zn^{2+} is able to influence platelet responses in particular the cytoskeletal rearrangement that occurs during platelet shape change upon platelet activation. We hypothesis that during platelet activation, Zn^{2+} is released from intracellular stores to modulate platelet responses.

PB 1337 | Role of Heterotrimeric G Proteins in Platelet Activation and Clot Formation in Platelets Treated with Integrin $\alpha_{IIb}\beta_3$ Inhibitor

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Background: Mechanisms of platelet activation are triggered by soluble agonists which induce signaling via heterotrimeric Gαq, Gαi, and Gα12/13 proteins.

Aims: To investigate the contribution of the G proteins to platelet activation and clot formation in the presence of eptifibatide, excluding outside-in signaling caused by integrin αIIbβ3 engagement.

Methods: The study was approved by the research ethics committee, and each volunteer signed a written informed consent. Blood was drawn and diluted by 40% with Tris-buffered saline. Platelet-rich plasma or reconstituted whole blood were prepared and pretreated with 25 μg/mL eptifibatide. Selective and combined activation of the G proteins was achieved by using combinations of platelet agonists and inhibitors. Platelet activation was evaluated by P-selectin expression using flow cytometry. Clot formation was assessed by rotation thromboelastometry.

Results: Selective signaling of Gαq or Gαi but not Gα12/13 promoted P-selectin expression. Further increase in P-selectin expression was achieved by ADP-induced combined signaling of Gαq and Gαi and to more extent by combined signaling of Gαq and Gα12/13. The maximal P-selectin expression was achieved by TRAP-induced combined signaling of Gαq, Gαi, and Gα12/13. In ROTEM, selective activation of Gαq, Gαi, or Gα12/13 failed to affect blood clotting. Combined signaling of Gαq and Gαi or Gαq and Gα12/13 or all three G proteins shortened the clotting time and stimulated clot strength. Pretreatment of platelets with acetylsalicylic acid did not change the effect of ADP but inhibited the effect of TRAP. Signaling of Gαq and Gα12/13 triggered by U46619 (1.5 μM) also stimulated clot formation.

Conclusions: Combined signaling of either Gαq and Gαi or Gαq and Gα12/13 stimulates maximal platelet activation and clot formation in platelets treated with inhibitor of integrin αIIbβ3. It could be suggested that outside-in signaling is not necessarily required to fulfill these platelet functions.

PB 1338 | *In vitro* Assessment of Catecholamines to Restore Platelet Aggregation in the Presence of Ticagrelor and Aspirin

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Background: Ticagrelor, a $P2Y_{12}$ antagonist increases bleeding. No antidote is available and platelet transfusion is probably ineffective. However, catecholamines, epinephrine (EPI) and norepinephrine (NOR) activate platelets, thus could be considered to restore antiplatelet agents-induced platelet inhibition.

Aims: To assess *in vitro* the efficacy of EPI and NOR to restore platelet aggregation in the presence of ticagrelor and aspirin (ASA).

Methods: Platelet rich plasma (PRP) from volunteers was incubated with ticagrelor (n=16) or ASA (n=16). Platelet aggregation (spectrophotometry-based technique) was assessed using various agonists: ADP (2μM), EPI (1nM to10μM) or NOR (1nM to 0.4mM), and a mix of ADP+EPI or ADP+NOR with ticagrelor, and arachidonic acid (AA) (1mM), EPI or NOR, and a mix of AA+EPI or AA+NOR with ASA. Results are expressed in percentage of maximal aggregation.

Results: Compared to control, ticagrelor and ASA inhibited ADP- and AA-induced platelet aggregation respectively (8 vs 60%, p< 0.01, 10 vs 95%, p< 0.01). EPI or NOR concentration-dependently induced platelet aggregation in samples incubated with ticagrelor. However, the level of aggregation didn't return to baseline corresponding to the ADP-induced aggregation in the absence of ticagrelor (38% and 40%, vs 60%, p< 0,01, respectively). A combination of EPI (1μM) or NOR (10μM), and ADP completely restored platelet aggregation, to a similar level as ADP-induced platelet aggregation in the absence of ticagrelor. Similar results were observed with ASA. Catecholamines also potentiated the effects of the other agonists: the resulting aggregation from catecholamines+ADP or AA was greater than the sum of the aggregation response induced by catecholamines and ADP or AA.

Conclusions: EPI and NOR, in combination with ADP or AA, completely restored *in vitro* platelet aggregation in the presence of ticagrelor or ASA, respectively. Thus, EPI and NOR could potentiate the effects of ADP and TXA2 *in vivo* and thereby circumvent the effects of ticagrelor and ASA.

PB 1339 | Emerging Role for TLT-1 at the Interception of Inflammation and Hemostasis

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Background: TREM like transcript 1 (TLT-1) is a membrane receptor released from platelet α-granules upon activation. *Treml1*$^{-/-}$ mice bleed in response to various immune challenges where their wild type (WT) counterparts do not.

Aims: This study aims to evaluate TLT-1's role in the modulation of inflammatory associated hemorrhage.

Methods: We used an acute lung injury model, transmigration assays, and the cremaster muscle model to evaluate TLT-1 function.

Results: Using a LPS derived acute lung injury model we can demonstrate that administration of lipopolysaccharide (LPS:10µg) by nasal inhalation causes bleeding in the lungs, delayed neutrophil transmigration, but increased neutrophil accumulation in the bronchoalveolar lavage of mice suggesting that the neutrophils from *treml1*$^{-/-}$mice may cause the bleeding problems at the endothelial barrier (n=16,p≤0.01). To evaluate neutrophil function, we used whole mount staining of the cremaster muscle treated with CXCL2. Our results demonstrate that neutrophils from *treml1*$^{-/-}$mice accumulate at the endothelial barrier where their WT counterparts transmigrate, supporting the idea that these neutrophils are delayed in transmigration (n=10, p≤0.001). *In vitro* transmigration assays using neutrophils from WT or *treml1*$^{-/-}$mice with platelets from *treml1*$^{-/-}$mice platelets demonstrate that these platelets have difficulty in releasing from the transmigrated neutrophil and increased cell death as confirmed by flow cytometry and propidium iodine staining (p≤0.001). Those neutrophils (WT and *treml1*$^{-/-}$mice) incubated with WT platelets transmigrate alone and with a greater survival percentage. Moreover, addition of soluble TLT-1 intra venously protected mice from inflammatory associated hemorrhage in the lung upon nasal LPS challenge (n=18, P< 0.001).

Conclusions: These data suggest a potential role for TLT-1 in mediating platelet derived modulation of inflammatory associated bleeding.

PB 1340 | Uptake of Influenza Virus by Platelets Occurs via Sialic Acid Binding

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Background: Thrombocytopenia is a common symptom during influenza virus infection. We have recently shown that influenza A/H1N1 virus load is significantly correlated with a lower platelet count.

Aims: In this study, the relationship between platelets and influenza infection was studied further *in vivo* and *in vitro*.

Methods: To study influenza induced thrombocytopenia *in vivo*, 120 ferrets were inoculated intratracheally (1x10E6 TCID50) with an influenza A/H3N2 (n=30), A/H1N1 (n=30), or A/H5N1 virus (n=30). Platelet counts were measured at day 1-4. To assess direct platelet influenza virus interaction *in vitro* electron microscopy was performed on isolated human platelets incubated for 1 minute with influenza virus (A/PR/8/34). Additionally, platelet characteristics after virus uptake were measured, including membrane expression of the GPIb-IX-V complex, CD62P, surface sialic acid and GlcNac using flowcytometry and functional capacity employing aggregometry using agonists collagen, thrombin and ADP. Finally influenza virus uptake was measured in platelets after neuraminidase treatment.

Results: All ferrets had thrombocytopenia at day 3 post infection. The decrease in platelet count was significant in the A/H5N1 infected ferrets (p< 0.05). In these animals, influenza virus antigen was also detected in the liver and spleen at day 3 after infection. By electron microscopy we observed platelets with virus containing vacuoles, suggesting that platelets had rapidly phagocytosed the virus. Uptake of influenza virus had no effect on expression of GPIba, GPIX, P-selectin, surface sialic acid and GlcNAc or functional capacity of these platelets. Finally, desialylated platelets could not take up influenza virus.

Conclusions: Our study shows that sialic acid is essential for uptake of influenza by platelets. This mechanism may play an important role in the occurrence of thrombocytopenia during influenza infection and may be part of the host response mechanism by which viruses are cleared in the liver.

PB 1341 | Platelets Express and Release Immune-modulatory CXCL14 upon Activation

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Background: Platelets are the source of a variety of chemokines with immune-modulatory activity. The chemokine CXCL14 is expressed in immune cells with pro-inflammatory atherogenic impact. Expression of CXCL14 in platelets was unexplored.

Aims: The current study investigated whether platelets express and release CXCL14 upon activation. Furthermore a possible autocrine, immune-modulatory paracrine function of platelet derived CXCL14 was explored.

Methods: Flow cytometry, immunoblot, ELISA, monocyte migration, phagocytosis, and endothelial scratch assays (HUVECs) were performed.

Results: Western Blot analyses show that human as well as murine platelets express the chemokine CXCL14. Upon activation with

agonists CXCL14 surface expression and milieu release were significantly enhanced on human platelets. Furthermore, activation of platelets with increasing concentrations of Collagen Related Peptide (CRP) corresponded with CXCL14 surface expression. Recombinant (r) CXCL14 had no effect on agonist induced platelet activation. CXCL14 is chemotactic for $CD14^+$ monocytes. Currently we observed that CXCL14 present in activated platelet supernatant induced monocyte migration, counteracted upon CXCL14 neutralization with a neutralizing antibody. Moreover, blocking the chemokine receptor CXCR4, but not CXCR7 reduced the number of migrated monocytes towards rCXCL14, suggesting the involvement of CXCR4 in the CXCL14 directed monocyte chemotaxis.

In addition, a scratch assay with HUVECs indicates that platelet-derived CXCL14 counteracts the angiogenic effect of VEGF on these endothelial cells, which might be of importance during wound healing and angiogenesis.

Conclusions: Platelets are a hitherto unrecognized source of CXCL14 and release it upon activation. Platelet derived CXCL14 induces monocyte migration and counteracts the angiogenic effect of VEGF on HUVECs.

PB 1342 | CD8 T Cells Are Important Mediators of Platelet Refractoriness

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Background: Platelet refractoriness remains a significant clinical problem, yet the mechanisms by which it occurs are multifactorial and incompletely understood. Immune-mediated platelet clearance by anti-platelet alloantibodies plays a significant role, and patients with detectable alloantibodies can be managed with transfusion of HLA-matched platelets. Still, many patients are platelet refractory even after receiving HLA-matched platelets. We have previously shown that CD8 T cells play a direct role in platelet clearance. Allogeneic platelets are cleared within 24 hours post transfusion in B cell-deficient μMT recipient mice (ie in the absence of anti-platelet alloantibodies), whereas syngeneic platelets are not. Platelet clearance is dependent on CD8 T cells, as CD8 depletion, but not NK cell depletion, prevents clearance.

Aims: However, whether minor antigen differences can mediate CD8 T cell-dependent platelet clearance remains unknown.

Methods: To test this possibility we examined platelet refractoriness using mOva and OT I transgenic mice.

Results: We found that transfusion of mOva platelets into OT I mice, whose CD8 T cell receptors recognize a specific ovalbumin peptide in the context of MHC class I H2Kb, results in significant platelet clearance as compared to transfusion with wildtype platelets. Clearance kinetics demonstrate platelet loss starting at 8 hours and peaking at 24 hours, and are similar whether OT I mice are naïve or previously primed with mOva splenocytes. In addition, OT I CD8 T cells show increased expression of activation markers at 24 hours post transfusion. Mechanistic studies examining the role of common cell-mediated cytolytic molecules, such as perforin and granzyme, are underway.

Conclusions: This work extends the ability of CD8 T cells to mediate platelet clearance to a minor antigen, providing insight into the mechanisms underlying platelet refractoriness with significant implications for treatment of non-antibody-mediated platelet clearance.

PB 1343 | Endothelial Expression of TLT-1 Binding Pockets Mediate Platelet-endothelial Interaction, Leukocyte Transmigration, and Angiogenesis

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Background: Trem like transcript (TLT)-1 is one of the most abundant platelet proteins, yet its true function remains an enigma. TLT-1 is a receptor, stored in the a-granules, and expressed and released as a soluble fragment (sTLT-1) upon activation.

Aims: Our recent characterization of TLT-1 antibodies showed that a greater amount of TLT-1 co-localized with pro-angiogenic VEGF-containing (31±9%) than anti-angiogenic endostatin-containing α-granules (8±4%), leading to the hypothesis that TLT-1 has angiogenic properties

Methods: To test this, we used HUVECs to demonstrate that sTLT-1 mediates filopodia formation and actin polymerization when compared to control proteins and that labeled sTLT-1 can be up-taken into the early endosomes as denoted by co-localization with the early endosomal marker EEA where labeled BSA is not.

Results: The functional consequences of sTLT-1 interactions with endothelial cells (ECs) were demonstrated by a marked increase of IL-6 ($p \leq 0.005$) and IL-8 ($p \leq 0.0005$) compared to controls. Soluble TLT-1 also markedly increased angiogenic structures of endothelial cells ($p \leq 0.005$). Both the increased cytokine production and angiogenic features induced by sTLT-1 were reversed in the presence of anti-TLT-1, suggesting that TLT-1 mediates EC activation. *In vivo* evaluation using the *treml1*$^{-/-}$ mouse and a Lewis Lung Carcinoma model demonstrated that while no growth differences were witnessed, *treml1*$^{-/-}$ mice (n=12) demonstrated greater survival compared to wild type (*wt*; n=13; 66.6% verse 23.1%; p≤0.023). *Treml1*$^{-/-}$ mice demonstrated a dramatic absence in pecam staining compared *wt* mice (7% vs 1% of total area). The addition of anti-TLT-1 to *wt* mice markedly decreased vessel formation while sTLT-1 significantly increased vessel formation in null mice, suggesting that TLT-1 affects the endothelium *in vivo*.

Conclusions: Use of a TLT-1 fc chimera demonstrates that endothelial cells express TLT-1 binding sites allowing us to conclude that ECs recruit platelets to mediate their function.

PB 1344 | Extracellular Vesicles from Activated Platelets: A Quantitative Cryo-electron Microscopy and Immuno-Gold Labeling Study

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Background: Upon activation, blood platelets release two types of extracellular vesicles (EV), namely µm-large EV named microparticles characterized by the presence of phosphatidylserine (PS) at their surface, which supports their role in hemostasis and in thrombosis[1], and small EV (50-100 nm) called exosomes characterized by the exposure of CD63[2].

Aims: This study aims at providing a quantitative description of the size, phenotype and relative amount of PS+, CD41+ and CD63+ EV, using cryo-electron microscopy (EM) and immuno-gold labeling[3].

Methods: Peripheral blood was collected over citrate from four healthy adult donors after informed consent. Activation of platelet rich plasma (PRP) was performed with thrombin, TRAP and CRP-XL. Gold nanoparticles conjugated with annexin-5 or with anti-CD41 or anti-CD63 antibodies were synthesized to label PS+ EV, platelet-derived EV and CD63+ EV, respectively[3]. Cryo-EM was performed as described[3].

Results: EV activated by the three agonists presented a similar size distribution, about 50% of them ranging from 50 to 400 nm. About 60% EV were found to expose CD41, a majority of them exposing also PS. Several mechanisms of EV formation are proposed to explain the presence of large amounts (40%) of CD41-negative or PS-negative EV of large size, as well as large EV containing organelles, principally mitochondria or granules[4] .

In addition, we found that the majority of EV in activated platelets exposed CD63. Two populations of CD63+ EV were distinguished, namely large EV with low labeling density and small EV, likely the exosomes, with high labeling density.

Conclusions: This study provides an unprecedented description of EV from activated platelets.

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2. Heijnen et al., *Blood* 1999,94:3791-9

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PB 1345 | Renal Denervation Decreases the Platelet Activation Status in Hypertensive Patients

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Background: Hypertension is associated with increased arterial thrombotic events. Increased shear stress and sympathetic tone can affect platelet activity, a key player in the pathophysiology of atherothrombotic complications. Renal denervation (RDN) reduces sympathetic activity and blood pressure (BP) in patients with resistant hypertension. However, its effect on platelet activity has not been investigated.

Aims: To determine whether RDN-induced reduction in sympathetic nerve activity affects platelet activation in patients with hypertension.

Methods: Ambulatory BP, and platelet activation markers P-selectin expression, glycoprotein αIIbβ3 (GPIIb/IIIa) activation (binding of antibody Pac-1), monocyte-platelet aggregate formation (MPA) and soluble P-selectin were measured at baseline, 3 months, and 6 months after RDN in 41 patients.

Results: RDN significantly reduced ambulatory BP at 3 months (150.6±11.2 / 81.0±11.3 mmHg to 144.7±11.8 / 77.9±11.0 mmHg; $P< 0.05$), which was sustained at 6 months (144.7±13.8 / 78.6±11.1 mmHg; $P< 0.05$). Expression of P-selectin on the platelet membrane, the most sensitive platelet activation marker, significantly decreased at 3 months ($P< 0.05$) and 6 months ($P< 0.05$), indicative of a reduction in platelet activation. Soluble P-selectin was also reduced at 3 months ($P< 0.05$) and 6 months ($P< 0.05$). MPA was reduced at 3 months ($P< 0.05$) after RDN. The GPIIb/IIIa activation status showed a non-significant trend of reduction. Notably, patients who received aspirin as part of their medication regime did not show reduction in P-selectin expression following RDN.

Conclusions: Our results indicate that RDN can effectively reduce BP and at the same time have an additional beneficial effect on platelet activation. In patients on aspirin medication, RDN does not show further reduction of the platelet activation status. These results provide additional mechanistic explanations by which RDN can have favourable results in reducing cardiovascular complications.

PB 1346 | Upregulation of Inflammatory Transcripts in Platelets of Patients with Nonalcoholic Fatty Liver Disease (NAFLD): Role of Platelets in the „Inflammatory Network“ of NAFLD

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Background: Non alcoholic fatty liver disease (NAFLD) and non alcoholic steatohepatitis (NASH) are associated with a significant increase of cardiovascular events that represent the major cause of mortality. The presence of a systemic proinflammatory/prothrombotic state, contributing to the cardiovascular events, has been hypothesized in these diseases.

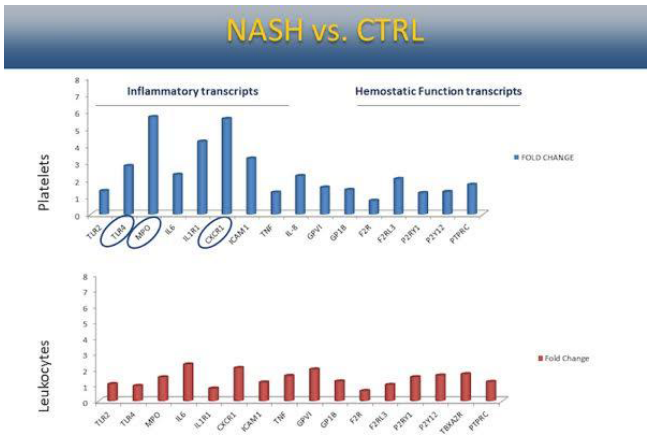

FIGURE 1 Fold changes of inflammatory and hemostatic transcripts in NASH vs controls. a) platelet b) leukocytes

Aims: The aim of this study was to assess whether chronic inflammatory stimuli in NAFLD are associated with proinflammatory/prothrombotic changes in platelets by investigating the expression of platelet inflammatory and hemostatic transcripts.

Methods: Twenty patients with histologic diagnosis of NASH, 10 patients with NAFLD and 15 healthy volunteers were recruited. The differential expression of hemostatic (GPVI, GPIB, F2R, F2RL3, P2Y1, P2Y12, TBXA2R) and of inflammatory transcripts (TLR2, TLR4, IL6, IL1R1, MPO, CXCR1-the IL8 receptor-, ICAM1, TNF) was investigated in platelets of patients and controls. Plasma levels of inflammatory cytokines TNF-alpha, leptin, IL6 and LPS were measured in the three groups.

Results: NASH patients had increased expression of CXCR1, TLR4, ICAM1, MPO and IL1R1 platelet inflammatory transcripts compared to controls. Expression of CXCR1 was significantly increased in NASH compared to NAFLD patients. The hemostatic transcripts of platelets were similar in the three populations. Surprisingly, the inflammatory transcripts were unchanged in leukocytes of NAFLD and of NASH subjects compared to controls. Plasma markers of inflammation, such as leptin and IL6, and plasma LPS levels were significantly higher in NASH and NAFLD patients compared to controls.

Conclusions: In NASH patients a pro-inflammatory phenotype of platelets was associated with elevation of systemic inflammatory markers and of LPS, suggesting that platelets play a role in the inflammatory network of the disease. The platelet pro-inflammatory phenotype might represent a link between inflammation and increased cardiovascular risk of NAFLD.

PB 1347 | Characterization of PF4-heparin Complexes by Photon Correlation Spectroscopy and Zeta Potential

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Background: Heparin-induced thrombocytopenia (HIT) is associated with antibodies to complexes between heparin and platelet factor 4 (PF4). HIT antibodies preferentially recognize macromolecular complexes formed between PF4 and polyanionic heparins over a limited range of molar ratios.

Aims: To investigate the complexes that human PF4 forms with heparins from various species and organs, along with different low molecular weight heparins (LMWHs) at several stoichiometric ratios to evaluate their sizes and charges by Photocorrelation Spectroscopy and Zeta Potential.

Methods: PF4, from human platelets (hPF4), was purchased from ChromaTec, Greifswald, Germany. The porcine heparin, Medefil (Glendale Heights, IL), Bioiberica (Barcelona, Spain) and Hepalink (Shenzhen, China), LMWH included enoxaparin (Sanofi Aventis, Paris, France) Lovenox; tinzaparin, (LEO-Pharma, Ballerup, Denmark) and dalteparin, (Pharmacia AB, Uppsala, Sweden). Bovine mucosal heparin was provided by Bioiberica (Madrid, Spain). Ovine heparin was provided by Kin Master (Passo Fundo -RS, Brazil). The pentasaccharide (fondaparinux) was from GlaxoSmithKline (Verona, Italy).

Results: The resulting data of the PF4 complexes with heparins (UFHs), LMWHs and their fractions, and components suggest that the size of aggregates is not only a simple function of average molecular weight (MW) but also of the MW distribution. At lower concentrations, ovine mucosal heparin formed large PF4/heparin complexes as compared to porcine and bovine heparin.

Conclusions: Generally the largest complexes occur at the molar ratios that lead to charge neutralization. Discrepancies are observed when samples are too small to bind more than one molecule of PF4 as in case of oligomers smaller than decasaccharides or when, because of polydispersion of the MW, small chains compete with longer chains for binding to PF4. The size of the aggregate is not a simple function of average MW but it is also affected by the MW distribution of the sample.

PB 1348 | Acetylsalicylic Acid Differentially Limits Activation and Apoptosis of Human Platelets Exposed to Various Staphylococcus aureus Strains

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Background: In addition to their haemostatic functions, platelets alter their inflammatory response according to the bacterial stimulus. *Staphylococcus aureus* is associated with exacerbated inflammation

during sepsis and poor prognosis as it induces thrombocytopenia. Acetylsalicylic acid and statins are active against platelet aggregation and decrease the mortality rate in sepsis patients.

Aims: Therefore, it is crucial to investigate these molecules, which have antiplatelet activities and protect platelets exposed to *S. aureus*, to limit thrombocytopenia and inflammatory responses.

Methods: Platelets were exposed to live clinical and reference strains of *S. aureus* in the presence or absence of acetylsalicylic acid or fluvastatin. Platelet viability, expression of activation markers, aggregation, and release of soluble CD62P, soluble CD40L, RANTES and GROα, which are the major pro-inflammatory platelet factors, were assessed. Apoptosis was evaluated by analyzing mitochondrial membrane potential, phosphatidylserine exposure and platelet microparticle release.

Results: All *S. aureus* strains induced platelet activation (increased expression of CD62P and CD63) and the release of RANTES and GROα; however, none altered platelet aggregation. Staphylococcal strains triggered platelet death via a decrease in the mitochondrial membrane potential and an increase in phosphatidylserine exposure, but no changes in platelet microparticle formation were found. We next observed that acetylsalicylic acid, but not fluvastatin, limited platelet activation and inflammatory factor release and restored platelet count by protecting platelets from *Staphylococcus*-induced apoptosis.

Conclusions: This study demonstrates that acetylsalicylic acid limits several of the effects induced by clinical strains of *S. aureus* on platelets in vitro. These results suggest new strategies to prevent bacteremia associated-inflammation.

PB 1349 | Tyrosine Kinase Inhibitor Pazopanib Inhibits Platelet Procoagulant Activity in vitro and in Renal Cell Carcinoma Patients

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Background: Pazopanib is an oral tyrosine kinase inhibitor (TKI) used for cancer treatment. Mild bleeding has been reported in patients treated with pazopanib.

Aims: As tyrosine kinases are essential for platelet signaling, the effects of pazopanib on platelet function were investigated in vitro and in renal cell carcinoma (RCC) patients.

Methods: Whole blood, platelet-rich plasma or isolated platelets from healthy volunteers (controls) were incubated with pazopanib or vehicle. In addition, blood was obtained from 6 patients diagnosed with metastatic RCC, both before and at 14 days on-treatment with pazopanib. Light transmission aggregometry, whole blood perfusion over collagen, flow cytometry, western blotting, and changes in $[Ca^{2+}]_i$ were measured.

Results: In control platelets, pazopanib reduced collagen- and ADP-receptor induced integrin activation and secretion, as well as aggregation and rises in $[Ca^{2+}]_i$, accompanied by diminished GPVI-dependent tyrosine phosphorylation of multiple proteins. After whole blood perfusion over collagen at high shear rate, thrombus height, size and phosphatidylserine (PS) exposure were reduced by pazopanib. Reduced GPVI-induced PS exposure by pazopanib was confirmed by flow cytometry. Pazopanib treatment
(14 days) moderately, but significantly decreased platelet count in the patients. In blood from patients on-treatment, thrombus formation under flow was altered by a marked reduction in PS exposure with limited effects on thrombus height and size.

Conclusions: Administration of pazopanib in vitro suppresses key GPVI-induced platelet activation responses, including thrombus formation, tyrosine phosphorylation, $[Ca^{2+}]_i$ rises and PS exposure. In treated cancer patients, pazopanib effects are confined to a reduction in GPVI-dependent PS exposure. Together with the mild reduction in platelet count, this may contribute to the higher bleeding tendency observed in patients treated with this TKI.

PB 1350 | Tunable Activation of Therapeutic Platelet-Rich Plasma by Pulse Electric Fields: Differential Effects on Clot Formation, Growth Factor Release and Platelet Morphology

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Background: Activation of platelet-rich plasma (PRP) by pulse electric fields (PEFs) produces gels and releases growth factors which promote wound healing.

Aims: To determine the parameters of platelet gel strength and the profile of released factors following activation of PRP with different PEF conditions and characterize the mechanism of PEF-induced factor release.

Methods: Concentrated PRP from normal donor blood (n=5) was activated by different PEFs (condition A or B) in the presence of $CaCl_2$. Clot formation was evaluated by thromboelastography (TEG), platelet surface exposure of markers of degranulation of α-granules (P-selectin) and T-granules (TLR9 and protein disulfide isomerase [PDI]) by flow cytometry, growth factors and soluble PDI by ELISA and morphology by transmission electron microscopy (TEM).

Results: By TEG, time to initial clot formation was shorter with thrombin (< 1 min) than with PEF A (4.4 min) and B (8.7 min, $p < 0.05$), but clot strength was greater with PEF B (80.7 mm) than with either thrombin (40.9 mm) or PEF A (40.6 mm, $p < 0.05$). Supernatants of PRP activated with PEF A had higher epidermal growth factor (EGF) levels than supernatants from all other conditions. Platelet surface TLR9 and PDI were higher after thrombin than after PEF A or B, whereas

the supernatant contained higher levels of PDI after PEF A than after thrombin. By TEM, platelets in PEF-treated samples had a distinct morphology and retained a subset of granules.

Conclusions: PEF conditions can be modulated to produce therapeutic platelet gels as strong or stronger those produced by thrombin, and this is tunable to produce growth factor profiles enhanced in specific factors important for different stages of wound healing. Moreover, the distinct morphology and the pattern of growth factor release suggest PEF initiates regulated release of contents rather than mechanical fracturing.

PB 1351 | Bacteria Educated Platelets (BEPs) - Altered RNA expression in Human Platelets Induced by Gram Negative Bacteria

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Background: Blood platelets contain ~9500 different mRNAs, different classes of non-coding RNAs and ~ 16 kb mitochondrial DNA. They are unable of de novo gene transcription, but contain mRNA splicing and translation machinery to produce functional proteins.

Aims: The aim of the study was to analyze the platelet transcriptome following exposure to E.coli bacteria, paying specific attention to changes, that could have a functional effect.

Methods: Citrated blood of healthy donors (n=4) was used to isolate platelets by OptiPrep density gradient centrifugation. Washed platelets were incubated with the non-pathogenic E. coli K12 and the pathogenic E.coli O18 strain in 1:5 or 1:1 platelet to bacteria ratio for 3 hours. Platelet RNA was isolated by the Trizol method and sequenced on the Hiseq 2500 Illumina platform. Platelet surface proteins were analyzed by flow cytometry on BD FACSCalibur using anti-CD41-APC, anti-P-selectin and anti- CD63 antibodies.

Results: Analysis of the intron-spanning reads (log counts per million > 3) allowed the identification of 136 genes that were differentially expressed in the K12 treated platelets, compared to the control ones. In the O18 treated platelets the expression of 176 genes was affected. (P-value < 0.05). Thirty-two genes overlapped between the two strains, the rest of them was strain specific. Most RNAs show a 4-8 log-fold decrease. Different RNA entities were affected by lower and high bacteria concentrations. Evaluation of pre-mRNA splicing is in progress in addition to detailed pathway analysis.

By flow cytometric analysis we found, that incubation of platelets (ratio 1:5) with K12 shows higher P-selectin level on the surface than after O18 incubation (~20 vs.~13%).

Conclusions: Pathogenic and non-pathogenic E.coli strains have common as well as strain specific effects on the platelet transcriptome. The effect is also dependent on the platelet to bacteria ratio. This could be an indication of a different role of platelets when exposed to lower or large number of bacteria.

PB 1352 | Ascorbic Acid on Platelet Activation. New Functions for an Old Drug

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Background: Early studies have shown that ascorbic acid (Asc), inhibits platelet (Plt) by reducing the levels of reactive oxygen species. However, recent studies revealed that excessive vitamin C consumption may aggravate cardiovascular diseases in susceptible populations.

Aims: We evaluated Plt responses at normal or low concentration of classical agonist.

Methods: Plt aggregation was quantified by LTA, fibrinogen binding and p-selectin externalization were evaluated by FACS. The level of HNO, an interesting reactive nitrogen specie, produced by Plt was determined by the classical spectral changes in a manganese(III) prophyrin and by a new HNO-selective electrode (based on a cobalt(II) porphyrin).

Results: Plt aggregation induced by AA 0.9mM or PAR1-AP 6μM were significantly inhibited when Asc 10mM was added to the aggregometer cuvette (49±10 or 24±9% of inhibition for AA or PAR1-AP, respectively). This effect was dependent on GPIIbIIIa activation, revealed by a similar inhibition of fibrinogen binding obtained in the presence of Asc. Moreover, p-selectin externalization was also abrogated by Asc. All the inhibitory effects on the Plt activation responses tested were dependent on the concentration of Asc.

Surprisingly, when Plt were stimulated with non-aggregating concentrations of the agonists, the presence of Asc resulted in an aggregatory response (60±5 or 45±6% of aggregation for AA 0.2mM or PAR1-AP 3μM, respectively, p< 0.05).

HNO was produced concomitantly with the aggregation induced by AA 0.9mM or PAR1-AP 6μM (290±20 or 216±36nM, respectively). Interestingly, the HNO production was significantly higher in the presence of Asc (963±146 or 980±126nM). However, HNO was not detected when Plt were stimulated by non-aggregating concentrations of agonists.

All these responses were dependent on the concentration of Asc.

Conclusions: Altogether, these results present Asc as a dual modulator for Plt aggregation and present evidence of HNO production in primary cell as a new endogenous negative regulator for Plt function.

PB 1353 | Lipopolysaccharide Do Not Affect Platelet Activation in vitro

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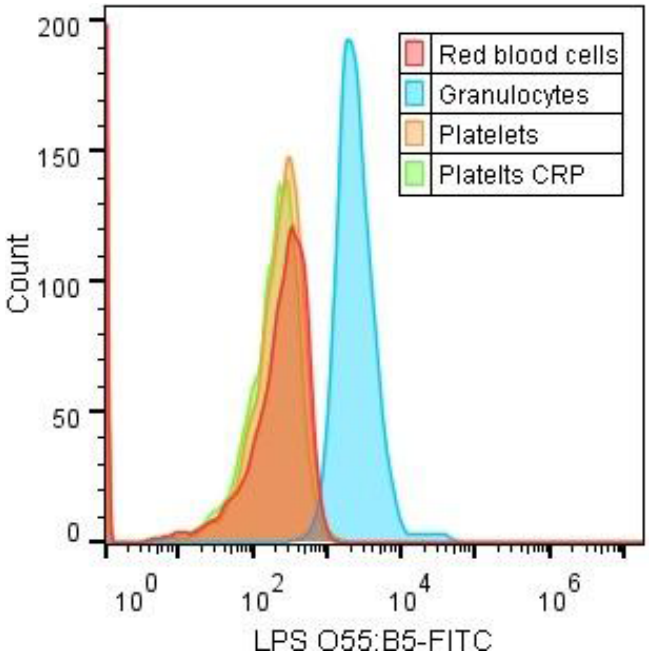

FIGURE 1 Binding of LPS by different cells

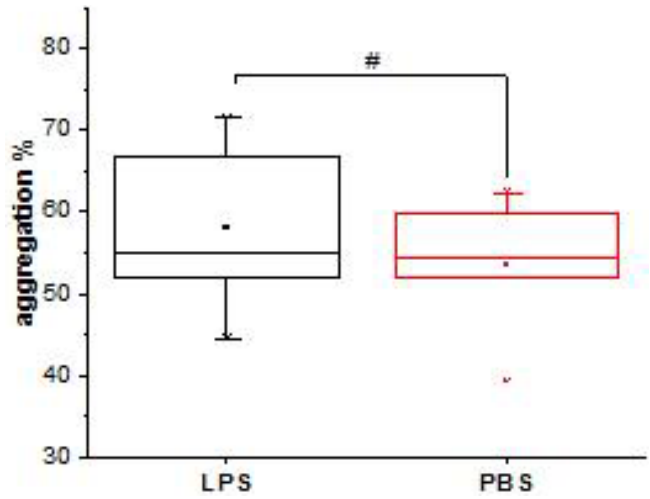

FIGURE 2 Aggregometry assay

Background: During severe infection bacteria get into the bloodstream where they can directly or indirectly influence coagulation system functioning. In the last decade many studies reported platelets interaction with bacteria through different pathways. One of the most controversial of them is activation of platelets through toll like receptor 4 (TLR4) by lipopolysaccharides (LPS) of gramnegative bacteria.

Aims: Throughout investigation of platelet pre-activation by bacterial endotoxins.

Methods: Platelet activation was assessed by aggregometry, spectrofluorimetry, flow cytometry and confocal microscopy of Fura-Red loaded platelets. Experiments were conducted with LPS O55:B5 or O111:B4 from Sigma. Also a non-pathogenic Escherichia coli XL was used as a model LPS-containing surface. Whole blood, platelet rich plasma (PRP) or washed platelets were incubated with 1-100 ug/ml LPS 0-2 hours with gentle mixing at room or body temperature prior to activation with 1-10 uM ADP or 0.03-10 ug/ml collagen related peptide. Computational modeling of platelet activation by LPS through TLR4 receptor was used to assess capabilities of this signaling pathway.

Results: LPS binding to platelets were not detected by flow cytometry (Fig.1). No significant difference of PRP treated/untreated with LPS was detected in aggregometry assay with ADP (Fig. 2). CRP - induced α- or δ-granule release, integrin activation or phosphatidylserine exposure were not significantly different for platelets incubated with LPS at all conditions. Platelets interaction with E.coli in confocal microscopy was not observed. Changes in form, behavior or cytosolic calcium concentration of immobilized on fibrinogen single platelets after incubation with LPS were not observed. Our calculations predict that due to very low concentration of soluble CD14 in healthy blood platelet activation by LPS should take up to 24 hours.

Conclusions: We are forced to conclude that there is no significant direct pre-activation of platelets by LPS in vitro.

PB 1354 | Intercellular Signalling between Platelets and a Myeloid Leukemic Cell Line

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Background: Platelets play a role in cancer metastasis however the mechanisms whereby they modulate circulating tumour cells remains unclear.

Aims: To explore the intercellular signalling between human platelets and a human megakaryocytic/erythroleukemic cell line (HEL) via changes in the ubiquitous second messenger, intracellular Ca^{2+} ($[Ca^{2+}]_i$).

Methods: $[Ca^{2+}]_i$ responses were measured in fura-2-loaded stirred suspensions of HEL cells and platelets using dual excitation spectrofluorimetry. Measurements were made from a pure population of each cell type and in a mixed population in which only one cell type was loaded with fura-2. Extracellular ATP was measured using a luciferin-luciferase luminescence assay. P2 receptor expression was determined using quantitative PCR.

Results: Thrombin evoked a transient increase in $[Ca^{2+}]_i$ in both platelets and HEL cells. Following co-incubation, the platelet $[Ca^{2+}]_i$ increase was unaffected while the HEL cell response was significantly potentiated (2.2 ± 0.23 fold). PAM_3CSK_4 (Toll-like receptor 2/1 agonist) caused a $[Ca^{2+}]_i$ increase in HEL cells only when co-incubated with platelets. Both agonists evoked ATP release from platelets, but not HEL cells, over a time course that mirrored the enhanced Ca^{2+} responses. HEL cells showed robust $[Ca^{2+}]_i$ responses to both ADP and ATP and expressed multiple P2 receptor subtypes (P2X4>P2Y11>>P2X1>P2X7>P2X6>P2Y12>P2Y13>P2Y2). The platelet-dependent HEL cell responses were unaffected by inhibition of P2X1 with NF449 but significantly reduced by the P2Y11 antagonist NF157. An increase in type VII apyrase (0.32 to 3.2U/ml) converted the platelet-induced

HEL cell response into a biphasic response, which can be explained by conversion of ATP to ADP.

Conclusions: These experiments demonstrate synergistic interactions between platelets and a myeloid cancer cell line at the level of Ca^{2+} signalling. Release of ATP and ADP followed by activation of P2Y11 receptors can at least partially explain the underlying mechanism.

PB 1355 | An Optimized Protocol for Platelet Rich Plasma Preparation to Improve its Angiogenic and Regenerative Properties

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Background: Although platelet rich plasma (PRP) is used for regenerative medicine as source of platelet-derived growth factors, its effectiveness remains controversial partially due to the absence of PRP preparation methods based on regenerative role of platelets.

Aims: We aimed to optimize PRP current protocols analyzing PRP dilution (to reverse the antiangiogenic effect of plasma factors); 4°C preincubation (as low T° increases platelet degranulation); and supplement with fibrin cryoprecipitate (as provisional matrix for regeneration) by evaluating angiogenic and regenerative responses. Optimization was compared with current PRP-preparation methods (37°C, no PRP dilution or supplements).

Methods: PRP pure or 25% diluted with saline, incubated at 37 or 4°C 30min, with or without supplementation with cryoprecipitate from frozen plasma were activated with $CaCl_2$ (22mM). Then, supernatants were used to induce: angiogenesis *in vitro* (HMEC-1 proliferation, migration and tubule formation), *in vivo* (chorioallantoic membrane, CAM) and skin mice regeneration of full-thickness excisional wounds (perimeter closure and histology). ANOVA, n=6-12, P< 0.05.

Results: We found that plasma-free platelets supernatants induced higher angiogenesis than PRP. The antiangiogenic effect of plasma was decreased by diluting PRP (Table). Moreover, angiogenesis was improved by both PRP preincubation at 4°C and by cryoprecipitate supplementation (Table). Combination of optimizing variables exerted an additive effect increasing angiogenesis not only *in vitro*, but also *in vivo* (optimized-PRP, Table). Furthermore, optimized PRP treatment induced a faster (3-7 days) and more efficient skin mice wound repair (determined by wound closure, angiogenesis, and epidermal thickness and granulation tissue reduction) compared to non-optimized PRP (7-14 days).

Conclusions: Our findings indicate that 25% PRP dilution, 4°C preincubation and cryoprecipitate supplementation improve angiogenic and regenerative properties of PRP compared to current methods.

PB 1357 | Treatment of Patients with Autologous Platelet Rich Fibrin is Dependent on the Composition of Peripheral Blood Cells: Dental Implant Stability is Associated with Red Blood Cells and Not with Platelets

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Background: Platelet Rich Fibrin (PRF) is a platelet-fibrin membrane clot clinically used to accelerate wound healing and bone regeneration. PRF membranes are prepared from the patient's own blood using a dedicated centrifugation protocol. In dental implantology PRF has been used to improve osseointegration of dental implants achieving implant stability.

TABLE 1 Angiogenesis mediated by supernatants from different PRP-preparation protocols

	Non-optimized PRP (100%, 37°C, without cryoprecipitate)	Washed Platelets	Diluted PRP (25%)	PRP (100%) 4°C preincubation	PRP (100%) supplemented with plasma cryoprecipitate	Optimized-PRP (diluted + 4°C preincubation + cryoprecipitate)
HMEC-1 Proliferation (pnpp)(in vitro)	1.7±0.2*	2.4±0.2*#	2.4±0.2*#	2.4±0.2*&	1.9±0.1*	4.2±0.2*Ŧ
HMEC-1 Migration (wound healing)(in vitro)	2.0±0.1*	4.0±0.5*#	3.5±0.3*#	2.5±0.3*	2.3±0.2*	5.1±0.3*Ŧ
HMEC-1 Tubule Formation (matrigel)(in vitro)	6.0±0.5*	8±1*#	8±1*#	6±1*	11±1*¥	12±1*Ŧ
CAM (in vivo) Blood vessel Branch Points	1.4±0.1*					2.4±0.2*Ŧ

Results are expressed as fold of unstimulated control. ANOVA n=6-12.*p<0.05 vs unstimulated; #p<0.05 vs PRP without dilution (or 100%); &p<0.05 vs 37°C; ¥p<0.05 vs without cryoprecipitate; Ŧp<0,05 vs non-optimized PRP.

Aims: We have introduced PRF for several years (N=250 patients) and we experience improved osseointegration of oral implants in patients treated with PRF. How PRF improves dental implantation is unknown and therefore we analyzed the influence of the composition of PRF on dental implant stability.

Methods: PRF membranes were prepared with different centrifugation protocols. The composition of PRF was analyzed with scanning electron microscopy. We included 90 patients to study the effect of blood cell- and fibrinogen levels on PRF. Correlation with clinical outcome was investigated in 20 patients by measuring the Implant Stability Quotient (ISQ) 17 weeks after treatment with PRF.

Results: We found that the PRF composition was influenced by the centrifugation method and the composition of peripheral blood cells. PRF membrane length was significantly inversely correlated to hemoglobin, hematocrit and erythrocytes, while leucocytes were not associated with PRF membrane length. PRF membrane length was increased with increased platelet count. Furthermore, we found that the ISQ value in patients, 17 weeks after implantation with PRF, was significantly correlated with increased red blood cells and not with fibrinogen, platelets and leukocytes.

Conclusions: The composition of PRF is significantly correlated to the composition of patient‘s blood. We show that red cell number was the dominant factor that determines implant stability. ISQ was not correlated with platelets, leukocytes and fibrinogen. Our findings give new insights into the mechanisms how PRF affects osseointegration and are the first steps to develop an optimal protocol for the use of PRF in oral implantology.

PB 2194 | Inhibitors of Apoptosis Signal-regulating Kinase 1 (MAP3K5), as Anti-thrombotic Agents

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Background: Recently, we showed that ASK1 is rapidly activated by physiological agonists in human platelets. Ablation of *Ask1* gene results in impaired platelet functions making it an important target for thrombosis.

Aims: To evaluate the effect of two structurally distinct ASK1 inhibitors; IPTB and GS-4997 on thrombosis.

Methods: ASK1 activation was determined by western blotting. Tail bleeding or laser-induced injury was used to assess hemostasis. $FeCl_3$-induced arterial thrombosis and pulmonary thromboembolism were used as thrombosis models.

Results: We found that GS-4997 and IPTB dose-dependently (100nM-5mM) inhibited thrombin-induced ASK1 and its downstream effector p38 MAPK activation, aggregation, and spreading of human platelets as well as clot retraction. Pretreatment of the whole blood with these inhibitors significantly (P=0.001) reduced thrombus formation under arterial flow without affecting platelet adhesion to collagen. *In-vivo* thrombosis assay revealed that C57BL/6 mice injected with IPTB (100mg/kg) or GS-4997 (100mg/kg), showed a significantly increased time of occlusion (P=0.028 and P=0.005 respectively). Furthermore, IPTB or GS-4997 protected mice against collagen/epinephrine-induced pulmonary thromboembolism. Out of 26 saline-treated mice only 3 survived whereas, 10 out of 11 mice treated with GS-4997 (100mg/kg) survived (P=0.0002). In case of IPTB 9 out of 12 treated mice survived (P=0.0028). Interestingly, hemostasis assays revealed that C57BL/6 mice treated with IPTB (1mg/kg) or GS-4997 (1mg/kg), had no effect on bleeding time or platelet accumulation as observed by tail bleeding or laser-induced injury model respectively, suggesting that both inhibitors had no effect on *in-vivo* hemostasis.

Conclusions: Our results strongly suggest that both IPTB and GS-4997 protect the mice from thrombosis without affecting hemostasis. Further development of these inhibitors as a potential therapeutic agent to combat thrombotic disorders is highly warranted.

PB 2195 | The Oral Bruton’s Tyrosine Kinase Inhibitor Ibrutinib Selectively Blocks Atherosclerotic Plaque-induced Platelet Thrombus Formation

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Background: Platelet glycoprotein VI (GPVI) interaction with plaque collagen is essential to trigger atherothrombosis under arterial flow conditions, whereas platelet integrin α2β1 interaction with native collagen is critical for hemostasis after vessel wall injury. GPVI signals through Bruton´s tyrosine kinase (Btk) activation. Btk can selectively and irreversibly be blocked by ibrutinib, approved as anti-proliferative drug with a remarkable long term safety profile.

Aims: We explored the potential of ibrutinib as an oral GPVI-targeting plaque-selective antiplatelet drug.

Methods: Ibrutinib was studied *in vitro* and in blood from patients on oral ibrutinib therapy in static platelet assays and arterial flow models of human atherosclerotic plaque- and collagen-induced platelet activation.

Results: Ibrutinib suppressed GPVI-mediated static platelet aggregation in blood exposed to plaque homogenate and collagen fibers by -84% and -92% at 1μM, respectively, and with similar IC_{50} values. In contrast, platelet aggregation stimulated by thrombin-receptor-activating-peptide (-31%) or ADP (-13%) was only slightly compromised. Under arterial flow conditions ibrutinib dose-dependently inhibited platelet thrombus formation onto plaque homogenate (-98%) and plaque tissue (-76%) but not onto collagen fibers (+8%). Ibrutinib did not impair integrin α2β1-mediated platelet adhesion to immobilised soluble collagen. Moreover, in patients taking ibrutinib platelet thrombus formation under arterial flow onto plaque homogenate (-100%)

and plaque tissue sections (-83%) but not onto collagen fibers (+8%) was suppressed. *In vitro* bleeding time was not significantly increased in patients on ibrutinib.

Conclusions: Btk inhibition by ibrutinib suppresses GPVI-dependent platelet thrombus formation on atherosclerotic plaque from flowing blood, but spares hemostatic platelet function. It holds promise as lesion-focused antiplatelet drug that may complement established dual antiplatelet therapy.

PB 2196 | The anti-asthma therapeutic zafirlukast is a broad spectrum thiol isomerase inhibitor that inhibits platelet function

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Background: Several members of the thiol isomerase family of enzymes are present in, and released by platelets, inhibition of these enzymes results in inhibition of platelet responses including aggregation, adhesion and thrombus formation. To date, few pan thiol isomerase inhibitors have been characterised. The most studied, bacitracin is known to be nephrotoxic which limits its therapeutic usage. Therefore it is desirable to identify novel broad spectrum inhibitors of these enzymes.

Aims: In this study we screened compound libraries of existing therapeutics to identify compounds which inhibit thiol isomerase activity, and determined their effects on platelet function.

Methods: 3641 compounds were screened for inhibitory effects on the redox activity of ERp5, PDI, ERp57, ERp72 and thioredoxin (TRX) in an insulin turbidity assay. The most potent compound, zafirlukast (ZFL) was selected for further investigation. Human platelets were incubated with ZFL (0.1-10μM) or vehicle for 5 minutes prior to stimulation with collagen or CRP-XL (1μg/mL). Platelet aggregation and granule secretion were measured by lumi-aggregometry and flow cytometry. Spreading and thrombus formation under arterial flow conditions were measured by confocal microscopy.

Results: Compound library screening revealed that the cysteinyl leukotriene receptor antagonist ZFL inhibits the enzyme activity of PDI, ERp57, ERp72, TRX and ERp5. When applied to platelets, ZFL caused a concentration-dependent inhibition of platelet aggregation and granule secretion. Platelet spreading on collagen and thrombus formation was also inhibited in the presence of ZFL.

Conclusions: We demonstrate that the anti-asthma compound ZFL is a potent broad spectrum platelet thiol isomerase inhibitor which broadens the repertoire of compounds available to further study the role of thiol isomerases in platelet function and may represent a novel antithrombotic compound. Investigations are underway to establish the effects of ZFL on thrombosis and haemostasis in mice.

PB 2197 | Potentiation of TRAP-6-induced Platelet Dense Granule Release by Blockade of P2Y12 Signaling with MRS2395

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Background: The release of ADP from platelet dense granules and its binding to platelet P2Y12 receptors is crucial for amplifying the initial hemostatic response and propagating thrombus formation. P2Y12 has thus emerged as a therapeutic target to safely and effectively prevent secondary thrombotic events in patients with acute coronary syndrome or a recent history of stent placement. However, the response to P2Y12-targeted anti-platelet therapy is variable and some patients are still at risk for cardiovascular events. Several mechanisms of resistance to anti-P2Y12 therapy have been proposed, including, paradoxically, the increased release of ADP by platelet dense granules in response to anti-P2Y12 therapy.

Aims: To explore whether targeting of P2Y12 ADP receptor results in the release of its autocrine ligand.

Methods: Human washed platelets were treated with MRS2395 or ticagrelor, two P2Y12 inhibitors, and assessed for agonist-induced platelet dense granule release and calcium generation by employing 96-well plate luminescence and fluorescence assays, respectively. The effect of MRS2395 and ticagrelor on agonist-induced platelet dense granule trafficking was visualized by Superresolution Structured Illumination Microscopy (SR-SIM). Platelet molecular pathways downstream of P2Y12 inhibition were studied via pharmacology- and biochemistry-based approaches.

Results: Our results show that in platelets activated with the PAR-1 agonist TRAP-6 (thrombin receptor-activating peptide), inhibition of P2Y12 with the antagonist MRS2395, but not ticagrelor, potentiated human platelet dense granule trafficking to the plasma membrane and granule secretion, intracellular cytosolic Ca2+ influx and phosphorylation of GSK3β-Ser9 through a PKC-dependent pathway.

Conclusions: These results suggest that inhibition of P2Y12 with MRS2395 may act in concert with PAR-1 signaling and result in the aberrant release of ADP by platelet dense granules, thus reducing or counteracting the anticipated anti-platelet efficacy of these inhibitors.

PB 2198 | Discovery and in vitro Pharmacology of BAY-386 - A Novel, Reversible PAR-1 Antagonist

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Background: Antiplatelet therapy represents a cornerstone in the prevention of ischemic cardio- and cerebrovascular events. Protease activated receptor-1 (PAR-1) is the dominant receptor for thrombin, the most potent platelet activator.

Aims: Our aim is to identify novel antiplatelet agents to overcome efficacy and safety limitations of standard therapy. Specifically, we set out to find a potent, selective and reversible PAR-1 antagonist with the potential for oral activity.

Methods:

- Ultra high-throughput chemical library screening (uHTS) using TRAP- (thrombin receptor activating peptide) induced calcium release in HEK293 cells
- High affinity TRAP radioligand binding assay
- Human platelet aggregation induced by TRAP, thrombin, PAR-4 agonist, ADP, and collagen
- High shear (1000/s) human whole blood perfusion over collagen; measurement of downstream platelet activation
- Thrombin-induced cytokine mRNA and protein expression on cultured human umbilical vein endothelial cells (HUVEC)
- In vitro drug metabolic and pharmacokinetic characterization (DMPK)

Results:

- uHTS: phenylpiperidine class of compounds further optimized to BAY-386 (IC_{50}: 0.01 μM)
- Specific binding to PAR-1 on human platelet membranes (IC50: 0.056 μM)
- Inhibition of human platelet-rich plasma (PRP) aggregation by BAY-386 at IC_{50} of 0.43 and 0.14 μM against TRAP and thrombin, respectively
- No inhibition of PAR-4 agonist- or ADP- or collagen-induced aggregation
- Abrogation of inhibition of PRP aggregation by a standard platelet washing procedure
- Complete prevention of downstream platelet activation in whole blood flowing at high shear over collagen by BAY-386 (IC_{50} 5.6 μM)
- Block of thrombin-induced pro-inflammatory cytokine mRNA and protein expression by HUVEC, including MCP-1 and CXCL1
- In vitro DMPK profile compatible with oral activity.

Conclusions: BAY-386 is a potent, specific and reversible PAR-1 antagonist with the potential for oral treatment of arterial thrombosis.

PB 2199 | Selective Serotonin Reuptake Inhibitors Inhibit Convulxin- and Fibrinogen-induced Phosphorylation of Syk in Human Platelets

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Background: We previously reported that selective serotonin reuptake inhibitors (SSRIs) reduce platelet aggregation induced by collagen. Collagen and convulxin induce platelet aggregation through glycoprotein VI (GPVI)-FcRγ chain-Syk signaling pathway. In addition, fibrinogen induces platelet activation through αIIbβ3 integrin-FcγRIIa-Syk signaling pathway.

Aims: To investigate the effects of SSRIs on GPVI- and αIIbβ3 integrin-mediated signaling pathway.

Methods: Citalopram and escitalopram, two relatively pure SSRIs, were used in this study. Platelet aggregation was measured by aggregometry. The expression of αIIbβ3 integrin was measured by flow cytometry. Fibrinogen binding-induced activation was examined by the spreading assay on immobilized fibrinogen. Signaling pathways were evaluated by immunoprecipitation and Western blotting.

Results: Citalopram and escitalopram both concentration-dependently inhibited convulxin-induced platelet aggregation and the activation of αIIbβ3 integrin. Convulxin-induced phosphorylation of Syk, LAT, and Akt was inhibited by citalopram and escitalopram. Citalopram inhibited the interaction between FcRγ and Syk, whereas the phosphorylation of FcRγ in response to convulxin remained unchanged. Further, citalopram inhibited the increase of the interaction between serotonin transporter and Syk induced by convulxin. In the presence of Mn^{2+}, escitalopram inhibited the formation of filopodia and lamellipodia on immobilized fibrinogen. Escitalopram did not influence the binding of fibrinogen to platelets. It inhibited the phosphorylation of Syk, LAT, and PAK2 triggered by the adhesion on fibrinogen.

Conclusions: Our data demonstrate that citalopram and escitalopram inhibit GPVI- and αIIbβ3 integrin-mediated platelet activation. The mechanism of the inhibitory effect of citalopram or escitalopram is not the influence on the activation of GPVI and the interaction between fibrinogen and αIIbβ3 integrin, but the interaction between Syk and its upstreaming molecules.

PB 2200 | Citalopram Inhibits Platelet Function through a SERT-Independent Mechanism

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Background: Selective serotonin reuptake inhibitor (SSRI) antidepressants reduce the rate of serotonin (5-HT) uptake by the serotonin reuptake transporter (SERT). Since platelets also express SERT, SSRIs may modify platelet function and influence myocardial infarction risk. However, the cardiovascular benefits and risks of SSRIs are poorly characterised and *in vitro* data is limited. The SSRI citalopram is a racemate, the (*S*)-isomer being a more potent SERT inhibitor. Although citalopram has been shown to inhibit platelets *in vitro*, it is unclear whether this is mediated via SERT blockade.

Aims: To determine if citalopram inhibits platelet function via SERT blockade.

Methods: Washed platelets (WP) were prepared from citrated blood from healthy volunteers.

Platelet aggregation was quantified using turbidimetric aggregometry. Static adhesion to various ligands was measured by detecting acid phosphatase from lysed adherent WP. SERT activity was quantified by adding exogenous 5-HT (1 μM) to WP and determining the fall in supernatant 5-HT concentration over time. 5-HT concentrations were quantified using high-pressure liquid chromatography.

Results: Citalopram and its isomers inhibited platelet aggregation (pIC_{50} ≈ 4.2) and static adhesion (pIC_{50} ≈ 3.9), with no significant difference in the *S*:*R* isomer potencies. By contrast, 5-HT uptake was more potently inhibited by (*S*)-citalopram (pIC_{50} ≈ 8.6) than (*R*)-citalopram (pIC_{50} ≈ 7.4), giving an *S*:*R* potency ratio of approximately 16-fold.

Conclusions: 5-HT uptake into platelets was completely blocked by both citalopram isomers at concentrations that had no effect on platelet aggregation or adhesion. Despite an *S*:*R* potency ratio of 20 for 5-HT uptake, (*R*)-citalopram was equally potent at inhibiting platelet aggregation and adhesion. These findings strongly suggest that inhibition of platelet function *in vitro* by citalopram is not mediated via SERT blockade. Alternative mechanisms of action are necessary to explain the pharmacological effects of citalopram on platelets.

PB 2202 | What is the Optimal Treatment Option for A Patient with X-linked Thrombocytopenia?

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Background: X-linked thrombocytopenia (XLT) is an allelic variant of the Wiskott-Aldrich syndrome (WAS) which presents with a milder phenotype with bleeding but not the immune dysregulation as demonstrated in patients with WAS. XLT patients have profound thrombocytopenia, small platelets, hemorrhage is a major problem. Stem cell transplantation at an early age is the treatment of choice for patients with WAS, therapeutic options for patients with XLT are controversial.

Aims: We asked whether eltrombopag, a thrombopoietic agent would increase platelet counts and / or reduce beeding in XLT patient.

Methods: We report the first patient with XLT in Slovak republic carrying a novel missense mutation p.Y88H (c.296T>C). Platelet (PLT) count was very low since the birth (6-29x10^9/l). Major problem of patients is bleeding, he received repeated platelet transfusion for severe traumatic hemorrhage. We asked whether a thrombopoietic agent-eltrombopag- would increase platelet counts, and/or reduce bleeding in WAS/XLT patients.

Results: Our patient was 6-yeard old and platelets were very low (PLT:6 x10^9/l) at the time of starting treatment. Eltrombopag (50 to 75 mg) was administered orally once daily and dosing was adjusted to maintain the platelet count >50×10^9/L. Eltrombopag treatment was well tolerated and resulted in an increased platelet count (in 8 week-PLT:45×10^9/l, in 16 week- PLT-84×10^9/l), but less that in control (5 pediatric chronic immune thrombocytopenia patients). Platelet activation was assessed by whole blood flow cytometry. Agonist-induced platelet surface activated glycoprotein GP IIb/IIIa were proportional to platelet size and therefore decreased compared with controls. Immature platelet fraction was significantly less increased compared with controls.

Conclusions: In XLT patients, eltrombopag may reduce the risk of hemorrhage by increasing the platelet count and/or platelet turnover until a more definitive therapy is available or while awaiting the effectiveness of other therapy.

PB 2203 | The Effect of Vorapaxar on Platelet-mediated Inflammatory Response

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Background: Apart from their important role in haemostasis and thrombosis, platelets also participate in inflammatory reactions, through mechanisms that may involve the CD40 ligand (CD40L), P-selectin and cell-to-cell interactions with leukocytes. Vorapaxar is a specific antagonist of the protease-activated receptor-1 (PAR-1) that potently inhibits platelet aggregation and thrombosis.

Aims: We investigated the possible vorapaxar effects on various platelet activation markers and on platelet-leukocyte interactions.

Methods: Whole blood of healthy volunteers was incubated with vorapaxar at various concentrations (0.1μM to 2μM) for 60min at 37°C and then activated with 50μM TRAP-6 or ADP. Platelet activation was evaluated by flow cytometry, determining the membrane expression of P-selectin and CD40L using the monoclonal antibodies, anti-CD61-PerCP, anti-CD154-FITC and anti-CD62P-PE. CD40L was expressed as the percentage of $CD61^+$/$CD154^+$ cells, whereas P-selectin was expressed as Mean Fluorescence Intensity. Formation of platelet-monocytes and platelet-neutrophil conjugates was determined by dual labelling with anti-CD61-PerCP and anti-CD14-FITC or anti-CD45-PE, respectively. The platelet-monocyte and platelet-neutrophil conjugates were assessed as the percentage of $CD61^+$/$CD14^+$ and $CD61^+$/$CD45^+$ particles, respectively.

Results: Vorapaxar significantly inhibited the TRAP-6-induced membrane expression of P-selectin and CD40L, in a dose dependent manner showing IC50 values of 0.80μM and 0.64μM , respectively. Furthermore, vorapaxar (1μM) reduced by 100% and 86% the formation of platelet-neutrophil and platelet-monocyte conjugates, respectively induced by TRAP-6. Vorapaxar did not significantly influence the ADP effect on all the above parameters.

Conclusions: Vorapaxar potently attenuates platelet-mediated inflammatory response followed by PAR-1 activation an effect that may contribute to the reduction of acute limb ischemia and peripheral revascularization observed in patients with peripheral artery disease.

PB 2204 | APAC, a Dual Antiplatelet and Anticoagulant Attenuates Collagen-induced Platelet Aggregation and Fibrin Elasticity in Blood

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Background: APAC, a semisynthetic mimetic of heparin proteoglycans, inhibits both collagen-induced platelet aggregation and procoagulant activity *in vitro* and *in vivo* (Lassila&Jouppila STH 2014). In two baboon models of arterial thrombosis, collagen-coated shunt (Oregon, USA) and 30-90% stenosis model of injured femoral artery (FA) (Bloemfontain, SA) APAC reduced platelet thrombus formation, and maintained vessel patency. In PET labeling APAC retained in rat FA anastomosis site for 48 -120h.

Aims: We further characterized the effects of APAC on platelet aggregation in whole blood and platelet-rich plasma (PRP), and in global coagulation (ROTEM®) *in vitro.*

Methods: APAC-spiked citrated blood of healthy volunteers was challenged to collagen (3.2 µg/ml), ristocetin (0.77 mg/ml) and ADP (6.4 µM) in Multiplate®, and to collagen (0.5 µg/ml) in PRP and in ROTEM® for INTEM, EXTEM, FIBTEM (with cytochalasin D) and HEPTEM (with heparinase) activation.

Results: Overall, APAC dose-dependently prolonged lag time and decreased slope of aggregation. In blood APAC (150 µg/ml) inhibited collagen- and ristocetin (but not ADP) -induced aggregation by 58 ±15% (mean ± SD) and by 25 ±2%, respectively, unlike UFH. In PRP APAC specifically inhibited collagen-induced aggregation already at 1 µg/ml by 55 ±31%, 3 µg/ml by 75 ±15%, and 30 µg/ml by 85 ±11%. The inhibition of maximal aggregation varied, but improved in all donors by an increase of APAC dose. In ROTEM® APAC (3 µg/ml) uniquely prolonged all EXTEM, INTEM and FIBTEM clotting times (CT), 4.5-8-fold, while UFH (3 µg/ml) prolonged mainly INTEM. However, APAC prolonged INTEM CT 2-fold in comparison to UFH. Anticoagulation was abolished in HEPTEM.

Conclusions: APAC, inhibits collagen-, but not ADP-induced, platelet aggregation in citrated blood and PRP. In all donors APAC attenuated the initial aggregation. Moreover, in ROTEM® APAC globally attenuates platelet procoagulant activity and fibrin elasticity.

PB 2205 | Citrated Iron Oxide Nanoparticles Inhibit Thrombin-evoked Ca2+ Signalling in Human Platelets

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Background: Previous work in our lab has suggested that platelet Ca^{2+} signalling is potentiated through a pericellular recycling system.[1,2] This allows Ca^{2+} removed into the Open Canalicular system (OCS) to accumulate and recycle back into the cytosol through Ca^{2+}-permeable ion channels potentiating Ca^{2+} signals.[2] Localised buffering of Ca^{2+} within the OCS could interrupt this system and provide a new method to inhibit agonist-evoked changes in platelet function.

Aims: Here we aim to assess whether the absorption of citrate onto the surface of the nanoparticles may inhibit agonist-evoked by providing localised Ca^{2+} buffering in the pericellular region of the platelet.

Methods: Platelets were isolated from healthy volunteers under informed consent and with local ethical committee approval. Thrombin-evoked changes in cytosolic- and extracellular Ca^{2+} concentration were monitored in Fura-2-loaded platelets and washed platelet suspensions containing 2.5 µM Rhod-5N salt respectively, using our previously published methodologies.[2] Thrombin-evoked aggregation was assessed using absorbance changes on a microplate reader.

Results: Human platelets preincubated with 300 µM citrated iron oxide nanoparticles could be observed to bind nanoparticles under light microscopy. This treatments also buffered thrombin-evoked rises in extracellular Ca^{2+} concentration (50.4 ± 14.9% of control; n = 6; $P < 0.05$). In addition, pre-treatment with nanoparticles also significantly inhibited thrombin-evoked rises in cytosolic Ca^{2+} concentration (41.8 ± 4.0% of control; n = 6; $P < 0.05$), aggregation (27.6 ± 8.1% of control; n = 6; $P < 0.05$) and clot retraction (51.0 ± 12.2% of control; n = 7; $P < 0.05$)

Conclusions: These studies provide further support for the pericellular Ca^{2+} recycling hypothesis[1,2] and suggest that Ca^{2+} chelator-loaded nanoparticles targeted to human platelets might provide novel antiplatelet therapies.

Refs: [1]Walford *et* al.,(2016). Br *J Pharmacol* **173**, 234-247. [2]Sage *et al*., (2013).*Physiol Rep***1**, e00085.

PB 2206 | Induction of Diabetes Attenuates the Antithrombotic Effect of Clopidogrel in Apolipoprotein E-deficient Mice

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Background: Several studies have demonstrated that patients with diabetes mellitus (DM) exhibit an impaired platelet inhibitory response to clopidogrel. This impaired response may contribute to their increased risk of recurrent atherothrombotic events, despite the use of dual antiplatelet therapy. However, the mechanisms for the impaired response have not been fully elucidated.

Aims: To compare the effects of clopidogrel on thrombus formation in wild type (WT), apolipoprotein E-deficient (apoE KO) and diabetic apoE KO mice.

Methods: DM was induced by injection of streptozotocin (STZ) at 55 mg/kg/d (i.p., x 5 days) in 9-week old mice. Antithrombotic effects of clopidogrel at 10 mg/kg/d (p.o., x 5 days) were determined in a carotid arterial thrombosis model induced by $FeCl_3$ at 21 weeks. Antiplatelet effects were also determined by flow cytometry.

Results: Similar antithrombotic effects of clopidogrel were observed in WT and apoE KO mice. However, in diabetic apoE KO mice, clopidogrel's effects were attenuated compared to WT and non-diabetic apoE KO mice: Percent inhibition of thrombus area (μm^2) by clopidogrel was 85.5% for WT, 75.0% for apoE KO and 1.9% for diabetic apoE KO mice. The time to occlusion and lumen stenosis also reflected significant losses of clopidogrel's antithrombotic effects in diabetic apoE KO mice. Ex vivo platelet reactivity, assessed by ADP-induced activated GPIIb/IIIa expression, was inhibited similarly and completely by clopidogrel in all three mice groups, whereas the effects of clopidogrel on ex vivo platelet P-selectin expression induced by PAR4-activating peptide were diminished in diabetic apoE KO mice compared to WT and non-diabetic apoE KO mice.

Conclusions: These data suggest that STZ-induced diabetic apoE KO mice would be a useful model to further study the impaired responses to clopidogrel in DM patients, whose condition may in part reflect a reduction of clopidogrel's effect on thrombin-induced platelet activation.

PB 2208 | With an ASA Dose that Inhibits Prostacyclin Formation, Clopidogrel Has a Lesser Antithrombotic Effect

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Background: Dual-antiplatelet therapy (DAPT), the standard-of-care in acute coronary syndromes, combines acetylsalicylic acid (ASA) and a $P2Y_{12}$ antagonist to inhibit thromboxane A_2 (TXA_2)- and adenosine diphosphate (ADP)-mediated platelet activation. Both agents also influence platelet levels of cyclic adenosine monophosphate (cAMP), an inhibitor of platelet activation. Higher doses of ASA inhibit endothelial prostacyclin (PGI_2) formation, which otherwise would increase platelet cAMP levels, while $P2Y_{12}$ antagonists block the inhibitory effect of ADP on cAMP formation. Thus higher doses of ASA may be less effective than lower doses of ASA when combined with a $P2Y_{12}$ antagonist.

Aims: To determine in the context of DAPT with clopidogrel, whether an ASA dose that preserves endothelial formation of PGI_2, but inhibits platelet TXA_2 formation, provides greater antithrombotic effect than a higher dose of ASA.

Methods: Mice were dosed orally with ASA, clopidogrel or both. Platelet aggregation was evaluated using the Multiplate Analyzer. The effects of ASA on TXA_2 and PGI_2 were monitored by measuring arachidonic acid (AA)-induced platelet aggregation and plasma levels of 6-keto-PGF1α, respectively. Platelet cAMP levels were measured by an ELISA. Thrombus formation was evaluated using the laser-injury cremaster arterial model.

Results: ASA at 10 and 40 mg (0.15 and 0.6 mg/kg body weight) inhibited AA-mediated platelet aggregation to a similar extent, but only ASA 10 mg preserved PGI_2 formation. PGI_2 elevated cAMP levels and reduced platelet aggregation in thrombin-stimulated, clopidogrel-treated platelets, but not in platelets not treated with clopidogrel. Thrombi in mice given clopidogrel alone or DAPT with 10 mg ASA were smaller than in mice given DAPT with 40 mg ASA.

Conclusions: We provide animal evidence against the use of higher doses of ASA in DAPT, which inhibit PGI_2 formation, which otherwise would favour cAMP-mediated platelet inhibition in presence of $P2Y_{12}$ antagonism.

PB 2209 | Inhibition of Human Platelet Aggregation by Some Newly Synthesized S-Esters of Thiosulfonic Acid

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Background: According to the current understanding, the hyperactivation of platelet may lead to increased intravascular coagulation and thrombosis. Today a relevant issue is the searches for new antithrombotic agents that are able to modulate the processes of platelet activation and aggregation. Previously, we described two low molecular weight compounds 1, 2 (Fig.1), which were effective inhibitors of rabbit platelet aggregation induced by ADP (IC_{50} ~ 12mM and 10mM, respectively).

Aims: The aim of this study was to investigate the effects of these thiosulfonate derivatives on human platelet aggregation.

Methods: All experiments using human subjects were approved by the ethical committee of Taras Shevchenko National University of Kyiv. Only healthy volunteers without taking any nonsteroidal anti-inflammatory drugs for at least 14 days were recruited and written informed consent was obtained before blood collection. Platelet aggregation was assessed within the first 3 hours after blood sampling using photo-optical aggregometer AT-02 (Medtech, Russia). In order to investigate the IC50 values of these compounds, their increasing concentrations (1-100 mM) were added to human PRP ($2,5\times10^5$platelet/ml) and incubated for 2 minutes prior to determination of ADP (5×10^{-6}M) or collagen (2 μg/ml) induced aggregation.

Results: The degree of inhibition exerted by these compounds was found to be proportional to their concentration. The identified dithiocarcamates were shown to have similar efficacy that was evidenced by their close IC50 values: 10-12 μM for ADP-dependent aggregation and 1.5-2.5 μM for aggregation induced by collagen.

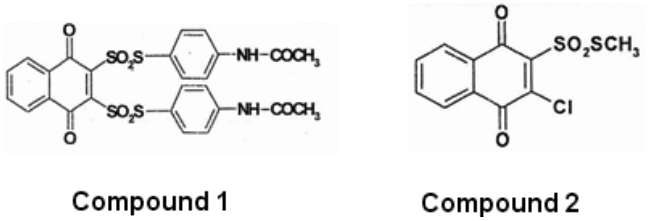

FIGURE 1 The structures of studied thiosulfonate derivatives

Conclusions: The findings suggest that these thiosulfonic acid S-ester derivatives are potent anti-platelet agents and the mechanism underlying their antiaggregation action needs more careful study.

PB 2210 | Looking for the Best Experimental Conditions for Studying the Effect of Aspirin on Murine Hemostasis

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Background: Aspirin (ASA) is an anti-inflammatory and anti-platelet agent that irreversibly inhibits the cyclooxygenase (COX) and, thus thromboxane (TX) generation. However, there are some discrepancies in pre-clinical studies regarding the ASA antiplatelet effect *in vivo* and *ex vivo*.

Aims: Define the appropriate experimental model (dose, route…) for the *in vivo* and *ex vivo* study of the effect of ASA administration on mouse hemostasis.

Methods: FVB and C57Bl6 mice were injected with ASA (10 or 100 mg/kg) or vehicle by oral route 30 min or 3 h before tail bleeding assay, or blood cardiac puncture to assess *ex vivo* washed-platelet aggregations (5 µg/ml collagen and 100 µM arachidonic acid (AA)) and TXB2 production (ng/10^9 platelets).

Results: Our results demonstrate that oral administration of ASA 10 mg/kg is not sufficient to obtain a maximum ASA-induced inhibition of hemostasis regarding bleeding time, TXB2 synthesis and collagen-induced aggregation. At ASA 100 mg/kg, platelet aggregation and TXB2 generation induced by collagen were significantly inhibited at 30 min (44% ± 12 and 69% ± 4, respectively) and at 3 h (51% ± 15 and 85% ± 5) after ASA oral administration. Paradoxically, no inhibition was observed on AA-induced aggregation, even if the TXB2 generation was fully inhibited (0,21 ± 0,05 for ASA vs 2,33 ± 1,13 for vehicle at 30 min post-ASA; 0,14 ± 0,02 for ASA vs 1,13 ± 0,48 for vehicle at 3h post-ASA). Interestingly, the effect of ASA on bleeding assay was more significant when performed 30 min after oral administration than after 3 h, and more specifically when considering bleeding time rather than blood loss.

Conclusions: Although the inhibitory effect of ASA (100 mg/kg) is observed *ex vivo* on collagen-, but paradoxically not on AA-, induced platelet aggregation at 30 min and 3 h after administration of ASA, its inhibitory effect *in vivo* is only observed at 30 min.

PB 2211 | Platelet Microvesicle Formation during the Hemostatic Response is Regulated by P2Y12 Signaling

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Background: Previous studies have shown that hemostatic plugs formed in the mouse microvasculature have a characteristic architecture in which the extent of platelet activation reflects gradients in platelet agonist distribution radiating outwards from the injury site. In that setting, there is minimal overlap of thrombin and ADP signaling.

Aims: Here, we sought to characterize the architecture of hemostatic plugs in large vessels and determine the role of $P2Y_{12}$ signaling in regulating architecture development.

Methods: Correlative microscopy integrating fluorescence with scanning electron microscopy was used to image hemostatic plugs in situ in mouse jugular veins from 1 to 20 min after needle injury.

Results: Our findings show in detail the intraluminal and the extravascular portions of the hemostatic plug, and reveal what could previously only be inferred (Fig. 1). Platelet size, morphology, and packing density varied remarkably depending on spatial localization within the platelet plug. The intraluminal portion was composed almost exclusively of platelets. Platelets closest to the injury edge had a highly activated morphology, including P-selectin surface expression, dense packing, and microvesicle formation. Platelets farther from the injury site often remained discoid. The extravascular portion was rich in densely-packed microvesicles intertwined with fibrin. The microvesicles were CD41, P-selectin, and annexin V positive, indicating they originated from highly activated platelets. In the presence of a $P2Y_{12}$ inhibitor, hemostatic plugs were significantly smaller, the platelet

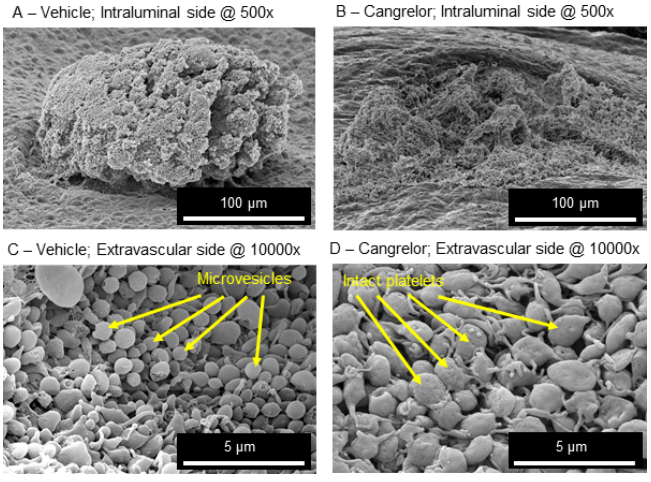

FIGURE 1 Jugular vein hemostatic plugs from mice treated with vehicle (A,C) or cangrelor (B,D) shown from the intraluminal or extravascular side

activation gradient was less apparent, and notably, microvesicle formation was greatly reduced.

Conclusions: Our findings indicate that

1) the development of a platelet activation gradient is conserved across vessels of different sizes,

2) platelet microvesicle formation is a prominent feature of the hemostatic response, and

3) clinically relevant platelet signaling pathways regulate the platelet activation gradient.

PB 2212 | Convection through Platelet Thrombi

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Background: Growth of macroscopic high shear thrombosis may stem from thrombi dominated by diffusion in the core or high-shear convection of whole blood reactants.

Aims: We measure the velocity of blood cells through the pores of the growing high-shear platelet thrombus.

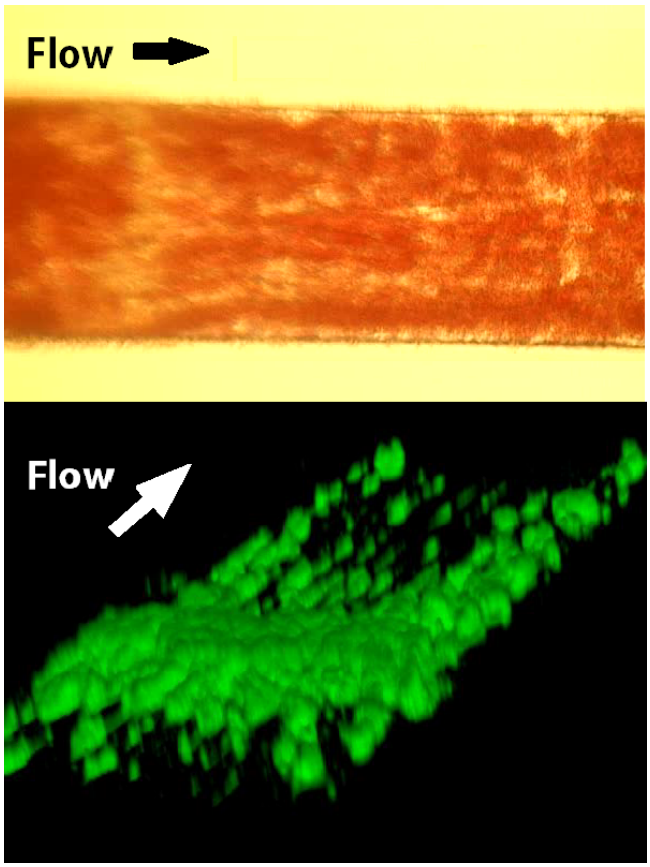

FIGURE 1 Microscopic images of test section. Upper: Picture of whole blood thrombus at 170s, Lower: Confocal image of thrombus at 170s (PRP)

Methods: Whole blood (WB) is perfused through a stenotic microfluidics chamber mimicking an atherosclerotic artery. Platelet adhesion and aggregation is imaged until full occlusion. A light microscope provides an *en face* image in real-time that is captured by a CCD video camera. Confocal images of mepacrine-labelled platelets are captured using a laser scanning microscope.

Results: A white thrombus forms under high shear rates (>3000/s). Platelet arrest on the collagen surface was instantaneous with little rolling. Rapid accumulation occurred between 30 and 200 seconds after perfusion began. Thrombus appeared in ridges in the stream-wise direction that were spaced approximately 30 μm peak to peak. Blood cell motion in the valleys proceeded at high velocity of >250 μm/s. The high velocities during growth imply a high Peclet number, low Damkohler number, and Reynolds number of 10^{-8}. At ~170 s, the velocities suddenly slowed to 50 μm/s with persistent velocities after 600s throughout the thrombus. Fluid motion indicates a volume porosity of 30% within the thrombus. As RBC velocities approached zero, red clot became prominent in the sections upstream and downstream of the stenosis, but less within the test section. Confocal imaging confirmed the 3-D structure of the thrombus as growing mountain peaks with interspersed valleys.

Conclusions: Thrombus formation forms by stream-wise ridges, with long porous valleys that create high velocity RBC passage long after thrombus fills the lumen. High resolution video can be used as a velocimeter to quantify convection within the platelet thrombus. The images of internal convection appear to contradict the stagnation build-up or core-shell hypotheses for growth suggested by some.

PB 2213 | Different Prothrombotic Phenotypes of Atherosclerosis-prone Apoe-/-and Ldlr-/- Mice

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Background: *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice have elevated plasma lipids, are prone to atherosclerosis and have a prothrombotic tendency. Feeding a western-type diet is crucial for the atherosclerotic propensity of *Ldlr*$^{-/-}$ but not *Apoe*$^{-/-}$ mice.

Aims: Given the suggested roles of platelets and coagulation in these processes, we determined the phenotype of *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, using multi-parameter tests of platelet/coagulant function.

Methods: Analysis of blood, lipids and coagulation parameters of wild type (C57BL/6), *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice with(out) high-fat diet. Thrombotic potential in whole blood flowed over collagen/tissue factor (TF). Platelet phenotyping of thrombi formed on collagen. Heat maps of normalized data of thrombus size and activation parameters. Assessment of coagulation in plasma by calibrated automated thrombin generation (TF stimulation) and thrombin-anti-thrombin(TAT)-complexes.

Results: Compared to blood from wildtype mice, blood from both *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice displayed an increased prothrombotic potential, measured as enhanced platelet-dependent clot formation under high-shear, arterial, flow conditions ($p < 0.05$). For both genotypes, the increased clot formation was independent of age (2-18 months) or diet (high/low fat). Coagulant activity was significantly increased in plasma from *Apoe*$^{-/-}$ but not *Ldlr*$^{-/-}$ mice (circulating TAT complexes and plasma thrombin generation). Multi-parameter assessment of platelet thrombus formation under flow (without coagulation) indicated a stronger gain-of-function of *Ldlr*$^{-/-}$ platelets than of *Apoe*$^{-/-}$ platelets compared to wild-type platelets.

Conclusions: The prothrombotic phenotypes of *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice are age- and diet-independent, and link to a different gain-of-function of platelet activity (*Ldlr*$^{-/-}$ > *Apoe*$^{-/-}$) and coagulant activity (*Apoe*$^{-/-}$ > *Ldlr*$^{-/-}$). This study provides novel insight into the mechanisms of linking atherosclerosis to thrombosis.

PB 2214 | PCSK9, Not only A Modulator of Cholesterol Levels but Also of Platelet Function

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Background: The serine protease Proprotein convertase subtilisin/kexin 9 (PCSK9) is a main player in cholesterol homeostasis inducing degradation of the LDL cholesterol receptor (LDLR). PCSK9 plasma levels predict recurrent cardiovascular events in stable angina (SA$^+$) patients. PCSK9 contribution to cardiovascular events might be mediated by mechanisms occurring via unknown LDLR-independent pathways. Platelets (PLT) play a key role in the acute complications of atherosclerosis and the hyperreactive phenotype of PLT in type-2 diabetes mellitus (DM) patients is well known. Interestingly PLT count is positively associated with plasma PCSK9 in SA$^+$ patients and increased serum PCSK9 is directly associated with PLT reactivity in acute coronary syndrome patients. No study has evaluated whether PCSK9 affects PLT function.

Aims: To evaluate whether PCSK9 modulates PLT activation and to assess whether PCSK9 is expressed by PLT from healthy subjects (HS) and SA$^+$ patients, DM$^+$ or DM$^-$.

Methods: The effect of PCSK9 (5μg/mL) on epinephrine(0.6μM)-induced PLT activation was assessed by aggregation assay and by whole blood flow cytometry (FC) evaluation of P-selectin, PAC-1, Tissue Factor (TF). PCSK9 levels in PLT lysates from 30 SA$^+$ (15 DM$^+$, 15 DM$^-$), 10 DM$^+$SA$^-$ patients and 10 HS, who gave informed consent, were assessed by ELISA.

Results: PCSK9 potentiates PLT aggregation induced by epinephrine (+40%AUC;+78%Slope;-15%LagTime;+15%MaxAggregation) and increases PAC-1, P-selectin and TF expression (+50%,+40%,+25%;$p < 0.05$). ELISA assay showed that PLT from SA$^+$DM$^+$ patients contained twice the amount of PCSK9 compared to the other groups (21.6±7.7pg/μg protein,$p < 0{,}001$). No difference in plasma PCSK9 levels were found among the groups.

Conclusions: These data show for the first time that PCSK9 is expressed in human PLT and significantly higher levels are found in SA$^+$DM$^+$ patients. Moreover PCSK9 plays a role in PLT activation and aggregation. These findings may help to understand the molecular basis of PLT hyperreactivity in SA$^+$DM$^+$ patients.

PB 2215 | Increased Platelet P2Y12 Expression in Diabetes and the Therapeutic Implication of Inverse Agonist for Antiplatelet Therapy

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Background: Platelet of diabetes patients are hyperactive, which contributes to not only the increased prevalence of cardiovascular complications, but also the inadequate response to currently available antiplatelet agents leading to increased cardiovascular death. The underlying mechanism is still not completely understood.

Aims: The aim of this study was to explore the mechanisms for increased platelet P2Y12 expression in diabetes patients and patients with upregulated $P2Y_{12}$ may gain more benefit from antiplatelet agents with potent $P2Y_{12}$ inverse agonist activity.

Methods: No clinical trial.

Results: Using real time PCR and Western blotting we show that platelet from diabetes patients express dramatically higher $P2Y_{12}$ than healthy subjects. $P2Y_{12}$ expression correlates well with ADP-induced platelet aggregation. Upregulated $P2Y_{12}$ of diabetic platelet is constitutively activated. Though both AR-C78511, a potent $P2Y_{12}$ inverse agonist, and cangrelor have similar antiplatelet efficacy on platelets from healthy subjects, AR-C78511 exhibits superior antiplatelet effects on diabetic platelet over cangrelor, a neutral $P2Y_{12}$ receptor antagonist we previously reported. Furthermore, we recapitulated the findings from diabetic patients using diabetic Goto-Kakizaki (GK) rats. Importantly, using two kinds of thrombosis models, we found that the superior antiplatelet effect of the inverse agonist can be translated into superior antithrombotic effect on diabetic GK rats. Using megakaryocytes from diabetic rats and high glucose-treated megakaryocytic cell line, we provided evidences suggesting that high glucose-ROS-NFκB pathway upregulates platelet $P2Y_{12}$ receptor in diabetes.

Conclusions: Platelet $P2Y_{12}$ receptor is significantly increased and constitutively activated in T2DM patients, which contributes to platelet hyperactivity and dampened antiplatelet efficacy in T2DM. $P2Y_{12}$ inverse agonists may have superior antiplatelet effects than cangrelor in diabetic patients, especially in patients with increased platelet $P2Y_{12}$ expression.

PB 2216 | Elevated Activity of Fibrinogen-like Protein 2, (FGL2/fibroleukin) in Platelets of Cancer Patients

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Background: Fibrinogen-Like Protein 2 (FGL2), a recently discovered trans-membrane procoagulant, is believed to play a central role in cancer development. Similar to factor Xa, FGL2 is capable of exerting prothrombinase activity however, independent of the classical coagulation pathway.
Aims: To investigate if platelets can directly induce coagulation via FGL2 activity and if FGL2 activity in platelets has the potential to be used as a malignancy biomarker.
Methods: Blood samples were collected from healthy individuals and patients with malignancies. Platelet rich plasma, were generated and washed to obtain factor X free samples. FGL2 activity, mRNA, protein levels and the expression of FGL2 Antigen on platelets were determined.
Results: We found a strong correlation between FGL2 prothrombinase activity and the number of platelets. Immunoprecipitation and immunoblotting analyzes proved the absence of factor X prothrombinase and presence of FGL2 in the samples. We showed that FGL2 is present in the membrane of the platelets as well as in peripheral blood mononuclear cells (PBMC). We demonstrated that FGL2 prothrombinase activity was higher in several malignancies compared to healthy controls. Moreover, remission in B cell lymphoma patients is correlated with reduced FGL2 activity levels.
Conclusions: We show for the first time that FGL2 prothrombinase is located in platelets as well as in PBMC. The exerted FGL2 activity is entirely dependent on platelets, despite being also expressed by T cells and macrophages. Therefore, we claim that platelets are capable of directly inducing coagulation, probably locally, via FGL2 activity beyond the classical coagulation route. In view of our findings, the measured activity of FGL2 in platelets has a potential to be employed as a malignancy biomarker. More study is needed to establish the role of FGL2 in thrombophilia-prone diseases.

PB 2217 | Dynamics of Single Platelet Dense Granule Release Induced by Various Concentrations of Thrombin and ADP

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Background: Primary hemostatic response to vascular injury involves platelet recruitment, aggregation and activation, the latter being strongly dependent on the action of ADP, thrombin and "outside-in" signaling through platelet integrins. However, spatiotemporal aspects of platelet activation, granule release, and distribution of agonists concentration during thrombus formation remain unclear. Platelet dense granule secretion is considered to be important source for ADP, though, the kinetics of dense granule release in response to thrombin and ADP remains a matter of controversy.
Aims: To assess the dynamics of dense granule release by single surface-attached platelet in response to various concentrations of thrombin and ADP.
Methods: We used confocal microscopy to image dense granules of platelets upon their loading with mepacrine (quinacrine). Briefly, citrated blood was perfused through the flow chamber, adhered platelets then were washed with buffer, followed by buffer with calcium (2 mM) and agonist. Experiments were performed under the wide range of conditions: on fibrinogen and/or von Willebrand factor-coated coverslips; at different thrombin and/or ADP concentrations (from 100 nM to 0.01 nM; from 100 uM to 0.1 uM, respectively, and combinations); in absence and presence of various shear stresses (from 100 to 1000 s-1); with platelets aggregated from blood, PRP or washed gel-filtered platelets.
Results: The kinetics of dense granule release depends on type and concentration of thrombin or ADP, the kind of surface protein and the shear rate. In particular, with decreasing concentrations of thrombin the number of granules released by platelets and the percentage of such platelets are decreased (from 5-8 to 1-2 and from 95-100% to 15-10%, respectively), while the mean time of granules release is increased (from 1-2 to 500-600 s).
Conclusions: Here we have shown for the first time concentration- and time-dependencies of single platelet dense granule release under a wide range of experimental conditions.

PB 2218 | Comparison of Reticulated and Non-reticulated Platelet Function and Releasates

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Background: Within the general platelet population there exists a subpopulation of platelets known as reticulated platelets, so called because of the presence of RNA within their cytoplasm. Reticulated platelets account for roughly 10% of the total platelet population in healthy subjects but can be markedly increased in specific patient groups. Critically to cardiovascular health, reticulated platelets are thought to be more reactive than their nonreticulated counterparts.
Aims: To isolate reticulated platelets and directly interrogate their functional capacity and lipid release profiles.
Methods: Thiazole orange (TO) staining of PRP coupled with flow cytometry was used to isolate reticulated platelets, intermediate platelets and nonreticulated platelets. mRNA content in each subpopulation was quantified using qRT-PCR. Platelet aggregate formation was assessed using imaging flow cytometry. To investigate

subpopulation releasates, mass spectrometry, ELISA and luciferase based assays were used.

Results: Reticulated platelets, defined as those with the highest TO staining intensity, corresponded with platelets containing the highest mRNA content. Aggregation studies revealed that reticulated platelets are disproportionally recruited into forming aggregates and locate towards the centre. In addition, reticulated platelets express a higher level of cell-surface P-selectin upon activation.

Conclusions: Reticulated platelets display increased reactivity and part-take more readily in aggregate formation than non-reticulated platelets. Thus this sub-population of platelets may be early drivers of thrombus formation and could be a contributing factor in the failure of anti-platelet prophylaxis.

PB 2219 | Platelet S1P: Evidence for the Involvement of the Membrane Transporter MRP4 during its Secretion, and Identification of Surface Receptors

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Background: Sphingosine-1-phosphate (S1P) is a bioactive lipid metabolite that plays key roles in vascular development and homeostasis. Most vascular roles of S1P have been attributed to activation of G protein-coupled S1P receptor (S1P1-5) signaling. In a previous study, we showed that sphingosine kinase deficiency in megakaryocytes and platelets impairs platelet activation and spreading, and reduces the availability of S1P for activation of vascular receptors during systemic inflammation. However, the mechanisms of S1P export from activated platelets and its mechanism of action on platelets remain to be fully defined.

Aims: To address mechanisms of S1P export from mouse platelets, and the expression and function of S1P receptors on mouse and human platelets.

Methods: S1P levels were quantified in serum, plasma and supernatants of activated platelets from MRP4-deficient mice and controls by LC-MS/MS. S1P receptor expression was assessed in wild-type mouse and human platelets by immuno-precipitation/-detection. The role of S1P signaling in platelet activation was addressed with pharmacological tools and in S1P1-deficient mouse platelets.

Results: While plasma S1P levels in MRP4-deficient mice were similar to wild-type controls, MRP4-deficient mice showed a significant reduction in S1P levels in serum and in supernatants from activated platelets. Immunodetection was most robust for S1P1 and S1P5 in mouse and human platelets, respectively. However, addition of S1P or S1P receptor agonists did not modify platelet aggregation. PAR4-ap-, collagen-, U46619- and ADP-induced platelet aggregation was not sensitive to S1P receptor antagonists and did not differ between platelets from platelet S1P1-deficient and littermate control mice.

Conclusions: Our results show that MRP4 contributes significantly although not exclusively to activation-induced S1P export from mouse platelets, but do not so far support a role for S1P signaling in mouse platelet activation.

PB 2220 | The Framingham Heart Study (FHS): Results from >900 Individuals with Detailed Platelet Function Testing

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Background: There are few large studies of platelet reactivity, limiting investigation of the genetic and environmental drivers in the general population.

Aims: We describe early results and analysis from an ongoing deep platelet study with respect to technical factors and cardiovascular risk factors (final projected n~3,800).

Methods: Fasting blood (sodium citrate+hirudin) is drawn. A bleeding history is taken by a modified ISTH Bleeding Assessment Tool (BAT). Whole blood platelet function measurements include impedance aggregometry (AA/ADP/collagen/ristocetin/TRAP-6 amide) and flow cytometry (FC) (CD61/CD63/CD62P/PAC1) after 20*u*M ADP. PRP is used for: (1) FC (as above), (2) traditional LTA (AA/ADP/collagen/epinephrine/ristocetin/TRAP-6 amide), and (3) Optimul absorbance assays (AA/ADP/collagen/epinephrine/ristocetin/TRAP-6 amide/U46619). Steps are taken to reduce variability (e.g., large agonist lots) and track technical factors (e.g., draw/test time). Detailed anthropometric measurements, biomarkers, clinical and sub-clinical measures are available on FHS participants.

Results: To date, of 939 participants with fasting blood draws (57.1% female, mean age 53.5 years), 60 (6.3%) were known diabetics. BAT results include: bleeding disorder (0.9%), discontinuation of medication for bleeding concerns (1.4%), serious bleeding issue after surgery (1.0%), some family history of bleeding (8.0%), and history of menorrhagia with medical attention (10.0% of women). In initial analysis, female sex was strongly associated with higher platelet reactivity across agonists, with other factors assessed showing much weaker or no association in multivariable models.

Conclusions: The Framingham platelet study will provide a previously unprecedented scale of population-level data on platelet function, bleeding history, and its relation to -omic and environmental factors. Systematic differences in platelet reactivity by sex require further study and may affect anti-platelet treatment responses in clinical settings.

PB 2221 | Polyphosphate Nanoparticles on the Platelet Surface Trigger Contact System Activation

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Background: Polyphosphate (polyP) is an inorganic polymer that has diverse effects on blood coagulation and fibrinolysis. The secretion of polyP by platelets is linked to thrombosis via factor XII (FXII) activation. However, the small polymer size of secreted platelet polyphosphate limits its capacity to activate FXII in vitro. This leaves the exact mechanisms by which platelet polyP and FXII interact in thrombosis unclear.

Aims: To investigate the nature of platelet polyP.

Methods: We studied subcellular localization of polyP in platelets under flow by live-cell imaging, confocal fluorescence microscopy and scanning electron microscopy (SEM). Biophysical characterization was performed by ultracentrifugation fractionation, dynamic light scattering and electrophoresis. Plasma contact system activation was studied in plasma with chromogenic substrates and specific ELISAs.

Results: Live-cell imaging studies revealed that platelets retain polyP on their surface under flow (Fig. 1A). This occurs when single platelets secrete their contents on von Willebrand factor or during aggregation on collagen. Ultracentrifugation fractionation studies show that cell-associated polyP forms stable insoluble spherical nanoparticles. Confocal microscopy and SEM confirmed the presence of these nanoparticles on the platelet surface after degranulation (Fig. 1B). The chelating agent EDTA disrupts polyP nanoparticles, indicating that divalent metal ions are critical for their stability. Electrophoretic studies suggest that the apparent polymer size of membrane-associated polyP exceeds that of secreted polyP. This is associated with calcium complexation. In contrast to short-chain soluble polyP, polyP nanoparticles strongly activate FXII and the contact system in plasma.

Conclusions: Membrane-associated polyP is present on the platelet surface in a nanoparticle state. We propose that these polyP nanoparticles mechanistically link the procoagulant activity of platelets with activation of coagulation FXII.

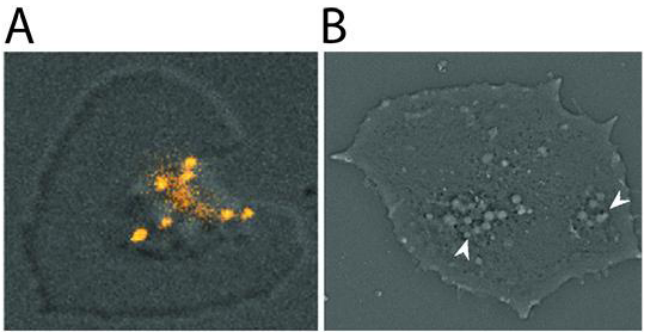

FIGURE 1 Polyphosphate nanoparticles on the platelet surface A) Fluorescence microscopy B) Scanning electron microscopy

PB 2222 | Analysis of Integrin β3 Clustering and Kindlin Association Using a Novel STORM Microscopy Method

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Background: Integrin αIIbβ3 activation and clustering is a vital part of platelet activation and is mediated in part through the association of kindlin with integrin β3.

Aims: To develop a Stochastic Optical Reconstruction Microscopy (STORM) method for measuring clustering in non-adherent platelets and investigate the time course of integrin β3 clustering and association with kindlin.

Methods: Platelet-rich-plasma was activated with thrombin (1U/ml) in the presence of GPRP (0.5mg/ml) for 0, 15, 30, 60, 90, 120, 180 and 300s, fixed with 2% (v/v) formal saline and permeablised with BD Phos Flow Perm Buffer III. Kindlin and integrin β3 were identified with primary and alexa fluor 647 and 555 labelled secondary antibodies, respectively. Labelled platelets were washed then adhered overnight to poly-D-lysine coated slides before 3D dSTORM imaging. Images were reconstructed using Nikon NIS Elements. The localization of integrin β3 and kindlin were analysed using the ImageJ plugin ThunderSTORM and a novel cluster analysis method that analysed the density of β3 molecules within 50nm of each β3 and kindlin molecule.

Results: The density of β3 molecules surrounding each β3 molecule (a measure of clustering) showed a biphasic pattern increasing from a low in resting platelets to peak at 60s, falling at 90s, then increasing again by 180s before again declining by 300s (P=0.026). The density of β3 molecules surrounding each kindlin molecule showed the opposite trend for the first 30s (decreasing from a high in resting platelets) and then followed the same pattern as β3 clustering (P=0.008). The kindlin-β3 and β3-β3 densities were positively associated after 30s stimulation (slope=0.32, r^2=0.22, P=0.047) but not in the first 15s (slope=-0.28, r^2=0.57, P=0.08).

Conclusions: We have developed a STORM microscopy method and used it to quantify kindlin-β3 and β3-β3 clustering in platelets. β3-β3 clustering was biphasic and associated with kindling-β3 clustering over time.

PB 2223 | Shear-mediated Platelet Activation (SMPA) Yields a Differing "Activation Signature" than that of Biochemical Agonists

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Background: Supraphysiologic shear stress is the major driver of platelet activation within mechanical circulatory support devices (MCSD), leading to postimplantation thromboembolic events.

Aims: To define and compare the effect of constant shear vs biochemical agonist stimulation on platelet phosphatidylserine externalization (PSE), α-granule secretion and procoagulant activity.

Methods: Human gel-filtered platelets were exposed to constant shear (30, 50, 70 dynes/cm^2 for 10 min) generated by a hemodynamic shearing device [Girdhar, 2012]. Biochemical activation and aggregation were induced by ADP, TRAP6, collagen, thrombin or arachidonic acid (AA). Aggregatory activity was evaluated via optical aggregometry. Platelet annexin V binding and P-selectin exposure were analyzed by flow cytometry, as PSE and α-granule exocytosis markers, respectively. Procoagulant activity was detected using the chromogenic platelet activation state assay [Jesty, 1999].

Results: ADP, TRAP6, collagen all induced P-selectin exposure and aggregation but did not affect platelet annexin V binding. Platelet stimulation with thrombin or AA led to massive α-granule secretion, annexin V binding and high-amplitude aggregation. In contrast, platelets exposed to high shear stress demonstrated high PSE levels and low secretory activity. The % of the platelet population binding annexin V grew quantitatively in parallel with the degree of shear exposure, while surface P-selectin barely increased. As well as PSE, thrombin generation was induced with shear (PAS assay), though with biochemical agonists was only noted with AA.

Conclusions: Constant shear, but not most biochemical agonists, induced massive PSE and subsequent thrombin generation on the platelet surface thus promoting the prothrombotic state formation. In contrast, SMPA was not associated with α-granule exocytosis, as was observed with biochemical activation. Our findings suggest a possible diagnostic signature for SMPA and provide data to further its mechanistic understanding in MCSD.

PB 2225 | Investigating Platelet Functional Heterogeneity Using Droplet Microfluidics

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Background: Platelets have long been known to be heterogeneous in size, volume and density. Functional heterogeneity has been suggested in several studies. However, there are currently no methods available to investigate platelet function on a single cell level. Single platelet research is needed to study intrinsic heterogeneity without the influence of adjacent cells and associated amplification mechanisms. Such a method should study platelets in isolation, with high throughput, to detect potentially rare phenotypes, without interfering with normal platelet function. To this effect, this study implements droplet microfluidics to investigate single platelet functionality.

Aims: The study aims to develop a high throughput droplet microfluidic protocol to investigate single platelet function to measure intrinsic functional heterogeneity.

Methods: The innovative droplet microfluidic protocol involves compartmentalizing and activating platelets without paracrine signalling. This is coupled with flow cytometry and imaging to quantify platelet response to agonists using three endpoints; integrin $\alpha_{IIb}\beta_3$ activation, degranulation and membrane inversion.

Results: Platelets are singularly encapsulated in 25-mm-diameter monodisperse (CV of 1-4%) water-in-oil droplets at a frequency of 4 kHz, with droplets containing a single platelet produced at a rate of 0.25 kHz (following a Poisson distribution). Isolated platelet stimulation excludes activation propagation by paracrine signalling between platelets, resulting in reduced active platelet numbers yet with enhanced activation levels caused by autocrine signalling. Platelet functional heterogeneity in response to agonists has a strong intrinsic heterogeneity component.

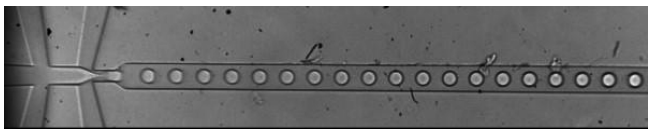

FIGURE 1 Monodisperse droplet formation

Conclusions: This study demonstrated the value of single platelet compartmentalisation in droplet microenvironments to determine system heterogeneity and ultimately unravel the intrinsic, pre-programmed states that drive platelet ensemble activation in health and potentially in disease.

PB 2226 | Sickle Cell Disease Patients under Regular Transfusion Program Have Decreased in vitro Platelet Aggregation

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Background: Sickle cell disease (SCD) results in chronic inflammatory response with frequent endothelium activation. Activated endothelial cells induce inflammatory pathway signaling with increased production of inflammatory mediators and expression of adhesion molecules.

Aims: This study aims to evaluate the adhesive and inflammatory properties of endothelial cells and platelets from SCD patients under regular transfusion, compared to healthy individuals.

Methods: Endothelial colony-forming cells (ECFCs) were isolated from peripheral blood mononuclear cells and seeded on collagen with conditioned media. Platelets were obtained by centrifugation of platelet rich plasma. ECFCs were incubated with or without platelets and in the presence or absence of TNF-α. Inflammatory and adhesion molecules levels were evaluated in culture's supernatants by Luminex (sCD40L, IL-1B, IL-8, PDGF-AA, PDGF-AB/BB, ADAMTS13, sICAM-1, sVCAM-1, sP-Selectin, thrombospondin-1). Endothelial adhesion molecules were analyzed by flow cytometry (VCAM-1, ICAM-1, P and E-selectin).

Results: SCD platelets under regular transfusion (N=4) showed a significantly decreased response to the aggregating agents ADP (p=0.003), epinephrine (p< 0.001) and ristocetin (p=0.001), compared to control platelets (N=7). The expression of adhesion molecules VCAM-1 and P-selectin on ECFCs (regardless of the group) increased significantly after co-incubation with control platelets (p< 0.001 and p=0.01, respectively). This co-culture supernatant also presented with higher levels of soluble VCAM-1 and P-selectin, compared to co-cultures with SCD platelets (p=0.003 and p=0.007, respectively). The addition of TNF-α lead to similar results. No differences were detected in the expression and release of adhesion molecules in control ECFCs (N=6) and SCD ECFCs (N=6).

Conclusions: Our results showed, for the first time, that regular transfusions in SCD patients can alter platelet function, reducing endothelial activation capacity and therefore, the risk of vaso-occlusive event.

PB 2227 | Microthrombocytopenia and Defective GPVI/ITAM Signaling in Mice Lacking the Small GTPase RhoB

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Background: Cytoskeletal rearrangements are critical for platelet production by megakaryocytes as well as for proper platelet function. We have previously shown that mice lacking the small GTPase RhoA in megakaryocytes exhibit a macrothrombocytopenia and reduced platelet activation in response to agonists coupling to G_{13}/G_q. In contrast, the role of the Rho subfamily member RhoB in megakaryocytes and platelets remains unknown.

Aims: We investigated the functional role of RhoB in platelets in vitro and in vivo.

Methods: Taking advantage of constitutive RhoB knock-out mice, platelet function and cytoskeletal rearrangements were assessed in vitro using biochemical, flow cytometric and microscopy based assays. Platelet in vivo function was studied by the tail bleeding assay and models of arterial thrombosis.

Results: In contrast to the macrothrombocytopenia observed in RhoA-deficient animals, loss of RhoB led to a decrease in both platelet count and size, indicating a distinct role for RhoB in platelet biogenesis. RhoB-deficient platelets displayed a selective activation defect downstream of the *immunoreceptor tyrosine based activation motif* (ITAM) coupled collagen receptor GPVI resulting in reduced activation of phospholipase (PLC) γ2 in line with impaired aggregate formation on collagen under flow. Interestingly, spreading on fibrinogen was unaltered in RhoB-deficient platelets, whereas F-actin assembly upon stimulation with collagen-related peptide and convulxin as well as microtubule reassembly were severely defective. *In vivo*, these defects translated into a marked protection from arterial thrombus formation and variable tail bleeding times.

Conclusions: Our results reveal an unexpected critical role of RhoB in platelet biogenesis and function in vitro and in vivo.

PB 2228 | Characterization of in Vitro Cell-free Induced Fibrillar Fibronectin: Morphology Dependent Effects on Platelet Function

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Background: Insoluble fibrillar fibronectin is the active form of fibronectin, an essential component of extracellular matrix that plays important roles in many cellular processes.

Aims: This study aimed to synthesize fibronectin fibrils under cell-free conditions and characterize the relationship between fibril morphology and platelet adhesion in hemostasis.

Methods: To induce fibrillogenesis, purified fibronectin at 4 concentrations (0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml and 1 mg/ml) were dialyzed against urea 2 M in 16 h followed by dialysis against PBS pH 7.3 to subsequently remove urea. Morphology of synthesized fibrils was characterized by fluorescence microscopy. To evaluate the effect of fibronectin fibrils on platelet function, platelet adhesion assays on fibronectin fibrils were performed.

Results: Microscopic images revealed that urea induced heterogeneous formation of fibronectin fibrils with various morphologies ranging from aggregation to fibrillar form. Fibrillar fibronectin that have diameter in range 30 µm - 130 µm tend to interact to form matrix while fibronectin aggregates with size around 50 µm suspend in solution. Our data showed that among 4 tested concentrations, 1mg/ml is the best condition for fibronectin polymerization which yields the most aggregated fibronectin and fibrillar matrix. At lower concentrations, less fibronectin fibrils were formed and they did not link together to form a matrix. Platelet adhesion assay indicated a stronger platelet adhesion on fibronectin fibrils coated surfaces than surfaces coated with untreated plasma fibronectin.

Adherent platelets clustered into small groups around aggregated fibronectin and separate fibrils. In contrast, platelets appeared to adhere much more on fibrillar matrix and link to one another forming a layer.

Conclusions: Mild denaturation of plasma fibronectin by urea induces irreversible heterogeneous formation of fibronectin fibrils. Microscopic analyses revealed that fibril morphology affects platelet adhesion.

PB 2229 | Extracellular Histones Stimulate Clot Retraction and Induce Resistance to Fibrinolysis

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Background: Histones possess prothrombotic properties among which platelet activation. Interaction between activated platelets and fibrin(ogen) underlies the process of clot retraction, which can contribute to thrombogenesis by increasing clot resistance to fibrinolysis.

Aims: To evaluate the influence of histones on clot retraction and lysability.

Methods: Platelet-rich plasma (PRP) was clotted with $CaCl_2$ ± calf thymus histones for 2 h at 37°C. Retraction was assessed by clot area decrease and fibrinolytic resistance by release of FITC-fibrin degradation products at 2 hours after addition of t-PA to the milieu surrounding the retracted clot.

Results: In PRP, histones increased clot retraction in a dose-dependent manner (EC50, 0.43 µg/mL), with maximal stimulation at 40 µg/mL (85.9±0.8% vs 47.4±3.9% in controls). A mixture of recombinant histone subtypes produced a similar effect. Enhancement of clot retraction by histones was independent of thrombin generation increase as it persisted with clots induced by high tissue factor (25 pM) or thrombin (1 U/mL). In the presence of the anti-GPIIb/IIIa antibody abciximab (5 µg/mL), clot retraction amounted to only 6.2±3% in control PRP and to 41.8±4.4% in histone-PRP ($P<0.01$), suggesting a histone-mediated resistance to anti-GPIIb/IIIa. The extent of t-PA-mediated lysis of histone-treated PRP clots was 39.7±2.7% as compared to 57.8±7.3% in control clots ($P<0.05$), indicating an increased resistance to lysis. No effect on fibrinolysis was seen when histones were incorporated into platelet-poor plasma clots. Histone/DNA complexes (40 µg/mL each) behaved like histones, whereas DNA alone had no effect on either clot retraction or lysis.

Conclusions: Enhancement of clot retraction and resistance to fibrinolysis may represent additional mechanisms accounting for the thrombogenic activity of histones. Moreover, resistance to GPIIb/IIIa inhibitors may have clinical and therapeutic implications.

PB 2230 | Evaluation of the Total Thrombus-formation System (T-TAS): Application to Human and Mouse Blood Analysis

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Background: The Total Thrombus-formation Analyser System (T-TAS) is a whole blood flow chamber system for the measurement of *in vitro* thrombus formation under different shear stress conditions.

Aims: To evaluate the utility of the T-TAS for the measurement of thrombus formation within human and mouse blood.

Methods: T-TAS microchips (collagen, PL chip; collagen/tissue thromboplastin, AR chip) were used to analyse platelet or fibrin-rich thrombus formation respectively. Blood from humans and wildtype (WT), CD148, CSK, and CsK/CD148 platelet-specific knockout (KO) mice were tested.

Results: Thrombus growth (N=22) increased with shear within PL (4:40±1.11, 3:25±0.43 and 3:12±0.48 mins [1000, 1500 and 2000s^{-1}]) or AR chips (3:55±0.42 and 1:49±0.19 [240s^{-1} and 600s^{-1}]). AUC on the PL chip was also shorter at 1000s^{-1} than at 1500/2000s^{-1}(260±51.7, 317± 55.4 and 301±66.2 respectively). In contrast no differences in the AUC between 240s^{-1} and 600s^{-1} were observed in the AR chip (1593±122 and 1591±158). The intra-assay CV (n=10) in the PL chip (1000s^{-1}) and AR chips (240s^{-1}) were T_{10}14.1%, T_{60}16.7%, $T_{10\text{-}60}$22.8% and AUC_{10}24.4% or T_{10} 9.03%, T_{80}8.64%, $T_{10\text{-}80}$23.8% and AUC_{30}5.1%. AR chip thrombus formation was inhibited with rivaroxaban (1µM), but not with the ticagrelor (10µM). PL chip thrombus formation was totally inhibited with the ticagrelor. T-TAS shows agreement with LTA in about 82% of patients tested so far (n=14). The onset (T_{10}) of thrombus formation in WT mice (N= 4) was shorter when compared to humans e.g. PL chip (1000s^{-1}) T_{10} were 02:02±00:23 and 03:30±0:45 respectively). All three KO mouse models exhibited no measurable thrombus formation in PL chips with delayed onset of thrombus formation in AR chips.

Conclusions: T-TAS measures *in vitro* thrombus formation within 2 different chips and can be used for monitoring antithrombotic therapy in patients. It can also be applied to investigating thrombus formation within patients and mouse models.

PB 2231 | The Mechanisms Underlying Core-and-Shell Architecture of Arterial Thrombus and it´s Dynamics Described with Computational Modeling

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Background: Under high shear conditions primary hemostatic response involves GPIb-mediated interaction between platelets and von Willebrand factor as well as platelet interaction through surface integrins which strongly depends on platelet activation. Despite the abundance of experimental data covering various features of those interactions, the origin of spatio-temporal heterogeneity of platelet thrombus and the mechanisms limiting its size are not clearly understood.

Aims: In order to get a deeper insight into complex mechanics of platelet thrombus formation and the origin of specific dynamics of thrombus shell observed in vivo we developed and analyzed the computational model of arterial thrombus formation.

Methods: We created a 2d computational model of arterial thrombus formation which considers two types of platelet interactions: through GPIb (vWF-mediated) and through αIIbβ3 integrins (fibrinogen and vWF-mediated). Platelets were represented by discoid particles 2 μm in diameter. Two types of inter-platelet interaction were described differently: interaction via GPIb and Von Willebrand factor was modeled by stochastic springs, while interaction via αIIbβ3 and fibrinogen/vWf was described using deterministic Morse potential. Platelet activation process was described with simple residence-time based model. Blood was considered as incompressible Newtonian fluid.

Results: The dynamics of thrombus formation described with our model has several features:

1) the height of the thrombus is limited by specific dynamics of its outer layers (shell) which flow pass the stable inner layers (core) due to transient nature of GPIb-vWF-mediated interactions and only partial integrin activation

2) the interplay between integrins activation kinetics and the dynamics of thrombus shell influences the evolution of thrombus core.

Conclusions: Our stochastic model of thrombus formation describes complex mechanics of thrombus shell and offers new mechanism of thrombus size regulation.

PB 2232 | Effects of pH and Concentration of Sodium Citrate Anticoagulant on Platelet Aggregation Measured by Light Transmission Aggregometry Induced by Adenosine Diphosphate

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Background: The 2013 ISTH-SSC guidelines for the standardization of light transmission aggregometry (LTA) were largely based on expert consensus, as studies directly comparing LTA methodologies were lacking.

Aims: We experimentally tested the cogency of ISTH-SSC recommendations pertaining to use of anticoagulant, in particular whether:

1) buffered citrate (BC) is preferable to un-buffered citrate (C);

2) the 2 recommended concentrations of sodium citrate (109 and 129 mM) are equivalent in terms of platelet aggregation (PA).

Methods: Blood from 16 healthy volunteers was collected into BC and C 109 and 129 mM. PA was measured by LTA in platelet-rich plasma (PRP) stimulated by ADP (2 μM) immediately after PRP preparation and up to 4 hours after blood collection; pH and platelet count in PRP were measured in parallel.

Results: pH in PRP increased with time up to about 8 for all anticoagulants, although it was lower in BC than in C at all times. In BC PA was lower at 45 min, but equivalent at all other times. PA was higher and more stable in sodium citrate 109 mM than in 129 mM at all times. The extent of PA did not change up to 2 hours since blood collection, and subsequently dramatically decreased.

Conclusions: In contrast with ISTH-SSC recommendations,

1) BC does not show advantages compared to C;

2) 109 mM citrate is preferable to 129 mM, because it better supports PA;

3) LTA studies should be completed within 2 hours of blood collection, instead of the recommended 4 hours.

PB 2233 | Idelalisib and Platelet Aggregation in Patients with Chronic Lymphocytic Leukemia (CLL)

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Background: Idealisib and ibrutinib are novel therapies for chronic lymphocytic leukemia (CLL). It has been reported that ibrutinib induces a platelet dysfunction that increases bleeding in 50% of patients. Knowledge about potential bleeding complications associated with the use of idelalisib are important for CLL patients management.

Aims: To assess the potential side effects of idelalisib on bleeding tendency in patients with CLL.

Methods: Ten patients with CLL (M/F: 6/4; median age 71 y) who started treatment with idelalisib were included in an observational prospective study. Bleeding Severity Score (BSS) was administered and primary and secondary hemostasis tests were performed before and after 1 and 3 months starting therapy with idelalisib.

Results: No bleeding episodes were observed in patients treated with idelalisib and BSS remainde below 5. All patients had normal coagulation tests at all three time points, while platelet count was below 100.000/mm^3 at baseline in 5 patients. At baseline, in 9/10 patients platelet aggregation were abnormal with at least 2 of the 4 aggregating agents tested and platelet secretion was impaired, particularly using ADP (8/10 patients). In 8 patients intraplatelet ATP/ADP ratio was abnormal, suggesting a delta storage pool disease. At 1 month, 5/9 patients with abnormal baseline platelet aggregation tests had an improvement of

platelet aggregation and one worsened. At 3 months, 3 further patients had an improvement of platelet aggregation. At 3 months, 7 patients reached complete remission and 2 a partial remission of CLL.

Conclusions: Idelalisib treatment appears safe in patients with CLL at increased bleeding risk. Platelet function tests improved after beginning of therapy.

PB 2234 | Platelet Investigations in Patients with Essential Thrombocytosis

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Background: Patients with essential thrombocytosis (ET) are characterized by an increased platelet count, and provide a biological model for studying platelet function in patients with accelerated platelet turnover. ET patients have an increased risk of thromboembolic events, partly explained by the increased platelet count. An increased proportion of reactive immature platelets may also be important.

Platelet function analysed by impedance aggregometry is dependent on platelet count but flow cytometry allows for evaluating of platelet function independently of platelet count.

Aims: To investigate associations between platelet count, platelet turnover and platelet function in ET patients.

Methods: In 24 ET patients, platelet aggregation was measured by impedance aggregometry using the Multiplate® Analyzer. Arachidonic acid (AA), Thrombin-Receptor-Activating-Peptide (TRAP) and adenosine diphosphate (ADP) were used as agonists. Platelet surface expression of P-selectin, CD63 and fibrinogen were measured with flow cytometry (Navios®) after activation with the same agonists. The results were reported as median fluorescence intensity of marker-positive platelets.

Immature platelet count (Sysmex®) was used as marker of platelet turnover.

Results: Platelet count correlated significantly with aggregation induced by AA (r=0.43, P=0.04), TRAP (r=0.46, P=0.03) or ADP (r=0.60, P=0.003).

After activation by AA and ADP, the median fluorescence intensity of expressed fibrinogen, CD63 and P-selectin were significantly increased in ET patients compared to healthy individuals (P< 0.03).

The immature platelet count was significantly higher in ET patients compared to healthy individuals (median 12.3 [IQR 9.8-18.7] vs median 6.9 [IQR 5.5-10.3], P< 0.0001).

Conclusions: ET patients have an increased aggregation potential compared to healthy individuals illustrated with a higher median intensity of activating-dependent surface markers. This is likely explained by an increased platelet count and increased proportion of immature platelets.

PB 2235 | Computational Analysis of Shear Stress Distribution during Thrombus Formation in a Blood Vessel and Parallel Plate Flow Chamber

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Background: Unrevealing the mechanisms governing the regulation of arterial thrombus size is one of the long-standing problems of biorheology. Studies on thrombus formation ex vivo usually involve perfusion of whole blood through especially designed flow chambers. In such experiments activators are usually applied to a sufficiently large surface area, leading to the formation of multiple thrombi which affect local hydrodynamics.

Aims: In order to identify the differences between local hydrodynamic conditions in case of thrombus formation in a blood vessel and during flow chamber experiment the computational fluid dynamics analysis was performed.

Methods: Thrombi in microvessel were represented by semi-ellipsoids of various sizes, the boundary conditions corresponded to constant pressure drop. The geometry of multiple platelet thrombi in the flow chamber was taken from experimental data obtained by whole blood perfusion over collagen at 1000 s-1. Platelet thrombi were represented by semi-ellipsoids, and the stationary flow fields corresponding to different time points of experiment were computed for constant inflow boundary conditions. The blood was treated as Newtonian fluid, while thrombi were considered as solid impermeable barriers.

Results: Computation results showed significant differences in the dynamics of shear stress magnitude at the surface of growing thrombi in the studied systems: the initial stage of thrombi formation inside the flow chambers was characterized by decrease of surface shear rate, while in the blood vessel shear rate at thrombus surface rapidly increases with initial thrombus growth. At later stages of thrombi formation within the flow chamber there is a strong decrease of shear rates in between the thrombi associated with redistribution of blood flow and increase of shear rates at thrombi apexes.

Conclusions: CFD analysis shows non-physiological dynamics of thrombus surface shear rate in parallel-plate flow chamber experiments.

PB 2236 | Platelet Function during Prolonged Targeted Temperature Management after Cardiac Arrest: A Randomised Clinical Trial

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Background: Current guidelines recommend to lower the body core temperature in patients resuscitated after out-of-hospital cardiac arrest. Previous studies investigating the effect of mild hypothermia on platelet function were small and showed conflicting results. Moreover, the platelet function during prolonged hypothermia in humans has not been investigated.

Aims: Investigate whether prolonged duration of targeted temperature management impaired the platelet function compared with standard duration.

Methods: We randomized 82 out-of-hospital cardiac arrest patients to either 24 hours (standard, n=42) or 48 hours (prolonged, n=40) of targeted temperature management at 33±1°C. Blood samples were obtained 22±2, 46±2 and 70±2 hours after targeted temperature management was reach. Platelet function was analysed by impedance aggregometry (Multiplate®Analyzer) using COL®test, TRAP®test, ADP®test and ASPI®test as agonists. Use of antithrombotic drugs was systematically registered from medical records. Informed content was obtained and the study was approved by the research ethics committee.

Results: The platelet function was below the reference interval at all time points, independent on the agonist used. No differences in platelet function were observed between groups in the 22-hour sample. In the 46-hour sample, where only the standard group had been rewarmed, the prolonged group had a 24% (95% confidence interval (-10%;-37%), p=0.002) decreased platelet function when using the COL®test compared with the standard group, but no difference were observed between groups when using TRAP®test, ADP®test, or ASPI®test. Moreover, no differences were observed between groups in any of the agonist used at the 70-hour sample where all patients had been rewarmed.

Conclusions: Prolonged duration of targeted temperature management did not induce a pronounced impaired platelet function when compared with standard duration.

PB 2237 | An Absence of Platelet Activation Following Thalidomide Treatment *in vitro* or *in vivo*

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Background: Increased risk of thromboembolism and platelet hyperreactivity has been reported in patients receiving thalidomide therapy. Whether thalidomide induces platelet activation directly or through other factors remains unclear.

Aims: The aim of this study was to evaluate the effect of thalidomide on platelet activation under resting conditions *in vitro* and *in vivo*.

Methods: Isolated human or mouse platelets were treated with different concentrations of thalidomide (10, 50 and 100 μg/ml) for 60 min at 37°C followed by analysis of platelet surface expression of platelet receptors GPIbα, GPVI, $\alpha_{IIb}\beta_3$ and P-selectin, and PAC-1 or fibrinogen binding, by flow cytometry and collagen- or ADP-induced platelet aggregation. In addition, thalidomide (200 mg/kg) was intraperitoneally injected into mice for analysis of the effect of thalidomide on platelet activation *in vivo*.

Results: No increased expression of P-selectin, PAC-1 or fibrinogen binding was observed in either human and mouse platelets after thalidomide treatment *in vitro* for 60 min at 37°C. Thalidomide treatment also did not affect expression of GPIbα, GPVI or $\alpha_{IIb}\beta_3$, nor did it affect collagen- or ADP-induced platelet aggregation at threshold concentrations. However, while mice injected with thalidomide displayed no increased surface expression of platelet P-selectin or $\alpha_{IIb}\beta_3$, there was a significantly shortened tail bleeding time, thrombin time, prothrombin time together with higher levels of Factor IX and fibrinogen.

Conclusions: Thalidomide at therapeutic doses does not directly induce platelet activation under resting conditions *in vitro* or *in vivo*, but results in increased procoagulant activity, which could explain the thalidomide-dependent prothrombotic tendency in patients.

PB 2238 | Reduced Left Ventricular Function Compared to Preserved Function after Myocardial Infarction is Associated with a Different Platelet Pattern

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Background: Heart failure is associated with increased risk of thromboembolic events such as stroke.

Aims: The aim was to compare the platelet activity in patients with reduced left ventricular ejection fraction (LVEF) and normal LVEF after a recent myocardial infarction (MI).

Methods: MI patients were included in an observation study 3-5 days after the index MI. Platelet-monocyte (PMA) and platelet-ganulocyte (PGA) complexes, defined as the amount of monocyte/granulocyte with surface expression of CD41 or CD62P, platelet derived microparticles (PMP) and the amount of single platelets with surface expression of CD42b, CD49b, CD62P were evaluated in whole blood at inclusion and after 3 months. LVEF was calculated (n=84) after the index MI before discharge and LVEF < 0.52 for men and < 0.54 for women were defined as reduced LVEF.

Results: Reduced LVEF was found in 26% (n=22) of the MI patients. The proportions of aspirin, P2Y12-inhibitor, anticoagulant and statin treatment were similar in the group with reduced compared to normal LVEF. After 3 months the CD41+MPs tended to persist lower in the reduced compared to normal LVEF group (p=0.090). No differences were found comparing the other platelet measurements after 3 months.

Conclusions: The observation that patients with reduced LVEF have lower concentrations of circulating PMPs and single platelets expressing adhesion proteins early after the MI might be explained by their adherence to the damage myocardium. Activated P-selectin expressing platelets forming complexes with monocytes induce production of

TABLE 1 Platelet markers at inclusion after MI separated by LVEF

	LVEF reduced (n=22)	LVEF normal (n=62)	p-value
CD41+MPs (n/10000 platelets)	2492 (1580-2985)	3086 (2318-4127)	0.018
CD49b (%)	7.10 (5.46-10.91)	10.70 (8.58-14.02)	<0.001
CD42b (%)	6.78 (5.10-10.44)	10.17 (8.29-13.72)	<0.001
CD62P (%)	14.26 (12.30-17.44)	12.58(8.64-18.70)	0.183
PMA CD41+ (%)	3.63 (2.88-4.25)	3.30 (2.73-4.08)	0.600
PMA CD62P+ (%)	4.71 (3.81-5.37)	3.98 (3.10-4.69)	0.015
PGA CD41+ (%)	4.11 (3.60-5.46)	3.94 (3.23-5.00)	0.302
PGA CD62P+ (%)	8.72 (6.58-10.20)	7.34 (5.62-9.31)	0.170

cytokines and tissue factor which could contribute to the increased risk of thromboembolism in patients with reduced LVEF.

PB 2240 | Lymphocyte-platelet Adhesion and Clusters Formation in Patients Infected with Influenza A/H3N2

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Background: Recent studies have accumulated evidence that platelets, besides their haemostatic activity, participate in inflammation, immunity and atherosclerosis. In a previous study we demonstrated that platelets interacted with T-lymphocytes and formed heterotypic aggregates. Also it support CD4+ lymphocyte adhesion to the subendothelial extracellular matrix under flow conditions by formation of heterotypic clusters that are dependent on platelet adhesion and aggregation and mediated by CD40L, PSGL-1 and dependent integrins. Previously, we found that the number of lymphocyte-platelet aggregates (LPA) increased in patients with influenza A/ H1N1 (2009).

Aims: The aim was to investigate LPA number and lymphocyte-platelet clusters (LPC) formation in patients infected with influenza A/ H3N2 in blood circulation.

Methods: In control group we used the blood of 14 healthy donors. The blood of patients was taken in the acute phase of the disease (1-2 days) and after antiviral and symptomatic treatment (5-6 days). Lymphocytes were isolated by ficoll-urografin gradient and then counted percent of LPA and number of LPC (rel. u.).

Results: The number of LPA in control was 11,7 ± 3,6%, and LPC 1,9 ± 1,03 rel. u. It has been found that the amount of LPA and LPC increased in the acute phase of the disease versus control (2.4 and 3.9 times, respectively, p < 0.001). Also the average numbers of platelets on lymphocytes surface increased in 2.7 times (p < 0.05). We didn't find differences in these parameters on 5-6 days of the disease.

Conclusions: Thus, lymphocytes increased ability to form heterotypic coaggregates and clusters with platelets in patients with acute phase of influenza A/H3N2. Probably the clusters forming process is mediated by platelet and leukocyte adhesion molecules.

PB 2241 | Detection and Characterization of Extracellular Vesicles at Different Stages of Platelet Concentrate Storage Using High-resolution Flow Cytometry

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Background: Platelet-derived extracellular vesicles are formed due to platelet activation. Increased level of these vesicles in the bloodstream is observed in various pathological processes.

Aims: To analyze platelet-derived extracellular vesicles in platelet concentrate samples at the different stages of storage using high-resolution flow cytometry.

Methods: 65 samples of platelet concentrates prepared from donors' blood according to the standard protocols were selected. Samples were fractionated by differential centrifugation and composition of the fractions was characterized by flow cytometer CytoFLEX/ BecmanCoulter using fluorescence-labeled antibodies against specific markers of different cell types-platelets(CD41,CD62P), erythrocytes(CD235a), leukocytes(CD45), endotheliocytes(VEGFR2).

Results: Analysis showed that the fractions contain minor amounts(< 0.01%) of objects carrying CD45 or CD235a and are substantially free of VEGFR2 carriers. Platelets were detected as objects of 1-2.5μm, bearing CD41(99% of all objects). Platelet-derived extracellular vesicles were defined as the objects, which bear CD41, and were represented by two populations of objects, which have size 0.1-1μm (71% of all CD41 positive objects with size < 1μm) and 0.03-0.1μm. The substantial proportion of platelet-derived extracellular vesicles (54%) was positive for surface marker of activated platelets CD62P/P-selectin. Levels of platelet-derived extracellular vesicles as well as extracellular vesicles from erythrocytes and leukocytes increased in platelet concentrate on the 7th day compared with the 2nd day.

Conclusions: Thus, during platelet concentrate storage the number of platelet-derived extracellular vesicles increases progressively, reflecting platelet activation and changing of platelet concentrate quality. The findings are of practical importance for blood banking and transfusion medicine, since they indicate the advantages of 2nd day versus 7th day concentrate. Supported by grant of Russian Foundation for Basic Research №16-04-01142.

PB 2242 | New Opportunities in Express-evaluation of Living Platelets

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Background: Platelets play a critical role in cancer progression, thrombosis, and markers of platelet-tumor cell interaction are candidates as biomarkers for cancerprogression and thrombosis risk.

Aims: The aim of our study was to evaluate living platelet size, function and morphology simultaneously in unactivated and activated states in patients with cancer using method of Quantitative Phase Imaging (QPI).

Methods: We examined 79 healthy volunteers and 75 patients (average age 67,3+/-9,1 years) with oropharynx cancer of I-II and III-IV stage, brain metastasis or glioblastoma. We observed the optic-geometrical parameters of each isolated living cell and the distribution of platelets by sizes to detect the dynamics of cell population heterogeneity using Computer-aided Phase-Inteference Microscope.

Results: Simultaneously we identified 4 platelet forms that have different morphological features and different parameters of size distribution. We found out that morphological platelet types correlate with morphometric platelet parameters. In the fluorescence microscope we observed the platelet concentration; the relative content of platelet granule (%); concentration of platelet granules; morphofunctional activity (in points); adhesive activity (in % or points); morpho-functional status of platelets (in points). In this method we used staining platelet which were painted with trypaflavine and acridine orange in Zerensena phosphate buffer (pH = 7.3).

We fixed the platelet activation using both methods. The degree of platelet activation is connected with initial tumor stage and metastases. The vital colorant allowed to obtain a differential glow of cell cytoplasm and granule in the fluorescence microscope. No light granules in platelets are associated with activation of cells or damage.

Conclusions: According to our results we suggest that both methods can serve criteria of the efficiency of the radio- and chemotherapy and can be express methods to detect the activation status of platelets.

PB 2243 | Comparing Platelet Reactivity in Patients with Chronic Kidney Disease to that in Healthy Volunteers

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Background: The 10-year mortality risk in patients with non-dialysis requiring chronic kidney disease (CKD) is 5 fold higher than the general population. This elevated mortality rate is strongly linked to atherothrombotic disease.

Aims: Here we aim to determine if changes in platelet reactivity may be a causative factor in the increased incidence of atherothrombotic disease in CKD patients.

Methods: Blood was obtained by venepuncture from healthy volunteers (n=20) and CKD patients (n=18, stage 4-5, eGFR=15±1) into 3.2% sodium citrate. Flow cytometry was used to determine P-selectin expression and platelet-leukocyte interactions in whole blood. Platelet rich plasma and platelet poor plasma were obtained by centrifugation and aggregation in response to arachidonic acid, collagen, TRAP-6 amide (ligand for thrombin receptor, PAR-1), U46619 (ligand for thromboxane A_2 receptor, TP), epinephrine and ristocetin, was determined using light transmission aggregometry (LTA) and Optimul aggregometry. Percentage aggregation was calculated and data were analyzed by ANOVA. Data is reported as mean±s.e.m.

Results: Basal P-selectin expression on platelets in whole blood was not different between CKD patients and healthy controls. LTA indicated increased platelet aggregation in CKD patients compared to healthy controls. For instance, aggregation to arachidonic acid (1mM) was 88±2% in CKD patients vs. 73±1% in controls; ADP (20 μM) 85±2% vs. 70±1%; collagen (10 μg/ml) 86 ±3% vs. 72±1%; TRAP-6 amide (25 μM) 87±2% vs. 71±1%; U46619 (10 μM) 87±2% vs. 72±1%; $p< 0.05$ for all.

Conclusions: Initial observations indicate increased platelet reactivity in CKD patients compared to healthy controls which may contribute to the increased risk of atherothrombotic disease.

PB 2244 | Disaggregation Following Agonist-induced Platelet Activation in Patients on Dual Antiplatelet Therapy

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Background: Light transmission aggregometry (LTA) is considered the historic gold standard method for the assessment of platelet function. The difference between maximal and final aggregation by LTA indicates the stability of platelet aggregates. A small difference means low disaggregation, whereas a great difference between the two values reflects high disaggregation, i.e. a low stability of platelet aggregates.

Aims: In this study, we evaluated the extent of disaggregation after platelet stimulation with 5 different agonists in patients on dual antiplatelet therapy.

Methods: LTA was performed in 323 patients with daily aspirin (100mg/d) and clopidogrel (75mg/d, n=256), prasugrel (10mg/d, n=47) or ticagrelor (180mg/d, n=20) therapy after angioplasty and

stenting using the following agonists: ADP (10μM), arachidonic acid (AA; 0.5mg/ml), collagen (190μg/ml), epinephrine (5.5μM) and thrombin receptor-activating peptide-6 (TRAP-6; 25μM).

Results: Significant differences between maximal and final aggregation values were observed with all agonists throughout the groups (all p< 0.001). Disaggregation was highest using AA (clopidogrel: 36.5% (18.2%-66.3%); prasugrel/ticagrelor: 100% (100%-100%)) and ADP (clopidogrel: 21.7% (5.1%-44.6%); prasugrel: 100% (81.3%-100%), ticagrelor: 100% (85.0%-100%)). In contrast, low disaggregation was observed after platelet stimulation with collagen and TRAP-6 in clopidogrel-treated patients (collagen: 8.8% (1.8%-28.9%); TRAP-6: 2.5% (0.7%-9.2%)), and after platelet stimulation with collagen (prasugrel: 3.0% (2.1%-5.5%); ticagrelor: 3.3% (2.2%-6.5%)) and epinephrine (prasugrel: 4.8% (0.0%-12.5%); ticagrelor: 5.0% (0.5%-17.4%)) in prasugrel- and ticagrelor-treated patients.

Conclusions: Pathways of platelet activation that are not inhibited by standard antiplatelet therapy allow persisting platelet aggregation and may at least in part be responsible for adverse ischemic events during antithrombotic secondary prevention.

PB 2245 | *In vitro* Study on the Effects of Ibrutinib (Ibr) on Platelet (Plt) Function via Light Transmission Aggregometry (LTA): Platelet Aggregation (PA) Results

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Background: Ibr is a first-in-class, once-daily inhibitor of Bruton's tyrosine kinase (BTK). BTK is expressed in human plts and is involved in signaling via collagen receptor glycoprotein VI. Bleeding events, mostly Gr 1/2 bruising and petechiae, are common with ibr (ibr PI, 2017) and other BTK inhibitors (Walter Blood 2016; Byrd NEJM 2016).

Aims: This in vitro study evaluated ibr effects on plt function.

Methods: Four cohorts (cht) of subjects (sbj) without known cancer were included: healthy, taking aspirin, taking therapeutic warfarin daily for ≥60 days, or with renal dysfunction on hemodialysis. Plt-rich plasma isolated from peripheral blood was incubated with 0-10 μM ibr and exposed to the following plt agonists: arachidonic acid (AA), adenosine diphosphate (ADP), collagen, ristocetin, and thrombin receptor-activating peptide 6 (TRAP-6). In vitro PA was measured by percent of maximum aggregation (% MA) obtained by LTA.

Results: Among 56 sbj, 8 per cht had evaluable samples. Only collagen-induced PA was meaningfully inhibited, occurring in a concentration-dependent manner with ibr 10 μM resulting in >50% PA inhibition in healthy, warfarin, and renal chts. In the aspirin cht, inhibitory effects of ibr on collagen-induced PA was also observed, but was less pronounced as collagen-induced PA was reduced in control (ie, without ibr). No measurable PA was noted to AA in aspirin cht control. In all chts, the reduction in PA was < 30% (ie, no appreciable inhibition) with ibr concentrations up to 10 μM for the remaining agonists.

Conclusions: In this in vitro study, ibr inhibited collagen-induced PA in samples from sbj who were healthy, on therapeutic warfarin, or had renal dysfunction. The magnitude of ibr inhibition of collagen-induced PA in sbj on aspirin was less pronounced since collagen-induced PA was already reduced in the aspirin control (ie, without ibr). PA induced by the agonists AA, ADP, ristocetin, and TRAP-6 were not substantially inhibited by ibr 10 μM.

PB 2246 | The Use of Laser Therapy for Muscle Bleeding in Glanzmann Thrombasthenia - A Case Report

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Background: Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder, caused by quantitative or qualitative defect of the membrane integrin αIIbβ3, a platelet receptor binding adhesive proteins in the initial stage of the coagulation proces. The primary objective of this study was to describe clinical effects observed in laser treatment in muscle bleeding in patient with GT.

Aims: A 13-year-old male with GT was admitted to the hospital due to a burgeoning bleeding in the right thigh caused by a fall from a bicycle. During physical examination, a haematoma was detected. The difference of 15cm in the circuit was observed between the lower limbs. The thigh was aching and an extensive bruise was noticeable. Platelet transfusions were ineffective so rFVIIa was successfully used to terminate the bleeding. Nonetheless the extensive, painful haematoma could have still been observed.

Methods: The biostimulation with *semiconductor laser generating a beam of monochromatic light (visible bright red) with the wavelength of 650 nm and the dose of 2J/cm* was used to accelerate the regression of hematoma. Furthermore, rehabilitation exercises were conducted in parallel.

Results: The reduction of hematoma and the cessation of patient's complaints were observed already after administrating the first dose. The *average reduction* in circumference per day was about 1cm. Adverse effects were observed as well though.

Conclusions: This case report describes the succesful use of laser therapy in the treatment of muscle haematoma in GT. The laser biostimulation is safe and can help to accelerate treatment and improve patient management.

PB 2247 | Amniotic Fluid-derived Mesenchymal Stem Cells (AF-MSCs) Decrease Agonists Platelet Response

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Background: When mesenquimal stromal cells are administered intravenously, they produce adverse events, mainly thrombotic disorders. These have been related to tissue factor (FT) expression, which gives them procoagulant activity. Moreover, these cells have the ability to interact with other cells, including platelets, key elements in haemostasis and homing.
Aims: The objetive was to evaluate in vitro, the effect of amniotic fluid-derived mesenchymal stem cells (AF-MSCs) on platelet function.
Methods: AF-MSCs were isolated from amniotic fluid obtained in deliveries by planned caesarean. They were cultured at 37 °C and 5% CO_2 with Amniomax medium supplemented with fetal bovine serum and antibiotics. AF-MSCs were tripsinized on passage 4-5 and mesenchymal markers (CD90, CD105, CD73) and FT expression was measured by flow-cytometry. Whole blood of control subjects was incubated *in vitro* with AF-MSCs (150.000/300.000 cell/mL, 15 min at 37 °C) and the platelet response was quantified by impedance aggregometry (multiplate; ADP and TRAP tests), flow cytometry (platelet activation marker P-selectin expression), and platelet function analyzer (PFA-100). Also, clotting tests were performed in plasma obtained from in vitro blood incubations.
Results: AF-MSCs cultures showed high proliferation and viability. The AF-MSCs expressed mesenchymal markers CD105 (88%), CD90 (60%) and CD73 (97%) and tissue factor (43%), being negative for the hematopoietic markers CD45 and CD34. The incubation of peripheral blood with AF-MSCs shorted PTTa; augmented EPI-collagen closure time and decreased impedance platelet aggregation response to ADP and TRAP (even in the presence of the antithrombin, hirudin), without modification of PTa, ADP-collagen clousure time, or P-selectin expression.
Conclusions: Platelet function is modified by AF-MSCs. The mechanisms involved will require further studies.

PLATELETS – CLINICAL

PB 690 | Obesity and ADAMTS13 Deficiency as Risk Factors for TTP

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Background: Thrombotic thrombocytopenic purpura (TTP) is caused by absence of ADAMTS13 activity. Thrombocytopenia is presumably related to formation of VWF and platelet rich microthrombi. Obesity may be a risk factor for TTP; it is associated with abundance of macrophages that may phagocytose platelets.
Aims: To evaluate the role of obesity and ADAMTS13 deficiency in TTP, and to establish whether macrophages contribute to thrombocytopenia.
Methods: Lean or obese ADAMTS13 deficient (*Adamts13*$^{-/-}$) and wild-type (WT) mice were triggered with 250 U/kg of recombinant human VWF, and TTP characteristics were evaluated 24h later. In separate experiments, macrophages were depleted in the liver and spleen of lean and obese WT or *Adamts13*$^{-/-}$ mice by injection of 10 µl/g body weight of clodronate-liposomes, 48h before injection of rVWF.
Results: Obese *Adamts13*$^{-/-}$ mice had a lower platelet count than lean mice (894±35x10^3 *versus* 1010±35x10^3 platelets/µL; p< 0.05) suggesting that they might be more susceptible to TTP development. Lean *Adamts13*$^{-/-}$ mice triggered with VWF did not develop TTP, while typical TTP symptoms developed in obese *Adamts13*$^{-/-}$ mice, including severe thrombocytopenia (35±13x10^3 *versus* 803±113x10^3 platelets/µL, p=0.0001) and higher LDH levels (630±123 *versus* 329 ± 57 mU/mL; p< 0.05). Removal of macrophages by clodronate injection in obese *Adamts13*$^{-/-}$ mice before treatment with VWF resulted in preservation of platelet counts 24h after the trigger (1327±80x10^3/µL *versus* 187±47x10^3/µL for control PBS liposomes; p=0.008). *In vitro* experiments with cultured macrophages confirmed VWF dose-dependent increase of platelet phagocytosis.
Conclusions: Obese *Adamts13*$^{-/-}$ mice are more susceptible to induction of acute episodes of TTP than lean mice. Phagocytosis of platelets by macrophages contributes to thrombocytopenia after VWF triggering in this model.

PB 691 | Acute Thrombotic Thrombocytopenic Purpura (TTP) - Diagnostic and Prognostic Implications of Highly Sensitive Troponin Assays, ECG and ECHO

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Background: Whilst cardiac involvement is a recognised feature of acute TTP, clinical symptoms are not consistent. The development of highly sensitive troponin (hs-cTnT) assays allows detection of subtle elevations with diagnostic and prognostic implications. Identification of myocardial injury is crucial as patients may benefit from early, aggressive treatment; however, the value of standard cardiac investigations is unclear.
Aims: To evaluate hs-cTnT levels and electro/echocardiographic changes in patients with acute TTP, and their correlation with disease severity and outcome.
Methods: A retrospective review of registry data from a Regional TTP centre and data collected on hs-cTnT levels, electrocardiogram (ECG), echocardiography findings in acute TTP.

Results: 29 patients with 32 hospital episodes (2011-2016) were reviewed. The M:F ratio was 1:1.4, mean age 52 years (28-83). 22/32 episodes had an abnormal hs-cTnT at presentation (>14ng/L). 18/22 episodes with an elevated hs-cTnT had an ECG, of which none demonstrated any acute abnormality. Echocardiography was performed in 13/32 episodes. 8/10 were performed in patients with an abnormal hs-cTnT but only 1/8 showed acute change - RV overload and dilated RV. 7/8 described chronic changes such as left ventricular hypertrophy.

Abnormal hs-cTnT levels were associated with a lower ADAMTS13 activity, higher ADAMTS13 IgG inhibitor level and lower presenting platelet count. Outcomes were poorer in those with abnormal hs-cTnT, including 4 deaths. All deaths were in patients with a hs-cTnT >100ng/L, irrespective of other cardiac investigation results (see table).

TABLE 1 HS-cTnT and TTP Severity/Outcomes

	Normal Presenting hs-cTnT (<14ng/L, n=10)	Abnormal Presenting hs-cTnT (>14ng/L, n=22)
Median presenting platelet count (x109/L)	22	11
Median ADAMTS 13 Activity (%)	26.5	<1
Median IgG ADAMTS13 Inhibitor (units)	8.5	61
Acute ECG/ECHO change	0	1
Deaths	0	4 (18%)

Conclusions: hs-cTnT assays allow early confirmation of myocardial involvement in acute TTP. Abnormal hs-cTnT levels correlate with disease severity and poor outcome. ECG and ECHO findings do not appear to correlate with hs-cTnT levels or represent acute cardiac involvement. Further study is required to determine the value of routine cardiac investigation and alternative cardiac imaging that may inform management.

PB 692 | Plasmin Cleavage of ADAMTS-13 Enhances its Activity

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Background: Thrombotic thrombocytopenic purpura (TTP) is associated with a deficiency of ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), which degrades von Willebrand Factor (VWF) multimers to control thrombogenicity. Plasmin has protective properties in TTP: it degrades platelet-VWF complexes and stimulation of plasmin activity has therapeutic value in a TTP mouse model. However, prolonged exposure (hours) of ADAMTS-13 to plasmin destroys it. This leaves the overall contribution of plasmin in thrombotic microangiopathy unclear.

Aims: To investigate the short-term effects of plasmin on ADAMTS-13 in plasma.

Methods: We studied how plasmin influences ADAMTS-13 activity in plasma using the FRETS-VWF73 assay. Direct binding studies were performed to investigate the influence of plasmin on the binding properties of ADAMTS-13 to immobilized VWF.

Results: Plasminogen activation in plasma (30 min.) increases ADAMTS-13 activity as measured by FRETS-VWF73 (25.0% ± 3.6%, p=0.0625) (Fig. 1A). This is unrelated to direct effects of plasmin on this substrate, as inhibition of plasmin prior to measurement still results in increased ADAMTS-13 activity (21.2% ± 6.9%, p=0.0313). We found that increased ADAMTS-13 activity is accompanied by plasmin-mediated cleavage of ADAMTS-13. We next investigated the influence of plasmin on the binding of ADAMTS-13 to immobilized VWF. Normally, a single species of ADAMTS-13 binds to VWF (Fig. 1B). After plasmin activity, full-length ADAMTS-13, multiple disulfide-linked cleaved forms-, and a truncated form of ADAMTS-13 are captured (Fig. 1C).

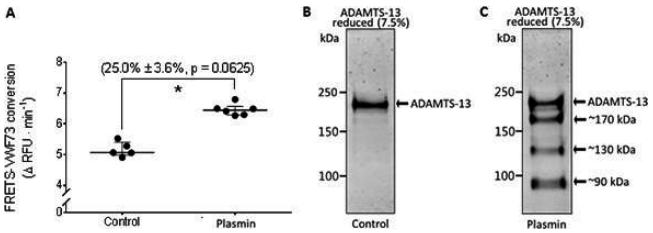

FIGURE 1 A) The effect of plasmin on ADAMTS-13 activity. B,C) Binding of ADAMTS-13 to immobilized VWF before (B) or after (C) plasmin activity.

Conclusions: We report the unexpected finding that plasmin stimulates ADAMTS-13 activity in plasma, which is accompanied by binding of cleaved forms of ADAMTS-13 to VWF. We propose that cleavage or truncation of ADAMTS-13 by plasmin alters protein conformation with functional consequences. This could reflect a physiological rescue mechanism to enhance ADAMTS-13 activity.

PB 694 | Unraveling Immunoprofiles of Acquired TTP Patients Using Anti-idiotypic Antibodies

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Background: In acquired (a)TTP patients, anti-ADAMTS13 autoimmune responses are polyclonal but with clear immunodominant epitopes in the ADAMTS13 spacer domain. A detailed analysis of immunoprofiles in aTTP patients is not available yet. However, insight into immunoprofiles of patients with other autoimmune disorders provided crucial diagnostic and prognostic information.

Aims: By using anti-idiotypic Abs that recognize specific anti-spacer autoAbs, we aim at getting insight into the anti-spacer immunoprofiles in aTTP patients.

Methods: Mice were immunized with three different categories of cloned human anti-spacer autoAbs: autoAb1, 2 and 3 with a strong, weak or no inhibitory effect on ADAMTS13 function respectively, to generate anti-idiotypic Abs. Fifty-six plasma samples of acute aTTP patients were analyzed via ELISA where the three anti-idiotypic Abs were coated to capture their respective category of anti-ADAMTS13 autoAbs. In this way, anti-spacer immunoprofiles were determined.

Results: We successfully developed three anti-idiotypic Abs that specifically captured autoAb 1, 2 or 3. Screening aTTP patient plasma on these three anti-idiotypic Abs revealed that 43% (24/56) of the patients had autoAb1 group Abs, 41% (23/56) had autoAb2 group Abs and 30% (17/56) had autoAb3 group Abs. Identifying which autoAb groups are present in the plasma of individual aTTP patients allowed us to setup immunoprofiles. Immunoprofile 1 (autoAb1 group Abs) was present in 7.14% of the patients, profile 2 (autoAb2) in 14.29%, profile 3 (autoAb3) in 5.36%, profile 4 (autoAb1 and 2) in 10.71%, profile 5 (autoAb1 and 3) in 8.93%, profile 6 (autoAb2 and 3) in 0%, profile 7 (autoAb1, 2 and 3 group) in 16.07% and profile 8 (none of the autoAb groups) in 37.5%.

Conclusions: We have developed a powerful tool to determine anti-spacer immunoprofiles in aTTP patients. We now will determine whether specific immunoprofiles present in aTTP patients would allow to identify prognostic factors to predict death and disease recurrence in these patients.

PB 695 | The in vitro and in vivo Effects of Streptokinase in a Papio *ursinus* Baboon Model of Acquired Thrombotic Thrombocytopenic Purpura (TTP) - A Pilot Study

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Background: The accumulation of large VWF multimers due to absence/inhibition of ADAMTS13, is central to the pathogenesis of TTP. Recently, plasmin has been identified as a back-up for ADAMTS13, suggesting utility of streptokinase in the treatment of acquired TTP, possibly at lower doses than used for fibrinolysis. A *Papio ursinus* baboon model of acquired TTP has previously been used for the preclinical study of novel therapies in TTP.

Aims: To determine the in vitro and in vivo effects of streptokinase in a *Papio ursinus* baboon model of acquired TTP.

Methods: VWF activities & multimer patterns, and thromboelastograms, were assessed (amongst other parameters) after spiking citrated baboon blood specimens with increasing concentrations of streptokinase. After induction of TTP with an anti-ADAMTS13 mAb, escalating doses of streptokinase (ranging from 50 000 to 900 000 IU) were administered intravenously to a 13 kg baboon, and the effects of streptokinase assessed on (amongst others) peripheral blood counts, fibrinolysis, VWF activities & multimer patterns, and thromboelastograms.

Results: After spiking, fibrinolysis with loss of large VWF multimers, was observed at a concentration of 2200 IU/mL - roughly equivalent to a dose of 1 500 000 IU in a 10 kg baboon. Escalating intravenous streptokinase doses (maximum: 900 000 IU) had no effect on platelet counts, or VWF activities & multimer patterns. Doses above 700 000 IU did however lead to activation of fibrinolysis as evidenced by increasing plasmin-antiplasmin complexes and fibrin(ogen) degradation products, and declining plasminogen levels.

Conclusions: Although intermediate dose streptokinase does activate the fibrinolytic system in a *Papio ursinus* baboon model of acquired TTP, it has no effect on the TTP phenotype. As suggested by in vitro data, further in vivo studies in this model would require higher streptokinase doses to confirm proof-of-concept, before it can be considered a therapeutic option in acquired TTP.

PB 696 | mRNA Identification of a Novel Isoform (ISF) and ISF3 from ADAMTS13 in Different Types of Cells

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Background: ADAMTS13 is a plasma metalloprotease that cleaves von Willebrand factor (VWF).

Alternative splicing (AS) is a common feature of ADAMTS gene expression, in ADAMTS13 this process could product cleave the genomic sequence in different positions and causes several potential variants. Shonrom (2009) described mRNA of ISF1 in cancer lines and ISF1, ISF2 and ISF3 of Hep3B. We described previously (ISTH 2013) mRNA of ISF1 and ISF2 in Plts, HUVEC and ISF2 in two breast cancer lines (MDA-MB231, MCF7).

Aims: Determine the presence of ISF3 in Plts, HUVEC and cancer cell lines, characterize and identify a potential ADAMTS13 ISF.

Methods: Total RNA was isolated by TRIZOL method. The integrity was verified by 260/280 optical density ratio. mRNA was reverse transcribed using special primers. We designed primers to amplify the sequence of ADAMTS13 to differentiate Iso1 (421bp) and Iso3 (334bp). β-actin was used as control. Hep3B ISFs were used as control. The PCR products were analyzed on agarose gel containing SYBR Safe. IQTL software was used to quantitative analysis (Pixel intensity values=PIV).

Results: All PCR performed, except the negative controls, were positive for β-actin and negative control did not present bands.

A particular novel ISF (ED7-8) and ISF3 were observed in all cells studied: Plts, HUVEC, Hep3B, MDA-MB231 and MCF7 (Figure 1).

The evaluation of PIV was made respect to ISF1 of each cell. The mean and standard deviation (SD) of three assays showed in Table I.

The PIV results of ISF3 and ED7-8 respect to Hep3B were not statistically significant for all the samples except for HUVEC. A large number of Plts PIV measurements would be necessary to decrease the high standard deviation obtained.

TABLE I ISFs1 and ED7-8 PIV results respect to Hep3B in studied cells

	ISF 3			ED7-8 ISF		
	Mean (%)	SD	p-value	Mean (%)	SD	p-value
Hep3B	11.8	6.2	1.000	30.0	5.5	1.000
Plts	2.8	0.5	0.061	116.0	82.6	0.148
HUVEC	1.8	0.4	0.043	8.6	7.6	0.017
MDA-MB231	8.1	8.8	0.557	54.0	30.0	0.246
MCF7	12.6	11.0	0.897	56.5	23.2	0.121

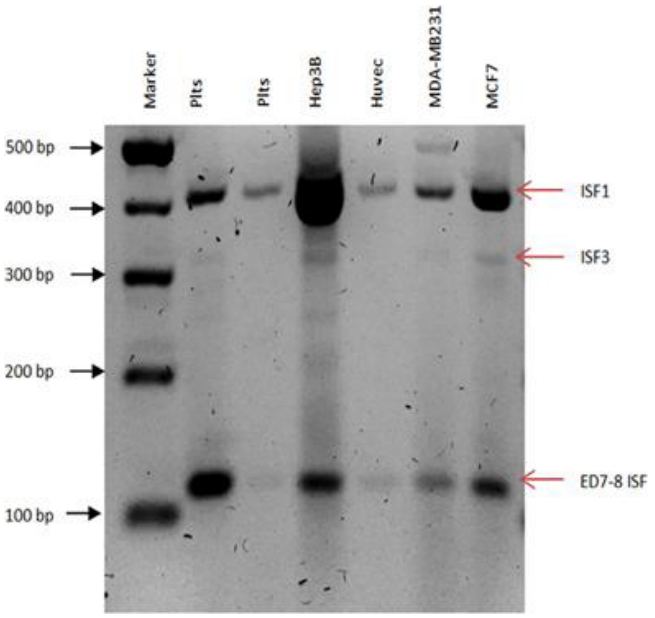

FIGURE 1 Differentiation of ISFs 1, 3 and novel ED7-8 on agarose 2%

Conclusions: The ISF 1, 3 and ED7-8 were identified by sequence analysis and quantified in relative form.

Additional studies are also required to elucidate the relationship between the presence of the ADAMTS13 ISFs, translational regulation, protease activity and cells proliferation.

PB 698 | Splenic Anti-ADAMTS13 Response in Relapsing Thrombotic Thrombocytopenic Purpura (iTTP) Patients Mirrors the Acute Immune Response in Plasma

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Background: Anti ADAMTS13 autoantibodies (ATS13-Abs) are a hallmark of iTTP. Long-lasting clinical remission in 90% of relapsing iTTP patients can be achieved by splenectomy (S).

Aims: To confirm the high number of pathogenic, ATS13-Abs producing B-cells in the spleen in relapsing iTTP found previously (Schaller et al. Blood, 2014) by a new approach.

Methods: ATS13$^+$ splenic mononuclear cells of 4 relapsing iTTP patients (A, C, E, F) were single cell sorted. RNA of individual ATS13$^+$ B-cells was reverse transcribed and Abs amplified by nested PCR. Selected Abs of pat E (relapsing iTTP with non-inhibitory ATS13-Abs, relapse 4 months after S, in remission after rituximab) and pat F (severe, relapsing iTTP with persistence of ADAMTS13 < 5% and a strong inhibitor, in remission after concomitant S and rituximab) were cloned into plasmids containing constant IgG4 regions and transfected into

HEK293T cells. Abs in the supernatant were screened for binding to ATS13 by ELISA and their inhibitory potential in a Bethesda-like assay.
Results: Sequencing revealed 80 ATS13-Abs clones with enrichment of IGHV1-69 encoded Abs (15% versus 1% among all splenic B-cells). ATS13 Abs were encoded by gene families IGHV3 (43,6% and 29.2%), IGHV4 (33.3% and 16.7%), IGHV clan I (IGHV1, 5 or 7; 23% and 54.2%) in pat E (n=39 Abs) and F (24 Abs), respectively, and among clan I Abs 2.6% and 16.7% employed IGHV1-69. Cloning of ATS13-Abs sequences resulted in 7 (pat E) and 2 (Pat F) IgG, of which 6 and 1 were ATS13 binding. In line with the plasma-phenotype of acute TTP episodes all ATS13-Abs of pat E were non-inhibitory, and a strong functional ATS13 inhibitor in pat F.
Conclusions: The spleen is an important reservoir of pathogenic B-cells in relapsing iTTP, and mirrors the ATS13-Abs response in plasma during acute TTP episodes. Different mechanisms may elicit distinct tolerance breaks, as inhibitory and non-inhibitory ATS13-Abs show different gene usage preferences.

PB 699 | The Incidence of Thrombotic Thrombocytopenic Purpura in Israel, as Determined by ADAMTS-13 Activity and Anti-ADAMTS-13 Antibody Levels

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening disease. Measurement of ADAMTS-13 activity and anti-ADAMTS-13 antibody (antibody) levels may advance the TTP diagnosis and therapy efficacy monitoring.
During 2005-2013, the Hematology Laboratory at Rambam was the central and sole facility in Israel to perform ADAMTS-13 assessment.
Aims: To estimate the incidence of TTP in Israel based on the measurement of ADAMTS-13 levels.
Methods: During 2005-2013, 393 patient blood samples were received for TTP diagnosis. ADAMTS-13 activity and antibody levels were measured using the TECHNOZYM ADAMTS-13 ELISA kits. Acquired TTP was defined by the presence of anti ADAMTS-13 antibodies and low activity during the acute episode. Congenital TTP was defined as ADAMTS-13 activity < 10% without the presence of antibodies.
Results: Out of the 393 samples, 117 (30%) were diagnosed as acquired TTP, with average levels of ADAMTS-13 activity 11±18 % and antibodies 56±34 U/ml. Nine patients (2.3%) had an ADAMTS-13 activity level between 1-10% and 10 patients (2.5%) < 1%; in none of them anti-ADAMTS-13 antibodies were found.
Female patients accounted for 64% of acquired and 80% of congenital TTP; in 8% of them the acute episode occurred during pregnancy.
Median age of patients at first episode of acquired TTP was significantly higher than congenital TTP (41 vs. 27 years, P=0.04).
No increased frequency of TTP diagnosis across the four seasons was observed.
The annual incidence of newly diagnosed acquired and congenital TTP in Israel was found to be 2.0 and 0.3 patients, respectively, per million population.
Therapy response monitoring by ADAMTS-13 levels was performed in 44% of acquired and 37% of congenital TTP patients.
Conclusions: The incidence and characterization of TTP patients in Israel is comparable to other worldwide TTP registries.
Measurement of ADAMTS-13 activity and antibody levels can advance TTP diagnosis and treatment efficacy monitoring.

PB 700 | A Rapid and Simple Assay for the Determination of ADAMTS-13 Activity

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Background: The standard laboratory test for ADAMTS-13 Activity is an ELISA, which requires at least 4 h to process. It tends to be performed by specialized laboratories only. Therefore, for a physician to receive a patient's ADAMTS-13 activity result can range from 24 h to as much as one week. This delay in reporting could have a negative impact on patient care.
Aims: We have developed a rapid and simple assay which would allow the physician to have an initial reportable result for ADAMTS-13 Activity within 1 hour from the blood draw.
Methods: The assay is based on flow through technology, which allows for simple, rapid processing. Plasma samples are pre-incubated with a substrate which is cleaved by ADAMTS-13 present in the plasma. This pre-treated sample is applied to the test unit which contains a membrane with immobilized antibodies specific for the cleaved substrate. As the sample flows through the membrane into an absorbent pad the cleaved substrate binds to capture antibody. A secondary antibody is used to detect the membrane bound complex. Using a gold conjugate a red colour is produced. The colour intensity is proportional to the level of ADAMTS-13 activity in the plasma sample and compared with a colour standard card, allowing semi-quantitative analysis of the patient sample. The assay can be performed at room temperature without specialized laboratory equipment. The assay processing time is under 30 minutes. The test is designed for evaluation of a single patient sample, with a positive control run in parallel.
Results: The designed assay cut-off value of 10% ADAMTS-13 Activity is clinically relevant for differentiation of TTP patients. A 100% correlation was observed when TTP patient samples (n=20) were run in the TECHNOZYM ADATMS-13 ELISA and this assay.
Conclusions: This rapid and simple assay is versatile and suitable as a cost effective screening assay enabling quick turnaround for ADAMTS-13 activity reporting.

PB 701 | Retrospective Analysis of the Relapse Rate in Patients Surviving Acute Acquired Thrombotic Thrombocytopenic Purpura (TTP) Treated with or without Rituximab

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Background: Acquired TTP is caused by autoantibody mediated severe ADAMTS13 deficiency leading to microangiopathic hemolytic anemia (MAHA) and thrombocytopenia with or without ischemic organ involvement. 80-90% of patients survive with plasma exchange (PEX), fresh frozen plasma replacement and corticosteroid (CS) treatment; survivors are at high risk for relapse. The anti-CD20 monoclonal antibody rituximab is increasingly added to conventional PEX and CS in plasma-resistant and/or relapsing patients.

Aims: We analysed the relapse rate in survivors of acute TTP treated with or without rituximab added to PEX and CS.

Methods: 88 patients treated at or referred for consultation to our center between disease onset and November 2014 were evaluated. Number, duration, clinical manifestations, laboratory data and treatment of each bout were documented. Diagnostic criteria of acute TTP were thrombocytopenia, MAHA, increased LDH and (since 2004) severe ADAMTS13 deficiency (< 10%).

Results: 88 (female n=70; male n=18) patients had a total of 253 acute episodes (median 2, range 1-21/patient) over a median observation period of 8.1 years (range 0.03-32 years). Median duration of the acute episodes was 29 days. The relapse rate of the whole group was 2.6% per month. Women had a relapse rate of 2.3% per month and men of 3.7% (p=0.001). Information on therapy was evaluable in 232 acute TTP episodes. Rituximab was administered in 76 of those in 46 patients. Relapse rate after episodes not treated with rituximab (n=156) was not significantly higher (p=0.874) than after episodes where rituximab was added.

Conclusions: This retrospective analysis does not show a significant reduction of acute TTP relapses by rituximab given during an acute bout. A selection bias favouring the application of rituximab in plasma-refractory or recurring disease may exist. Therefore, only prospective evaluation of rituximab, ideally by a randomized controlled trial, will better define its role in acquired TTP.

PB 702 | Epidemiology of Thrombotic Microangiopathy in Malaysia

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Background: Thrombotic thrombocytopenic purpura (TTP) is rare and severe form of thrombotic microangiopathy (TMA) with severe deficiency in ADAMTS-13 protein. In Malaysia, there has not been a study addressing the epidemiology of TMA as yet.

Aims: To study the epidemiology of TMA and the overall survival in relation to ADAMTS-13 activity.

Methods: The cases (year 2012-2016) are traced through the Ampang hospital electronic hospital information system (ehis) and the haemostasis laboratory archived records. The records are matched with the National Registration Department for outcome. The retrospective data is analyzed using SAS (Statistical Analysis System) version 9.4.

Results: There are a total of 227 cases with the mean age of 35 years and female predominance (61%). The young adulthood is the most prevalent group (36%), followed by 25% of middle adulthood, 20% of adolescent, 13% of children and 6% of elderly adulthood. The three main ethnic distribution namely the Malay-57%, Chinese-21%, Indian-9% and also the others-13%. There is 25% of ADAMTS-13 activity < 10% and the ADAMTS-13 activity < 5% is only 16% if more stringent cut-off point used for TTP. For the ADAMTS-13 inhibitor (n=163), 36% of the cases are positive while 7% of cases are borderline positive and 57% of cases are negative. The ADAMTS-13 Inhibitor is positive for 74% cases and 81% cases for patients with ADAMTS-13 activity < 10% and < 5% respectively, indicating acquired TTP (p< 0.0001). The hereditary TTP is possibly present in 18% of ADAMTS-13 activity < 10% and 11% of ADAMTS-13 activity < 5% with negative inhibitor testing. There is 19% mortality cases. The overall survival is lower in the ADAMTS-13 activity ≥ 5 % (OR: 10.14, *p*=0.0287, 95% CI: 1.34 - 76.21).

Conclusions: Most of the cases are not related to primary TTP. The secondary TTP has a significant lower OS than primary TTP. Therefore, secondary causes of TMA must be sought in order to prevent mortality. Further prospective study will be needed to verify this observation.

PB 703 | Highly Sensitive Fully Automated Chemiluminescent Immunoassay for Rapid Quantification of ADAMTS13 Activity

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a rare disease associated with a severe deficiency of the activity of the VWF-cleaving protease ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). ADAMTS13 activity assays have been shown to be useful for diagnosis, management, and prognosis in individuals with TTP. However, the utility of ADAMTS13 activity assays for initial diagnosis of TTP is currently limited by long turnaround times.

Aims: To evaluate the analytical performance of a chemiluminescent ADAMTS13 activity assay (Instrumentation Laboratory, Bedford, MA, USA) currently in development.

Methods: The ADAMTS13 activity assay is a fully automated 2-step immunoassay with a time to first result of 33 minutes. Reagents are standardized against the WHO 1st International Standard for determination of ADAMTS13 in plasma. Magnetic particles are coated with the VWF73 peptide substrate containing the ADAMTS13 cleavage site, and chemiluminescent detection is based on an isoluminol-labeled monoclonal antibody that reacts specifically with the cleaved peptide. A method comparison study was performed using 72 plasma samples compared against a commercial fluorescence resonance energy transfer (FRET) method.

Results: The method comparison study revealed comparable results with the FRET assay (r=0.94, slope 0.94 by Weighted Deming). The chemiluminescent ADAMTS13 activity assay demonstrated a linearity of 0.3%-150% with good within-laboratory precision (< 5.2 % CV) across the measuring range.

Conclusions: The chemiluminescent ADAMTS13 activity assay shows comparable results to a commercial FRET assay. The assay is automated, has a very low limit of quantification, and rapid time to first result allowing for reliable assessment of ADAMTS13 activity for levels < 10%.

PB 704 | Clinical Characteristics and Outcome of Patients with Thrombotic Thrombocytopenic Purpura at Siriraj Hospital

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Background: Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease characterized by microvascular platelet thrombi resulting in microangiopathic hemolytic anemia (MAHA) and thrombocytopenia. Therapeutic plasma exchange (TPE), the current standard of treatment, significantly improves the prognosis. However, the mortality rate remains over 10%.

Aims: To study the clinical features, laboratory characteristics, outcome, and mortality rate and determine factors associated with the mortality in TTP patients at Siriraj hospital.

Methods: The data of TTP patients at Siriraj hospital from 1999 to 2013 were retrospectively reviewed.

Results: Forty five patients (34 women) had a median age of 40.6 years (range 16.0-76.0). All patients presented with MAHA and thrombocytopenia, 88.9% had neurological symptoms, 46.7% had fever, and 33.3% had renal impairment. There were 5 patients with the classical pentad of TTP. 30 patients (66.7%) were identified as idiopathic TTP. Secondary causes of TTP included 9 SLE, 2 cancer, 2 HIV infection, one psoriasis, and one renal transplantation. Plasma ADAMTS13 activity was determined in 17 patients in which 12 patients had severe ADAMTS13 deficiency (< 10%). 41 patients underwent TPE, 29 patients (71.1%) achieved response outcome with an average of 5 cycles of TPE (range 1-21). Response rate in idiopathic TTP was 80%. Adjunctive therapies included fresh frozen plasma transfusion prior to initiation of TPE, glucocorticoid therapy, and cytotoxic agents. Overall mortality rate was 24.4%. In multivariate analysis, male sex (odds ratio [OR] 48.0; 95% confidence interval [CI] 2.3-1024.7) and fever (OR 36.4; 95% CI 1.2-1145.7) were associated with increased mortality rate. Four patients had TTP relapse, however, there was no mortality in TTP relapse group.

Conclusions: Mortality rate of TTP at Siriraj hospital was 24.4%. Factors associated with increased mortality rate were male sex and fever.

PB 705 | Characteristic of ADAMTS13 in Patients with Acute Phase of Kawasaki Disease

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Background: Kawasaki Disease(KD) is an acute febrile vasculitis with vascular endothelial cell (VEC) injury occurs to small and medium arteries, most commonly coronary arteries. ADAMTS13 is known as a VWF-cleaving protease. Inflammatory cytokines and VEC injury stimulate the release of unusually large VWF multimers(UL-VWFM). The accumulation of high plasma UL-VWFM results in low enzyme-to-substrate ratio and leads to the needless formation of platelet thrombi, termed TTP. In addition, ADAMTS13 is reported to be available to modulate the shear-induced inflammation by VWF.

Aims: To know the characteristics of ADAMTS13 in acute phase of KD.

Methods: ADAMTS13 activity and VWF antigen(VWF:Ag) of 38 patients with KD(21 males, 17 females) were measured before, 1 week, and 1 month after treatment. Univariate and multiple linear regression analyses were performed to determine the relationship with clinical and biochemical variables.

Results: ADAMTS13 activity was 79.5±27.5%, 123.2 ±52.4%(p 0.001), and 121.7±37.1%(p 0.001), respectively. VWF:Ag was 169.3±49.3%, 150.7±54.3%(p=0.129), and 110.1±32.5%(p 0.001), respectively. The ratio of VWF:Ag to ADAMTS13 activity was 2.51±1.43, 1.64±1.40(p=0.011), and 1.02±0.55(p 0.001), respectively. Before treatment, in univariate linear regression analysis, ADAMTS13 activity was correlated with the white blood cell counts(WBC), platelet count, and albumin(Alb), VWF:Ag with sodium(Na), and potassium,C-reactive protein(CRP), and the ratio of VWF:Ag to ADAMTS13 with WBC, Alb, Na, and CRP, respectively. In multivariate linear regression analysis, Alb was for ADAMTS13 activity, CRP was for VWF:Ag, and Alb and Na were predictors for the ratio of VWF:Ag to ADAMTS13 before treatment.

Conclusions: In acute phase, the low ADAMTS13 activity and the high ratio of VWF:Ag to ADAMTS13 were revealed. These were independently associated with parameters of VEC injuly and got normalized with treatment. Subclinical platelet thrombi formation and shear-induced inflammation should be cared.

PB 706 | Acquired TTP in Prague: More than 20 Years Single Centre Experience

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is a rare but life threatening condition caused by production of autoantibodies against metaloproteinase ADAMTS13 and its severe deficiency results in microangiopathic haemolytic anemia (MAHA) and thrombocytopenia, neurological symptoms, renal impairment and fever.
Aims: Evaluation of our patients with diagnose of TTP including demographic, clinical and laboratory data.
Methods: From 1993 to 2016 we have treated 34 patients: 26 women (76%) and 8 men (24%). Median age was 40 years (16-63) in female and 45 years (25-57) in male patients. At diagnosis: all of patients presented with MAHA and thrombocytopenia, 90% had neurological symptoms, 55% fever and 26% renal impairment. The complete „pentade syndrome" occurred in only 7% of patients. Bleeding was present in 65% of patients.
Results: Diagnostic testing revealed mean initial ADAMTS13 activity of 3% (0-8%) and inhibitor value was 94IU/ml (44-164) without previous treatment (two patients received FFP before established diagnose).
The standard first line treatment comprised of therapeutic plasma exchange (TPE) and corticosteroids. This treatment led to TTP remmission in 18 ptn. (58%). The need for extending immunosuppressive therapy with prolonged TPE was in 16 ptn. (42%) with single or combination of rituximab, vincristine, immunoglobulins or cyclophosphamide. Median number of TPEs in one single episode was 28 (3-95).
The remission of TTP was achieved in all our patients. The median duration of the first remission was 56 months (2.5-200). Twelve patients have relapsed (5 repeatingly). In the whole cohort only 4 patients have died: 2 because of TTP relapse, and 2 of non-TTP cause.
Conclusions: Early diagnose with an aggressive and adequate treatment leads to an excellent outcomes and durable responses in our center. These results are comparable and consistent with literature and treatment results in other hematology centers.

PB 707 | Complements are Activated to a Similar Level in both Thrombotic Thrombocytopenic Purpura and Atypical Hemolytic Uremic Syndrome

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Background: Uncontrolled complement activation has a major role in the pathogenesis of atypical hemolytic uremic syndrome (aHUS) and the restraint of this process by eculizumab is life saving. However, the evidence of complement dysregulation in the pathogenesis of thrombotic thrombocytopenic purpura (TTP) is still unclear.
Aims: In this study we examined the presence of complement activation biomarkers in patients with aHUS and TTP and the levels were compared to normal healthy controls.
Methods: Patients with thrombotic microangiopathic thrombocytopenia diagnosed either as TTP or aHUS were chosen from the Korean TTP registry. Prospective plasma and serum samples prior to intervention were collected from newly diagnosed patients with TTP (n=48), aHUS (n=50), and 40 healthy controls and frozen at -70^{0}C. Complement activation products were measured by ELISA.
Results: The level of alternate (C3a, C5a and factor Bb) and terminal markers (C5b-9) were significantly increased in patients with aHUS or TTP compared with controls. There were no significant differences in complement activation product levels between the patients with TTP and aHUS except that the level of factor Bb was higher in aHUS patients than those with TTP.
Conclusions: Complement biomarkers are activated to a similar level in both newly diagnosed cases of TTP and aHUS.

PB 708 | aPreliminary Report on the Genetic Background and Clinical Course of Upshaw-Schulman Syndrome in Polish Patients

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Background: USS is a form of congenital thrombotic thrombocytopenic purpura characterized by hemolytic anemia with red cell fragmentation, thrombocytopenia and organ ischemia due to thrombosis in microcirculation. The underlying cause of USS is an inherited deficiency of von Willebrand factor cleavage protease - ADAMTS13.
Aims: To characterize the causative mutations in the *ADAMTS13* gene and the clinical course of USS in Polish patients treated in the reference Centre in Warsaw.
Methods: We measured ADAMTS13 activity, antigen and inhibitor using chromogenic, enzyme-linked immunosorbent assay (Technoclone, Vienna) and performed Sanger sequencing of all 29 exons and flanking intronic regions of the *ADAMTS13* gene.
Results: The study comprised 10 symptomatic patients (7 families) with undetectable baseline ADAMTS13 activity and antigen. ADAMTS13 inhibitor test was negative. Molecular analysis of *ADAMTS13* gene showed 7/10 patients (4 families) to be homozygous

for the well-known frameshift mutation c.4143dupA in exon 29. Three patients were compound heterozygous for the following mutations:
1) c.[4143dupA(;)2455delG];
2) c.[2272T>C(;)3178C>T];
3) c.[3178C>T(;)4143dupA].
In the study patients the first symptoms of USS occurred at the median age of 4 whereas the diagnosis was made much later at the median age of 21. A specific case is the patient with c.[2272T>C(;)3178C>T] genotype who presented USS at the age of 52 (ischemic stroke). Five experienced at least one ischemic stroke, 7 had at least one episode of renal failure. In two patients the disease was triggered by pregnancy. Five patients required regular FFP infusions to control the hemostatic balance through ADAMTS13 supply. USS-related deaths were reported in two families.
Conclusions: The mutation spectrum in Polish USS patients does not differ from those of the populations already characterized. The clinical course of USS in our study group was severe. The significant time interval between onset of the first symptoms and the definitive diagnosis is of special concern.

PB 709 | A Murine Anti-ADAMTS13 Monoclonal Antibody against the Disintegrin-like Domain Inhibit the Enzyme Activity

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Background: ADAMTS13 (adisintegrin and metalloprotease with a thromboSpondin type 1 motifs 13) is a plasma metalloprotease that proteolytically regulates von Willebrand factor (VWF), a critical mediator of platelet tethering and primary haemostasis. The physiological importance of the ADAMTS13-VWF axis is highlighted by cases of either inherited or acquired ADAMTS13 deficiency, which is associated with life-threatening thrombotic thrombocytopenic purpura (TTP). Preparation of murine monoclonal antibody against human ADAMTS13 is crucial for further study for the function of ADAMTS13.
Aims: Preparation of murine monoclonal antibody against human ADAMTS13 is crucial for further study for the function of ADAMTS13.
Methods: Balb/c mice were immunized by purified recombinant ADAMTS13 truncated eukaryotic protein (ADAMTS13-T7) without TSP-1 8 and CUB domain. Murine anti-human ADAMTS13 monoclonal antibody (McAb) was developed by standard hybridoma technology and identified by ELISA. The recognition of McAb with full-length recombinant ADAMTS13 protein was identified by Western blotting. In function assay, the influence of McAb on the proteolysis of VWF by ADAMTS13 was observed. Moreover, a series of small fragment of ADAMTS13 were prokaryotically expressed and purified, which were to confirm the domain McAb recognized.
Results: A murine anti-ADAMTS13 McAb SZ-169 (1G11) was obtained. In ELISA, SZ-169 (1G11) showed the higher affinity to ADAMTS13-T7 compared with full-length ADAMTS13. The results of Western blotting demonstrated that SZ-169 (1G11) could both recognize full-length ADAMTS13 and the disintegrin-like domain. In addition, under static (denatured) conditions, SZ-169 (1G11) could inhibit the cleavage of VWF by ADAMTS13 dramatically in increasing concentrations of the McAb.
Conclusions: Our results further indicated that the disintegrin-like domain of ADAMTS13 is critical to efficient substrate cleavage. McAbs SZ-169 (1G11) could be provided as the useful tool for further study of mechanism of ADAMTS13.

PB 710 | Relationship between ADAMTS13 Antigen (Ag) and Free IgG Anti-ADAMTS13 Antibody (Free IgG ab), Functional ADAMTS13 Inhibitor (FI) and Circulating Immune Complexes (CIC) Using two ELISA Techniques, in Patients (P) with Acquired Thrombotic Thrombocytopenic Purpura (aTTP)

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Background: TTP is caused by the absence of functional ADAMTS13. The deficiency may be congenital or acquired. Thomas (2015) suggests that enhanced ab-mediated clearance provides a major pathogenic mechanism in aTTP, corroborated by the reduced levels of circulating Ag in P with non-inhibitory ab.
Aims: The aim of this study was to evaluate the relationship of ADAMTS13-measurements in 64 P, [(55 in the acute phase of their first episode (ap) and 9 in non-acute phase (nap)] at first consultation.
Methods: Activity (Ac), Ag, FI and free IgG ab were evaluated by ELISA. CIC by ELISA using ab: clone 20A5 (A) or monoclonal from Ag kit (B).
Results: The P were 46 female/ 18 male. The median age was 37±19 (SD).
Inter-assay and intra-assay variation were acceptable for CIC in A and B. The cut off for normal levels of ADAMTS13-specific CIC was 0.501, n=28 in A and 0.503, n =25 in B. The medians of A and B were not significantly different p=0.1069 ($p < 0.05$). A is the elected ELISA. CIC median of all P was 0.42, n=60 and CIC median of P with higher levels was 0.55, n=16.
All ADAMTS13-related measurements were in Table I.
In ap P, there was a very weak correlation found between Ag and CIC (Spearman's coefficient r= -0.017, n=51), and between Ag and FI (r=-0.061, n=40). There was a moderate correlation (r=-0.477, n=55) between Ag and free IgG ab (including two P with high titre ≥850 U/

TABLE I ADAMTS13-related parameters

ADAMTS13-related parameters	ap P (% of total)	nap P (% of total)
Ac (U/mL)	49 (94%)	-
severely reduced (<10%)	3 (6%)	3 (25%)
reduced (10%-40%)		5 (50%)
normal (>40%)		1 (25%)
Ag (μg/mL)	-	-
undetectable (0.010 μg/mL)	5 (9%)	-
severely reduced (<0.100 μg/mL)	16 (29%)	1 (8%)
reduced (0.100-<0.600 μg/mL)	28 (51%)	6 (50%)
normal (0.600-1.600 μg/mL)	6 (11%)	2 (42%)-
FI (negative<30%)	-	-
detectable	37 (86%)	8 (83%)
undetectable	3 (14%)	1 (17%)
Free IgG ab (U/L)		-
detectable(>12 U/mL)	47 (85%)	8 (83%)
undetectable (≤12 U/mL)	8 (15%)	1 (17%)
CIC	-	-
detectable	14 (27%)	2 (22%)
undetectable	37 (73%)	7 (78%)

mL). The 95th percentile said that the free IgG ab was below 318 U/mL, then there was a strong correlation (r =-0.643, n=53).

Conclusions: The prevalence (pr) of ap P with aTTP who had CIC pr=27% was lower than that found by Lotta (2014) and Mancini (2016), pr=47% and pr=39% respectively, although we substituted the ab and doubling the number of ap P of our previous report (pr=33%). Thomas (2015) said that not all anti-ADAMTS13 ab promote clearance, as free IgG ab did not correlate with Ag, exemplified by two P in remission with high-titre ab. In our study, only 95% of free IgG ab (without high titres) might be a variable with prognostic relevance.

PB 711 | 'Predisposed' Hemophagocytosis in a Patient with Thrombotic Thrombocytopenic Purpura

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Background: Although the diagnosis of thrombotic thrombocytopenic purpura (TTP) may not be established before the determination of disintegrin and metalloproteinase with thrombospondin motif 13 (ADAMTS13) activity, therapeutic plasma exchange must be started immediately. Hemophagocytic lymphohistiocytosis (HLH) is a condition in which immune dysregulation causes hemophagocytosis and disseminated intravascular coagulation (DIC), thereby presenting or mimicking thrombotic microangiopathy, and compromising the distinction from TTP.

TABLE 1 Laboratory Data

Variable	Reference Range	On the First Admission	On the Second Admission
Peripheral Blood Smear		minimal	3+ schistocytosis
Lactate Dehydrogenase (U/liter)	119 - 229	1950	6090
Ferritin (ng/ml)	10 - 260	> 40000	72878
Triglycerides (mg/dl)	30-149	233	405
Fibrinogen/Fibrin Degradation Product (ug/ml)	0 - 5	33.9	96.2
EBV DNA (copy/10*6 White Blood Cells)	< 20	94	Not detected
ADAMTS13 (%)		25.8	< 0.5
Inhibitor (Bethesda Unit/ml)	< 0.5	<0.5	1.2

Aims: We present a case of TTP with previous HLH that demonstrated typical bone marrow hemophagocytosis at the time of diagnosis.

Methods: Case presentation. A Japanese woman in her thirties was admitted to our hospital because of fever and severe thrombocytopenia. Generalized lymphadenopathy, hepatosplenomegaly, and bone marrow hemophagocytosis were present. Other test results are shown in the table. Anemia had progressed. Epstein-Barr virus (EBV)-encoded RNA 1 positivity in the biopsied cervical lymph node and elevated viral load supported the diagnosis of EBV-HLH. Dexamethasone administration induced clinical remission. After more than a year, she was re-admitted for abdominal pain with acute oliguric renal failure. Although similar thrombocytopenia with DIC and hemophagocytosis were observed, marked schistocytosis of more than 20% and extremely high levels of lactate dehydrogenase, as shown in the table, prompted us to perform therapeutic plasma exchange, which brought quick clinical improvements.

Results: We evaluated the plasma ADAMTS13 activity and the inhibitor for the enzyme at the time on both admissions, and confirmed the loss of the proteinase activity by the inhibitor only in the second admission as shown in the table.

Conclusions: Recurrent bone marrow hemophagocytosis after the definitive HLH does not preclude a diagnosis of TTP. This further emphasizes the importance of plasma exchange therapy at the time of presumptive diagnosis of thrombotic microangiopathy.

PB 712 | Technozym® ADAMTS-13 Activity Assay for Determination of Inhibitory Antibodies against ADAMTS-13

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Background: Acquired TTP is frequently caused by inhibitory autoantibodies against ADAMTS-13. Determination of ADAMTS-13

inhibitors therefore provides a valuable tool for diagnosis and patient follow-up in TTP. ADAMTS-13 antibodies can be detected in vitro either as IgG by ELISA, or functionally in a Bethesda based assay due to their inhibitory effect on ADAMTS-13 activity.

Aims: Aim of this study was to evaluate the usability of TECHNOZYM® ADAMTS-13 activity ELISA in combination with a Bethesda based assay for detection of inhibitory antibodies against ADAMTS-13.

Methods: We determined ADAMTS-13 activity in plasma samples from patients clinically diagnosed with TTP and in normal plasma as negative control. The inhibitory antibodies against ADAMTS-13 were determined by a Bethesda-type method similar to the one used to analyze inhibitory FVIII antibodies. Patient plasma was heat-treated at 56°C for 30 min to eliminate endogenous ADAMTS-13 activity. A serial dilution was made before mixing 1+1 with normal human plasma (NHP). ADAMTS-13 activity of all dilutions was determined with TECHNOZYM® ADAMTS-13 activity ELISA.

Results: Residual activity in % was calculated from the results obtained in IU/mL for all patient plasma dilutions and the control mixture. The residual activity of patient plasma between 25% and 75% was used to further calculate the Bethesda Units (BU).

The results for inhibitory auto-antibodies were considered to be positive if the result of patient plasma was found to be >0.5BU.

All normal plasma samples were found to be below the 0.5BU limit. The inhibitor titer in TTP samples ranged from 0.64 BU/mL to 8.24 BU/mL.

Conclusions: In this study we demonstrate that combining TECHNOZYM® ADAMTS-13 activity ELSIA with a Bethesda based assay setting provides a good method for measurement of functional inhibitors. That allows differentiation of different forms of TTP and may enable close monitoring of inhibitor titer changes in the course of the disease and in response to treatment.

PB 713 | Evolution of ADAMTS13 Activity in Patients with Relapsing Acquired Thrombotic Thrombocytopenic Purpura

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Background: The diagnosis of acquired Thrombotic thrombocytopenic purpura.

(TTP) is based on the study of ADAMTS13 (activity and antibody), acquiring special importance in establishing the risk of relapse.

Aims: To record the levels of ADAMTS13 in patients with relapsing episodes.

Methods: 13 patients diagnosed with PTT in our center, 5 patients (38%) were followed up, 4 women and 1 male from 2002 to the present with recurrent episodes. They presented an average of 3 episodes.

We studied the activity of ADAMTS13 and anti-ADAMTS13 IgG by ELISA technique.

The treatment of these cases was followed by the common protocol: plasma exchange, prednisone and rituximab in cases of more severe or relapsing / refractoriness.

ADAMTS13 activity was measured at diagnosis, three months after the complete response and one year after diagnosis.

Results: Patient 1 presented three episodes with persistence of severe deficiency of ADAMTS13 and anti-ADAMTS13 despite treatment. Patient 2 presented five episodes, but only was recorded ADAMTS 13 activity in both fourth and fifth episodes, with severe deficiency but achieving antibodies negativization.

Patient 3 presented two episodes with persistent presence of antibodies anti-ADAMTS13 and severe deficiency despite treatment . Is currently in folow-up after third episode.

Patients 4 and 5 had two episodes and they achieving antibodies negativization after second episode with rituximab, plasma exchange and prednisone treatment.

Conclusions: Patients with severe deficiency at presentation and persistence of anti-ADAMTS13 developed relapsing disease, whereas patients without severe deficiency and anti-ADAMTS13 disappearance rarely relapse . Measuring the activity of ADAMTS13 is critical to define the long-term prognosis and follow-up of patients with acquired TTP. More prospective studies should be done to establish the relationship between risk of relapse or recurrence and activity of ADAMTS13 and anti-ADAMTS13.

PB 714 | Evaluation of Sample Interferences on ADAMTS-13 Activity Measurement

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Background: Beside it's ability to directly inhibit plasma ADAMTS13 activity, unconjugated bilirubin interferes with certain fluorogenic assays with major impact on clinical diagnosis of TTP and treatment monitoring.

Aims: Aim of this study was to evaluate the possible interference of unconjugated bilirubin and other potential interfering substances with TECHNOZYM® ADAMTS-13 activity ELISA.

Methods: To investigate the assay performance in the presence of interfering substances plasma samples were spiked with increasing amounts of this substances and ADAMTS-13 activity was determined. 3 samples with different ADAMTS-13 activity levels have been analysed. A deviation of up to 15% from the un-spiked sample was regarded as acceptable.

Results: For hemolysis no interference was observed with samples containing up to 200 mg/dL haemoglobin. For lipemia, samples containing at least 500 mg/dL Intralipid or samples containing up to 20 mg/dL unconjugated Bilirubin no interference was observed.

A possible interference of rheumatoid factor was evaluated and no interference was detected up to 40 U/mL.

Within the spectrum of therapeutic intervention of TTP CD20 antibodies, like rituximab, were described to be successful especially within refractory patients. Thus, we also tested for interfering potential of CD20 antibodies in ADAMTS-13 activity assay. No interference was observed up to a level of 200 µg/mL of CD20 blocking antibodies, which corresponds to the upper level of serum concentrations found after Rituximab administration.

Conclusions: In this study, we demonstrate that with TECHNOZYM® ADAMTS-13 activity ELSIA no significant interferences could be detected for all tested interfering substances, allowing an accurate clinical diagnosis of TTP and treatment monitoring.

PB 715 | Early Detection of Refractoriness and Silent Relapse in Acquired Thrombotic Thrombocytopenic Purpura

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Background: Acquired thrombotic thrombocytopenic purpura (PTT) is a primary thrombotic microangiopathy. The diagnosis is based on a decreased activity of ADAMTS 13 due to autoantibodies. The standard therapy for this disorder is the combination of corticosteroids and plasma exchange until clinical and biological response. After dischard, there is no protocol for monitoring these patients.

Aims: To develop a protocol in order to detect risky situations.

Methods: After clinical response our patients are monitored by measuring the activity levels of ADAMTS 13 (normal> 40%) and autoantibodies (normal < 10 IU / mL).

If these parameters are pathological, the determination is repeated weekly. Patelet count and clinical monitoring are done. If the parametrs remain pathological, even with normal patelet count, we consider it as a refractory patient and we proceed with second line treatment (Rituximab).

All patients are also monitored biannually. In case of detecting pathological values, we make a close follow up. If relapse is confirmed, we proceed to the second line treatment.

Results: We have follow up fifteen patients since January of 2015 to date. Four silent or subclinical relapses were detected. Three of them recieved Rituximab and they maintain an optimal biological response. One patient refused the treatment and now she is been closely monitored. One early detection of refractoriness was detected and treated successfully.

Conclusions: Early monitoring of ADAMTS 13 after clinical response allows the detection of refractory patients and subclinical relapses.

Biannual monitoritation also allows the detection of risky or silent relapses.

With our protocol four patients have been benefited from biannual monitoring and one from early monitoring.

PB 716 | Upshaw-Schulman Syndrome: The Importance of the Modulating Effect of the Different Genetic Variations

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Background: Congenital Thrombotic thrombocytopenic purpura, also known as Upshaw-Schulman syndrome (USS), is a rare disorder classified as a type of primary thrombotic microangiopathy. It is caused by an inherited deficiency of ADAMTS-13 without an inhibitor. Low ADAMTS13 levels result in increased ultra-large von Willebrand factor multimers which induce platelet adhesion and thrombosis. We describe a late-onset USS in a healthy man patient with three single nucleotide polymorphism (SNP), previously reported *in vitro*.

Aims: To demonstrate the genetic origin of the disorder and the effect of the interaction between the SNPs.

Methods: ADAMTS-13 activity was analyzed by a chromogenic test measured with ELISA. Quantification of antibodies IgG against ADAMTS-13 was obtained by ELISA with IMUBIND. PCR of ADAMTS13 coding sequence was performed. *In silico* prediction of functional effects were performed using Polyphen-2.

Results: We present a case of a 58 years old male who presented in 2009 a platelet count of 5 x10^9 /L, hemolytic anemia, neurogical symptoms and schistocytes. He has suffered from six unspecified acute episodes to date with late-onset, mild symptomatology and good response to therapy.

He had a very low ADAMTS13 activity (< 10%) and no evidence of inhibitor measured in several occasions both in relapse and remission conditions.

Genetic sequence of ADAMTS13 gene showed three variants. Heterozygous c.1852C>G p. (Pro618Ala) has a negative effect on ADAMTS13 function and secretion levels, which is weakened by the association of two more variants: heterozygous c.19C>T p. (Arg7Trp) and homozygous c.1342C>G p.(Gln448Glu).

Conclusions: In our patient the phenotype is correlated with his genotype: late onset, mild symptomatology, relapses with moderate thrombopenia and a quick response to plasma infusion.

SUS is a rare disease with great genetic and clinical polymorphism. It is important to emphasize the modulating effect of the different genetic variations. In our case the variations have caused an attenuating effect.

PB 717 | Characterization of a Hereditary Thrombotic Thrombocytopenic Purpura (TTP) Misdiagnosed as HELLP Syndrome

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Background: Hereditary thrombotic thrombocytopenic purpura (TTP) is characterized by abnormally disseminated thrombus due to the

mutations of ADAMTS13, which cleaves its substrate von Willebrand factor(VWF) in normal conditions.

Aims: To characterize the clinical and genetic molecular features of a pregnant patient with congenital TTP.

Methods: ADAMTS13 activities were analyzed by residual collagen binding assay (R-CBA) plus FRET-VWF substrate. And the inhibitors of ADAMTS13 were analyzed by 1:1 mixture of patient and pooled normal plasma followed by R-CBA. And all exons of the ADAMTS13 gene, including the intron-exon boundaries, were PCR-amplified with the corresponding primers, and the PCR products were directly sequenced.

Results: The patient with the second trimester of pregnancy, was admitted to hospital intensive care unit due to the high TBILL (33, normal range:5-21), high LDH(420, normal range:0-248), high ALT(48, normal range 0-35) and the low platelet count number(20×10^9) although all her coagulation indexes were normal. She was discharged from hospital after abortion. However, the following routine blood test demonstrated that her platelets had cyclical fluctuation almost every two weeks, and the congenital TTP was suspected because her ADAMTS13 activities were less than 5% (both R-CBA and FRET-VWF substrate) and had absence of ADAMTS13 inhibitors. The corresponding heterozygous mutations were found in this patient, including R594W and IVS3+1G>A.

Conclusions: The clinical HELLP syndrome in the patients with pregnancy sometimes resembles the features of TTP, and it is difficulty to differentiate these two distinct diseases clinically. ADAMTS13 activity and corresponding inhibitor assay are helpful to dissolve this problem in clinic.

PB 718 | The Next Case Report of Thrombotic Thrombocytopenic Purpura (TTP) after Influenza Vaccination

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Background: Thrombotic thrombocytopenic purpura (TTP) is a very rare disease belongs to the group of thrombotic microangiopathy (TMA). TTP is characterized by microangiopathic haemolytic anaemia, consumptive thrombocytopenia, and significantly reduced activity of metalloprotease ADAMTS13 (< 10%) and leads to significant damage of vital organs and death. Secondary TMA can be induced also after using some drugs.

Aims: Description of TTP case induced most likely after influenza vaccination.

Methods: -

Results: Case report. A 38-year-old woman was admitted from an external hospital due to breathlessness, faintness, fevers, dark urine, severe non-immune haemolytic anaemia (HGB 66 g/l) and thrombocytopenia (PLT $4x10^9$/l). Suspected TTP was confirmed by undetectable low ADAMTS13 activity and extremely high titre of inhibitor of ADAMTS13 (> 100 U/ml). The cause of this secondary TTP was most likely influenza vaccination 10 days before the development of the first symptoms. No other cause has not been detected by detailed testing. Immediately was started a series of exchange plasmapheresis and immunosuppressive corticotherapy. The platelet count rose very slowly in the following days (PLT $75x10^9$/l), there was still elevated schistocytes count and activity of lactate dehydrogenase (7.7 ukat/l). Because of the persisting symptoms weekly rituximab therapy was indicated (375 mg/m^2) for four weeks. After the second dose of rituximab application the platelets has normalized with continuing mild anaemia, and normal level of lactate dehydrogenase. There was a remission of the disease after rituximab therapy.

Conclusions: This case report highlights another rare case of a few published cases of TTP after administration of influenza vaccine, and presents ADAMTS13 deficiency with extremely high inhibitor. The patient will be monitored for recurrence or relapse of this rare disorder.

PB 719 | Thrombotic Thrombocytopenic Purpura as an Initial Presentation of Systemic Lupus Erythematosus in a Thai Child: A Case Report

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Background: Distinguishing between systemic lupus erythematosus (SLE) and thrombotic thrombocytopenic purpura (TTP) is challenging as both diseases share the same clinical features. The delayed diagnosis of TTP is associated high mortality, especially in SLE-associated TTP. The presentation of TTP as the first manifestations of SLE was mainly reported in adults.

Aims: To report a case presented with fever, acute renal failure and alteration of consciousness and was diagnosed with TTP before the diagnosis of SLE.

Methods: A girl was investigated for the cause of rapidly progressive glomerulonephritis (RPGN). All clinical data were recorded.

Results: A 15-year-old previously healthy girl manifested with fever for 3 weeks and developed petechiae with facial swelling for 1 day. On admission, she had marked pallor, tachycardia and right pleural effusion without skin lesion. Later, she developed alteration of consciousness. The computed tomography (CT) of brain showed diffuse brain edema. Blood tests showed Hb 4 g/dL, platelet count 15,000/mm^3, creatinine 1.5 mg/dL and observed hemoglobinuria, but normal coagulogram and fibrinogen level and negative Coombs' tests. She was diagnosed with RPGN and treated with methylprednisolone but no improvement. The blood smear showed microangiopathic hemolytic anemia (MAHA), then TTP was suspected. The ADAMTS-13 was < 3% and inhibitor was 3.3 units/mL. She was treated by fresh frozen plasma (FFP) transfusion and plasmapheresis for two episodes, then was gradually improved. The rest of tests later showed positive

antinuclear antibody (ANA), low serum C3 and C4 levels at 247 and 31.5 mg/dL, respectively, but negative anti-double stranded DNA antibody. She was diagnosed with SLE and received steroid and cyclophosphamide to maintain her in remission.

Conclusions: The clinical suspicious and blood smear are the key for the early diagnosis of TTP. The further tests should be performed to determine the definite diagnosis and treatment. TTP can be the initial symptom of SLE.

PB 720 | A Case of SLE with Different Types of Thrombotic Microangiopathy during the Course

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Background: Thrombotic microangiopathy (TMA) is a pathologic condition leading to microangiopathic hemolytic anemia (MAHA) and thrombocytopenia. There are many underlying causes for a TMA, including thrombotic thrombocytopenic purpura (TTP). Since the diagnosis and management of TMA is very challenging, it is very important to understand the mechanisms for TMA. We present a case of SLE with two types of TMA during the course.

Aims: To understand the various underlying mechanism that lead to TMA for appropriate treatment.

Methods: We measured plasma levels of ADAMTS13 (A13) activity, inhibitor titers to A13, and non-neutralizing biding antibody to A13 (NNAb) by ELISA.

Results: A 26-year-old female was admitted to the N hospital with systemic edema for 1 month and fever. They diagnosed her as TTP (first TMA) with thrombocytopenia (platelet count: $8X10^6$/L), severely reduced levels of A13 (< 0.5%), and renal dysfunction. After 4 times PE, the platelet count and A13 activity was improved to $80X10^6$/L and 80%, respectively. But one week later, her platelet count dropped again and showed exacerbation of MAHA despite enough levels of A13, that is second TMA, then she was referred to our hospital for further investigation of TMA. On admission, we diagnosed her as SLE with nephrotic syndrome and TMA, and started methylprednisolone pulse therapy. To investigate the cause of first TMA, we examined her plasma on admission to the N hospital. We found no inhibitors to A13 but increased levels of NNAb, indicating that first TMA may be caused by NNAb. Several methylprednisolone pulse therapies were given, each time her second TMA (MAHA, thrombocytopenia and increased percentage of schizocyte) responded only for a few days, suggesting that steroid after pulse therapy cannot suppress SLE vasculitis mediating second TMA. Then we started giving her 6 times cyclophosphamide pulse therapy, leading complete remission of SLE and TMA.

Conclusions: It was very important to understand the underlying mechanisms for appropriate TMA treatment.

PB 721 | Congenital Thrombotic Thrombocytopenic Purpura without Nephropathy and Neuropathy

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Background: Congenital TTP is due to an inherited deficiency of ADAMTS13, but acquired immune TTP is due to the reductionof ADAMTS13 by autoantibodies directed against ADAMTS13. Other clinical forms of thrombotic microangiopathy (TMA) occur in the absence of severe deficiency.

Aims: We present a patient with TTP without without nephropathy and neuropathy.

Methods: A 5-day-old girl was admitted to our hospital with fatigue. Physical examination revealed pallor and hepatomegaly. A blood smear showed hemolysis with 10% schistocytes and polychromasia. He had anemia with 5.5 g/dL hemoglobin level and 14.6% reticulocyte level. He had also thrombocytopenia (18,000/mm3) and elevated lactate dehydrogenase (LDH) as 856 U/L. A direct Coombs test was negative. The other blood parameters of the patient were as follows: haptoglobin < 5 mg/dL, C3 122 mg/dL, creatinine level 0.5 mg/dL, blood urea nitrogen 12 mg/dL, and indirect bilirubin 7 mg/dL. Urine analysis was normal. On the second day of admission, the patient's thrombocyte count dropped to 38,000/mm3, his LDH level remained elevated, and schistocytes were still present on his peripheral blood smear.

Results: Her gene analysis was performed to confirm whether the patient had aHUS or not.

Conclusions: We present a patient with TTP without without nephropathy and neuropathy.

PB 722 | Thrombotic Microangiopathies (TMA): First Report of 250 Cases from a Single Institution Experience in Latin America

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Background: TMA are characterized by microangiopathic hemolytic anemia (MAHA), thrombocytopenia and organ failure. Worldwide, patients' cohort studies have been of great interest to understand pathological mechanisms and develop methods to differentiate entities, specifically between atypical Hemolytic Uremic Syndrome (aHUS) and adquired or congenital Thrombotic Thrombocytopenic Purpura (aTTP/cTTP).

Aims: For the first time, we described laboratory and clinical features of consecutive TMA patients studied in our department.

Methods: A prospective study (n=250) from January 2013 until June 2016, requiring patients' informed consent, was approved by the institutional Ethical Committee. Laboratory (Technozyme® ADAMTS13 activity and IgG anti-ADAMTS13, platelets, creatinine, LDH) and clinical parameters were recorded. Mann-Whitney U-test was used to compare parameters across groups ($p< 0.05$).

Results: The two major TMA groups were aHUS (n=94) and TTP (n=57). In both groups, women were more frequently affected than men (% aHUS: 38♂ vs 62♀ ; % TTP: 25♂ vs 75♀), particularly in the non-pediatric population (% aHUS: 17♂ vs 40♀ ; % TTP: 15♂ vs 65♀). Cases of aHUS (n=26) and aTTP (n=17) studied during remission or treatment were excluded from the analysis. Mean of ADAMTS13 activity was significantly lower in TTP than in aHUS patients. The presence of IgG anti-ADAMTS13 confirmed aTTP diagnostic. Patients with deficient ADAMTS13 activity presented a more severe thrombocytopenia than patients with normal activity. While LDH level was slightly higher in aHUS than in TTP groups, creatinine was significantly increased in aHUS compared to aTTP subjects. Rates of MAHA observed in all 3 entities were superior to 70%. In aHUS, 29% (n=27) of cases were associated with chronic kidney disease compared to only 12% (n=7) in TTP, a disorder more frequently related to neurological alterations (51% vs 28%).

Conclusions: The first description of a TMA cohort in Argentina confirmed the importance of ADAMTS13 analysis to differentiate TTP from aHUS.

TABLE 1 TMA disorders divided into subgroups and characterized by patients' sex distribution

SUBGROUPS	Number of males (%)	Number of females (%)	Total number n=250 (%)
aHUS	36 (38)	58 (62)	94 (38)
- aHUS acute phase (ap)	28 (77)	40 (69)	68 (72)
TTP	14 (25)	43 (75)	57 (23)
- aTTP acute phase (ap)	6 (43)	23 (53)	29 (51)
- aTTP in remission	3 (21)	14 (33)	17 (30)
- cTTP	5 (36)	5 (12)	10 (17)
- TTP during pregnancy	-	1 (2)	1 (2)
STEC(Shiga toxin-producing Escherichia coli)-HUS	0	1	1 (0.5)
Secondary TMA	15 (45)	18 (55)	33 (13)
- Cancer	2 (13)	7 (39)	9 (27)
- Renal Transplant	7 (47)	2 (11)	9 (27)
- HIV	1 (7)	1 (6)	2 (6)
- Others	5 (33)	8 (44)	13 (40)
Other TMA (confirmed by renal biopsia)	2 (33)	4 (67)	6 (2)
Preeclampsia/HELLP	-	15	15 (6)
Other non TMA	4 (22)	14 (78)	18 (7)
Not defined	2 (28)	5 (72)	7 (3)
Relatives of aHUS and TTP patients	7 (37)	12 (63)	19 (7.5)

TABLE 2 Laboratory features of aHUS and TTP patients during acute phase (ap). p: p-value; ns: non-significant

LABORATORY FEATURES	aHUS (ap)	aTTP (ap)	cTTP
ADAMTS13 Activity mean (%±SD) Severe deficiency <10% Normal activity >40%	86 ± 24	7 ± 12 p<0.005	4 ± 3.8 p<0.005
IgG anti-ADAMTS13 mean (U/mL±SD) Negative <12	4.2 ± 3.4 p<0.005	105 ± 171	3.2 ± 2 p<0.005
Platelet count ($x10^9$/L±SD) Normal range:150-400	58 ± 45	21 ± 15 p<0.005	32 ± 26 p<0.05
LDH (IU/L±SD) Normal range:105-333	2325 ± 2076	1615 ± 752 ns	736 ± 294 p<0.05
Creatinine (mg/dL±SD) Normal range:0.5-1.2	3.6 ± 3.1	0.9 ± 0.4 p<0.005	2.3 ± 2.8 ns

PB 723 | Successful International Standardisation of the ADAMTS13 Assays - Results from the APMAT (Asia Pacific Microangiopathic Thrombocytopenia) Network

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Background: An ADAMTS13 level of < 10% in patients with microangiopathic thrombocytopenia (MAT) confirms the diagnosis of thrombotic thrombocytopenic purpura (TTP) and differentiates TTP from aHUS and other MAT causes. However, routine ADAMTS13 laboratory testing is challenging due to differences in the methodology of ADAMTS13 assays, poor turnaround-time and little standardisation of results between laboratories. The APMAT Network recently implemented standardised ADAMTS13 testing in select Asia Pacific laboratories in Australia, New Zealand, China, Korea, Japan, Taiwan, Hong Kong, Malaysia, Thailand,

India and Singapore and sought to conduct an external quality assessment study of assays performed at these centres. The results were compared to an established ECAT survey on the same samples.

Aims: To establish and assess standardised ADAMTS13 testing in the APMAT Network.

Methods: Two surveys were conducted 6 months apart at 23 laboratories across 11 countries. Each survey utilised two blinded plasma samples of known activity. Laboratories performed ADAMTS13 activity and inhibitor titre assays using the same chromogenic ELISA (Technoclone GmbH); the results were compared to the ECAT analysis from the same samples.

Results: The APMAT survey samples mean and standard deviation were slightly higher when compared to the ECAT cohort, but still within the expected variation (< 20%). Similarly, inter-laboratory variations were within an acceptable range; samples with low intrinsic ADAMTS13 activity exhibited higher variation (CV=30% to 47%) than samples with high activity (CV=18%). ADAMTS13 inhibitor survey results produced smaller CVs (18% to 34%) but had a wider range and contained more outliers (5-10%). 100% of laboratories achieved a passing Z Score (-3< Z-Score< 3) for the activity assay, and 90-95% achieved a passing Z-score for the inhibitor assay.

Conclusions: ADAMTS13 assay standardisation is feasible in a international multicentre setting and has potential to expedite correct MAT diagnosis.

PB 724 | Treatment of Thrombotic Thrombocytopenic Purpura and Hemolytic Uremic Syndrome in Pediatric Patients - A Single Center Experience

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Background: Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome (TTP/HUS) are rare entities in children. Therapeutic plasma exchange (TPE) is recommended treatment.

Aims: To report ten-year experience in the treatment of TTP/HUS in children with TPE from pediatric tertiray care center.

Methods: We treated two patients with TTP (median age of 17 y) and three with HUS (5±3.6 y) from 01.01.2007 to 31.12.2016. Efficacy of TPE was estimated measuring the platelet (PLT) count, reticulocytes, LDH, haptoglobin in patients with TTP and diuresis, serum creatinine in patients with HUS.

Results: Patients with TTP had the mean PLT count 28.4±17.1x10^9/l, Hb 81.3±2.4 g/l. Boy with TTP suffered CVI. Patients with HUS had oliguria, proteinuria, Hb 85.5±3.53 g/l and one patient of them had diarrhea and convulsions. We done 82 TPE, 62 in patients with TTP and 20 TPE at HUS. The frequency of TPE was daily, with 1.5 total plasma volumes (TPV). The plasma cryoprecipitate removed and fresh frozen plasma (FFP) were used as replacement fluids. The apheresis system was filled with allogeneic blood in one patient with low body weight (10 kg). For TTP patients TPE is performed until the platelet count was > 150x10^9/l for three consecutive days, for patients with HUS to the establishment of diuresis. Average of quantity replaced plasma was 3754.5 ml (835.6 to 6286), the blood flow was 32.3 ml/min (9.8 to 44) and the duration of the procedure was 215 min (157.8 to 289.5). One patient with TTP had two exacerbations, another patient two relapses. We continued TPE daily with a good final response. Steroids, folic acid, vincristine and LMWH or aspirin also included in the treatment in our patients. Patients with TTP had mild allergic reactions to plasma proteins (FFP without cryoprecipitate).

Conclusions: Four our patients were successfully treated with TPE. Immediate initiation of TPE, with additional forms of treatment was crucial for favourable outcome.

PB 725 | Successful Treatment of Renal Infiltration Complicated by Atypical Hemolytic Uremic Syndrome in a Child with Acute Leukemia

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Background: Renal infiltration occurs in 7% to 42% of children with leukemia; however, it has rarely been described in association with atypical hemolytic uremic syndrome (HUS). A literature search revealed only one case of simultaneous occurrence of atypical HUS and ALL to date.

Aims: We report successful treatment of a child with ALL who had renal infiltration complicated by atypical HUS.

Methods: A 9-year-old boy presented with oliguria, hypertension, and periorbital edema. He was diagnosed with t(8;14)(q24;q32) positive pre-B ALL with meningeal involvement and tumor lysis syndrome (TLS). Anemia, thrombocytopenia, elevated uric acid and lactate dehydrogenase were identified. Steroid treatment was initiated. The patient's electrolyte imbalance resolved but his urine output remained inadequate despite effective hydration and hemodialysis for TLS. Radiological imaging and urinalysis suggested renal infiltration secondary to leukemia. While on steroid therapy, the patient's LDH and creatinine levels increased markedly again and his anemia and thrombocytopenia worsened. His ADAMST13 activity was normal. He developed atypical HUS in the first week after diagnosis.

Results: Renal failure did not respond to plasma infusion and steroid administration. Therapeutic plasma exchange was administered for 2 consecutive days, after which clinical and laboratory assessments revealed a dramatic response. Induction chemotherapy was continued.

At the time of writing, the patient was in remission and had experienced no signs of recurrence.

Conclusions: Atypical HUS is a rare but serious life-threatening complication in patients with malignancy. Although anemia, thrombocytopenia, and elevated LDH are common abnormalities in patients with leukemia, coexistence of second unexplained LDH elevation with anemia, thrombocytopenia, and renal dysfunction should alert the physician to the likelihood of atypical HUS development. Plasma exchange seems to be a better treatment for renal infiltration complicated by atypical HUS in leukemia.

PB 726 | Transplant/Imunosupressor Related Thrombotic Microangiopathy

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Background: De novo HUS (Haemolytic Uremic Syndrome) can affect 3 to 14 percent of kidney transplant recipients. Calcineurin Inhibitors, namely Cyclosporine or Tacrolimus and Mammalian target of rapamycin (mTOR) inhibitors (sirolimus, everolimus) seem to be related with cases of HUS culminating in loss of kidney transplant. However antibody-mediated rejection may also play a role in kidney transplant failure, leading to difficulties in understanding which process was standing first when renal function started to deteriorate.

Aims: The author describes a case of thrombotic microangiopathy in a patient with renal transplant on Tacrolimus therapy.

Methods: Case report: Woman, 48 years old, with history of chronic renal insufficiency due to Membranous Glomerulonephritis diagnosed in 1986, who undergone kidney transplantation in 2009. This patient was admitted to the nephrology ward because of sudden renal graft function deterioration (creatinine 4.9mg/dL) in December of 2016.

Results: Blood tests showed signs of haemolytic anaemia (haemoglobin 7,7g/dL with rare schizocytes, associated with very low level of haptoglobin and high unconjugated bilirubinaemia) with thrombocytopenia. Immediately the hypothesis of HUS had to be taken into account.

Conclusions: The Laboratory has an important role in the prompt detection and diagnosis of these disorders, as analytical data, including blood smear observation, is a key sign for suspicion. Secondary thrombotic microangiopathy should be assumed when blood signs are present and therapeutic approach managed, in order to save the transplanted organ. Even though, the most likely etiology of HUS was related to the transplant and imunosuppressor therapy, it is known that some patients seem to have a genetic or acquired predisposition to this event, which under the right stimulus, can trigger a thrombotic microangiopathic event. HUS associated with transplant is a potential life threatening situation that, if not recognized soon, can lead to loss of the transplanted organ or even death.

PB 727 | A Heterozygous Mutation of G-protein-Coupled Receptor 25 in a Family with inherited Thrombocytopenia and Thrombosis

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Background: Inherited thrombocytopenia is associated with bleeding tendency and no association with thrombotic tendency has been reported. We found a family with autosomal dominant form of thrombocytopenia associated with arterial and venous thrombosis.

Aims: To identify a causative gene for hereditary thrombocytopenia with thrombosis.

Methods: A propositus was a 42-year-old Japanese woman with thrombocytopenia and deep vein thrombosis (DVT). She had normal activities of antithrombin, protein C, and protein S, and negative results for lupus anticoagulant and anti-cardiolipin antibodies. She has suffered from thrombocytopenia (20 - 50 x 10^9/L) for at least 20 years. Six members among her family pedigree had thrombocytopenia, and also suffered from thrombosis including mesenteric artery thrombosis, young-onset myocardial infarction, femoral artery thrombosis, repeated cerebral infarction, DVT, and pulmonary embolism. Importantly, the family members with these thrombotic disorders had exclusively thrombocytopenia. Whole exome sequencing was performed with venous blood from the 6 affected and 6 non-affected family members to identify a causative gene for thrombocytopenia.

Results: We identified a heterozygous change (c.764G>T:p.G255V) in *GPR25* gene, an uncharacterized G protein-coupled receptor. The mutation was completely linked with thrombocytopenia. The mutated residue was located at the transmembrane domain of GPR25. We generated anti-GPR25 monoclonal antibodies and confirmed that GPR25 was expressed on the platelet surface. The patient's platelets showed the same level of PAC1 (a monoclonal antibody recognizing the active conformation of integrin αIIbβ3) binding and P-selectin expression as platelets from healthy subjects. ADP-induced aggregation of patient's washed platelets was enhanced as compared with normal washed platelets.

Conclusions: We identified a novel gene mutation responsible for inherited thrombocytopenia and thrombosis that may induce platelet hyper-reactivity.

PB 728 | Seven Years' Experience of Healthcare Cards Delivery from the French Reference Center on Inherited Platelet Disorders (CRPP)

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Background: A French Plan for rare diseases allowed to structure healthcare for patients with inherited platelet disorders (IPD) through a territory network referred as the Reference Center on inherited Platelet disorders (CRPP).

Aims: To estimate the distribution of IPD based on healthcare cards delivery since October 2009.

Methods: Requests for cards are subjected to the coordinating center for a review process. It issues a numbered card after checking the diagnosis and eliminating duplicates.

Results: 352 healthcare cards (249 families (F)) for thrombocytopenia (T) and 283 (197 F) for platelet dysfunction (PD) have been delivered. Among T, 134 are explained by MYH9 (median age [range]: 34 [0.7-82] yrs) and 56 by GPIBA/GPIBB/GP9 variants (10 pseudoWillebrand, 31 Jean Bernard-Soulier Syndrome (JBS), 2 di-george syndrome and 13 mono-allelic JBS). The remaining corresponds to TUBB1(8), FLNA(4), ACTN1(5), ITGB3(2), PRKACG(1), TRPM7(1) variants. Nineteen are gray platelet syndrome of which 14 correspond to NBEAL2 variants (29 [16-71] yrs). Three are due to FLI1 mutation and 3 correspond to TAR, Paris-Trousseau and Lowe syndromes. Forty-four are forms prone to leukemia: ANKRD26(22), ETV6(14) and RUNX1(8). Nine X-linked T (4 Wiskott Aldrich Syndrome, 3 XLT and 2 GATA1 variants) have been evidenced. For 63 T (18%; 54 F) genetic diagnosis remains unknown. Among PD, 159 patients (101 F) carry Glanzmann Thrombasthenia (28 [1.9-89] yrs) and 70 suffer a dense granule defect (55 F) (43 [3.9-75] yrs), including 3 Hermansky-Pudlack syndrome. Six patients (4 F) display inside out signaling defect (1 FERMT3 and 5 RASGRP2 variants). Five patients (4 F) carry P2RY12, 2 GP6 variants or defect in collagen response, 1 suffers a Scott syndrome. For 41 patients (14%; 27 F) etiology remains unknown.

Conclusions: This is the first effort to estimate the distribution of IPD in France. Data collected are being used to describe the prevalence and epidemiology.

PB 729 | Novel RUNX1 Mutations in Families with Inherited Thrombocytopenia

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Background: Familial platelet disorder with propensity to acute myeloid leukemia (FPD/AML) is a rare autosomal dominant inherited thrombocytopenia (IT) caused by mutations in the hematopoietic transcription factor RUNX1 and associated with an increased risk of developing myeloid neoplasms, such as AML and myelodysplastic syndromes (MDS). It is characterized by impaired megakaryopoiesis and moderate thrombocytopenia, with normal-sized and dysfunctional platelets.

Aims: To unravel the molecular basis of ITs and to improve our knowledge on the molecular basis and clinical-laboratory picture of FPD/AML.

Methods: Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT after the diagnostic workup based on the must updated diagnostic algorithm for ITs (Clin Genet 2016;89:141). *RUNX1* variants detected by WES were confirmed by Sanger sequencing in the propositi and all available family members, which also undergo clinical and laboratory characterization.

Results: We identified 3 pedigrees with different *RUNX1* heterozygous mutations: the novel variants c.578T>A and c.967+2_5del, and the known c.351+1G>A. The 13 affected subjects had mildly reduced platelet count, normal platelet size, variable expression of other morphological/functional platelet defects of alpha-granules content, glycoprotein expression and *in vitro* platelet aggregation. Three patients from 2 families developed AML, with a prevalence lower than reported in literature, probably because of a different criteria of enrolment (*RUNX1* is usually analyzed in ITs associated with AML).

Conclusions: FPD/AML lacks of pathognomonic criteria and is characterized by normal platelet size as the other ITs predisposing to hematological malignancies (*ANKRD26* and *ETV6*-related thrombocytopenias). Given the importance of recognizing these diseases for patients´ counseling, follow-up, and therapeutic approach, we recommend a systematic screening for *RUNX1*, *ANKRD26*, and *ETV6* mutations in all patients with an autosomal dominant IT and normal platelet size.

PB 730 | A Novel Mutation in *TBXA2R* Impairs the Expression of the TPβ Isoform of the Platelet Thromboxane Receptor and Associates with Bleeding Diathesis

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Background: The platelet thromboxane A_2 receptor (TP) is a G protein-coupled receptor playing an essential role in hemostasis by binding thromboxane A_2 (TxA_2). Two isoforms of TP, TPα and TPβ, are generated by alternative splicing from the TBXA2R gene. While the expression and function of TPα in platelets is well established, that of TPβ is still a matter of debate. The TP defect is a very rare autosomal dominant bleeding disorder characterized by mild mucocutaneous bleeding caused by mutations in *TBXA2R*, with 5 mutations, all affecting TPα, in a few families described.

We have studied a woman with a lifelong bleeding diathesis and platelet dysfunction in response to TP agonists, characteristics that oriented towards a TP defect.

Aims: To characterize the platelet dysfunction, to confirm TP defect by molecular genetic analysis, to investigate the impact of the mutation on the TP protein.

Methods: Platelet function was studies by light transmission aggregometry (LTA), flow cytometry and lumiaggregometry, TBXA2R gene was analyzed by PCR and direct sequencing. TP isoform expression was assessed by real time PCR and Western blotting (WB) using antibodies selective for the two TP isoforms.

Results: Platelet LTA was defective in response to arachidonic acid, U46619, U44069 and secondary wave to ADP and epinephrine. Sequencing of *TBXA2R* revealed a novel heterozygous A>T substitution at g.A11765T, not present in healthy controls, corresponding to the alternative splice site of TPβ. We confirmed the expression of the TPβ isoform in ultrapurified platelets by real time PCR and WB. Real time PCR showed a 50% reduction of the mRNA coding for TPβ accompanied by a reduction of the TPβ protein by WB in patient platelets.

Conclusions: We describe a novel g.11765A>T variation in *TBXA2R* associated with a mucocutaneous bleeding diathesis hitting the splice site that regulates the expression of the TPβ isoform. These findings suggest that TPβ has role in platelet function.

PB 731 | Spontaneous Loss of Mitochondrial Potential and Phosphatidylserine Exposure in Fibrinogen-bound Platelets of Patients with Wiskott-Aldrich Syndrome

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Background: Bleeding in Wiskott-Aldrich syndrome (WAS) is due to thrombocytopenia of unclear origin. Our previous studies suggested that WAS platelets are more likely to expose phosphatidylserine (PS) possibly leading to their destruction by macrophages.

Aims: To investigate PS exposure of fibrinogen-immobilized WAS platelets.

Methods: Whole blood was collected into sodium citrate. All patients (n=8) and healthy donors gave their written informed consent, and the study was approved by the institutional ethics committee. Platelets were loaded with Fura Red, immobilized and incubated on a fibrinogen surface for 50 min, followed by real-time confocal microscopy. Annexin V was used for PS detection, while tetramethylrhodamine was added in some cases to detect mitochondrial potential.

Results: Fibrinogen-immobilized WAS platelets had a high tendency to undergo spontaneous activation with PS exposure in comparison with normal platelets(25% versus 6%, P< 0.05). Signaling events preceding PS exposure were similar to those previously reported for procoagulant platelet formation in normal subjects upon stimulation: a series of cytosolic calcium spikes followed by membrane potential loss in all mitochondria, sustained cytosolic calcium increase and PS externalization. Cyclosporin A (CsA), an inhibitor of mitochondrial permeability transition pore opening, decreased the PS-positive platelet fraction several-fold.

Conclusions: WAS platelets spontaneously expose PS via a mitochondria-dependent necrotic mechanism, which may contribute to their clearance and thrombocytopenia.

PB 732 | ACTN1 Macrothrombocytopenia: Deleterious Effects of Rod-domain ACTN1 Variants

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Background: Mutations of *ACTN1*, the gene coding for alpha-actinin isoform 1, have recently been identified as responsible for a mild form

of inherited macrothrombocytopenia (IMT). All the mutations already described except one are located in the two important functional domains (Calmodulin-like domain and Actin-Binding Domain) involved in its dimerization and binding to actin.

Aims: Molecular and functional characterization of newly *ACTN1* identified mutations.

Methods: *ACTN1* variants were identified by Sanger sequencing in patients with IMT after having excluded *MYH9* defects. Organization of actin filaments was examined by immunofluorescence staining in two cell-lines (CHO and HeLa cells) transfected with *ACTN1* variants. Phenotype data of the affected patients are reported.

Results: In 6 patients of 5 unrelated families, we identified five different variants in the 4 Spectrin Repeats (SR) of the rod-domain of ACTN1 affecting highly conserved amino-acid residues and predicted to be deleterious by Sift® and Polyphen2®*in silico* programs.

Compared to wild-type *ACTN1*, cells transfected with each *ACTN1* variant presented actin filament disruption, change of actin network with shorter and thicker actin fibers, and coarse distribution of unbound actinin in the cytoplasm especially in CHO cells.

Phenotype analysis showed a moderate thrombocytopenia (platelet count mean: 91 G/L), the presence of few giant platelets (defined as platelet size over than red cell one - mean: 6%) and numerous macroplatelets (defined as platelet size under red cell size and over ½ red cell one - mean ± SD:41±18 %). No bleeding tendency was reported.

Conclusions: We identified five novel *ACTN1* variants located in the SR domain causing actin network disorganization, indicating that the rod domain plays also a key molecular role, probably by modulating its dimerization or its binding to others proteins.

PB 733 | Complete Failure of Integrin αIIbβ3 Activation in Kinetic Assay Associates with Severe Bleeding Problems in a Patient with Kindlin-3 Deficiency

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Background: Activation of platelet fibrinogen receptor integrin αIIbβ3 by inside-out signaling is essential for platelet aggregate formation. Although it has been established that the direct interaction of talin and Kindlin-3 with β3 is critical for conformational change and activation of αIIbβ3, the details of inside-out αIIbβ3 activation is still obscure. Recently, we reported the patient with CalDAG-GFEI deficiency and the importance of CalDAG-GEFI for immediate αIIbβ3 activation kinetics associated with severe bleeding problems (Kato et al. Blood 2016).

Aims: To elucidate the role of Kindlin-3 in inside-out signaling, we analyzied platelets of a newly diagnosed Kindlin-3 deficient patient and compared with those of CalDAG-GEFI or P2Y12 receptor deficiency.

Methods: We analyzed platelets from a 8-month-old baby who showed easy bruising and prolonged bleeding time (>20min) by flow cytometry, western blotting, sequencing, and shear-induced thrombus formation after obtaining written informed consent.

Results: Peripheral blood platelet count and the expression levels of platelet surface glycoproteins in platelets of the patient were comparable to those of control. αIIbβ3 activation determined by activated αIIbβ3 specific antibody PAC-1 was strongly impaired in all agonist stimulation including PMA. Western blotting analysis found deficiency of Kindlin-3 in patient's platelets and the sequencing results revealed homozygous nonsense mutation W277X in Kindlin-3. In contrast to the delayed αIIbβ3 activation kinetics in CalDAG-GEFI deficient platelets and transient activation in P2Y12 deficient platelets, no αIIbβ3 activation was observed by "initial velocity assay" at any time points after PAR1-TRAP stimulation. In addition, shear induced thrombus formation on collagen was severely impaired than that of CalDAG-GEFI deficiency.

Conclusions: Unlike CalDAG-GEI or P2Y12 deficiency, Kindlin-3 deficiency resulted in the complete failure of αIIbβ3 activation in kinetic assay and associated with severe bleeding symptoms.

PB 734 | Identification of Two New *P2RY12* Heterozygous Mutations Responsible for Hemorrhagic Diathesis in Two Unrelated Families

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Background: Since 2001, eleven mutation of *P2RY12* gene have been described with, in almost all cases, a hemorrhagic diathesis related to the presence of either a biallelic mutation of the *P2RY12* gene or of a heterozygous mutation associated with another primary hemostasis anomaly.

Aims: Here, we report two new heterozygous mutations of *P2RY12* found in *propositi* from unrelated families in the absence of any other detectable primary hemostasis defect.

Methods: Blood platelet exploration were performed in two patients (KC and CJP) with a bleeding history with normal platelet count and without any coagulation or Willebrand disorders. We examined platelet aggregation by light transmission and granular content by HPLC and ELISA. $P2Y_{12}$ quantification was performed by radioligand binding studies using intact platelets and downstream signaling was assessed by cAMPc measurement using a radio-immunoassay (CJP only) and the VASP test. *P2RY12* gene was sequenced using in-house primers.

Results: Platelet aggregations performed in citrated PRP and washed platelets showed a severe defect of ADP-induced aggregation in both patients while nucleotides and serotonin granular content were normal. $P2Y_{12}$ expression was reduced in both patients (282 sites per

platelet in CJP, 218 in KC, vs 573 in the control). The ADP receptor reactivity assessed through the VASP assay was normal (> 70%) in both cases but a moderate decrease in inhibition of cAMP formation was found after stimulation of CJP platelets by low concentrations of ADP (1 to 5 μM). *P2RY12* sequencing revealed the presence of one unknown heterozygous mutation in each patient: p.Phe95Ser in KC and p.Tyr259Cys in CJP.

Conclusions: We identified two new $P2Y_{12}$ variants affecting two essential amino acids as predicted by recent crystal structure studies. These mutations seem to be sufficient to induce a bleeding tendency even if the mutation is heterozygous. Reproduction of each variant is ongoing in 1321N1 cells using HA-tag plasmid vectors.

PB 735 | Glanzmann's Thrombasthenia in China: New Insights into its Pathogenesis

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Background: Glanzmann's thrombasthenia (GT) is a rare genetic bleeding disorder characterized by defects of the integrin aIIβ3 on the platelet membrane. Most patients with GT were found in areas where consanguineous marriages are common. There has yet been no large clinical and genetic study of GT in China, however.

Aims: To determine the molecular basis of GT in patients from China and extend knowledge of its pathogenesis.

Methods: Clinical features of 89 patients with GT were evaluated. Mutations in 30 patients were detected by Sanger sequencing, while 15 patients were analyzed using a panel of 89 genes related to hemostasis by next-generation sequencing.

Results: Consanguinity of these patients is much lower (16.8%) than in GT-prone countries. Mortality was relatively high (7.4%). Fifty mutations were identified, among which 36 were new. Most patients were compound heterozygotes. In variant types of GT patients, three mutations were identified on ITGA2B, which are different from previous reports. p.R584X, p.Q891X, p.Q778P on ITGA2B and p.C400Y on ITGB3 were hot spot mutations. Among 36 novel mutations, there were 18 missense mutations, five nonsense mutations, seven splicing mutations, one insertion mutation and five deletion mutations. Molecular modeling revealed these novel mutations induced changes in aIIβ3 domain structures, thereby interfering with integrin activation and function. In 15 patients who underwent next-generation sequencing, one patient revealed no molecular genetic anomalies and three patients were only identified with one heterozygous mutation in ITGA2B or ITGB3, which could not explain the diagnosis of GT. However, mutations in other genes related to hemostasis were found in these patients. These data suggest that, besides ITGA2B and ITGB3, mutations in other genes involved in coagulation may contribute to the pathogenesis of GT.

Conclusions: Our results show unique clinical and genetic features of GT patients in China and highlight the necessity of next-generation sequencing in GT patients.

PB 736 | A New Form of Inherited Thrombocytopenia (IT) Due to Monoallelic Loss-of-function Mutation in the Thrombopoietin (THPO) Gene

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Background: The THPO-MPL axis plays a central role in platelet biogenesis: it activates the signaling cascade inducing megakaryocytes (MKs) differentiation from progenitor cells and regulates MK maturation, proplatelet extension, and nascent platelet release into the bloodstream.

Patients with inherited defects of MPL interfering with THPO binding present with severe congenital amegakaryocytic thrombocytopenia, evolving into trilineage bone marrow aplasia; similarly, a homozygous loss-of-function variant in the *THPO* gene was responsible for recessive aplastic anemia in a Micronesian family. At the opposite, gain of function mutations in *MPL* and *THPO* cause congenital thrombocytosis.

Aims: To unravel the molecular basis of ITs and to improve the clinical and laboratory characterization of the new ITs identified.

Methods: Whole exome sequencing was performed in 86 propositi with an unknown IT, part of our case series of 274 consecutive families, 151 of which without a definite diagnosis after the diagnostic workup based on the must updated diagnostic algorithm (Clin Genet 2016;89:141).

Results: The molecular analysis identified 2 unrelated individuals carrying the heterozygous variant c.91C>T (p. Arg31*), which is expected to result in mutant protein degradation and THPO haploinsufficiency. In each family the segregation with the disorder was confirmed analyzing one affected relative. Thrombocytopenia was mild, bleeding tendency absent in all cases. Platelet size was normal or slightly enlarged. *In vitro* platelet aggregation and major surface glycoproteins were normal. Serum THPO levels were at the extreme lower limit of the normal range, confirming that *THPO* mutation was responsible for haploinsufficiency.

Conclusions: The p. Arg31* mutation in THPO causes a new autosomal dominant form of mild, non-syndromic thrombocytopenia. This innocuous disorder is relatively rare (1.3% of families of our case series) but it must be differentiated from ITs with normal-sized platelets that predispose to hematological malignancies.

PB 737 | MYH9 Variants Defined by Next-generation Sequencing: Predicting Variants Likely Causing MYH9-related Disorder

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Background: Variants in the *MYH9* gene, coding for the heavy chain A of non-muscle myosin class II, are associated with the *MYH9*-related disorder (MYH9-RD) characterized by macrothrombocytopenia, Döhle-like inclusion bodies in neutrophils and ear, eye, kidney and liver involvement of various severity.

Aims: To screen cases with an *MYH9* variant within the Bleeding, Thrombotic and Platelet Disorders (BPD) enrolled in the BRIDGE-BPD and in the ThromboGenomics (Simeoni *et al.* Blood 2016) cohorts.

Methods: Patient DNAs were subjected to whole genome or targeted sequencing and clinical information collected using Human Phenotype Ontology (HPO) terms. Rare variants were defined after computational analysis excluding variants with minor allele frequencies >1/1000. Each variant was reviewed by a Multi Disciplinary Team and labelled with pathogenicity and contribution to the phenotype.

Results: 34 MYH9-RD cases with 22 different variants preferentially localized in 10 of the 41 exons of *MYH9* were found. We found 15 missense variations, 2 stop-gain, 3 frameshift, 1 insertion and 1 deletion. All cases had macrothrombocytopenia and 14 cases out of 34 had typical Döhle-like inclusion bodies. Variants involving the SH3/MD interface of MYH9 head, the coiled-coil and the tail domains were found in cases with a mild phenotype, confirming previous data (Pecci *et al.* Hum Mutat. 2014). Some degree of bleeding diathesis was present in 20 patients. In 10 out of 34 cases, MYH9-RD had not been suspected. Out of the 22 variants, 8 were not previously reported in the Human Gene Mutation Database (HGMD) and in the Exome Aggregation Consortium (ExAC) control datasets: 3 novel variants hit the SH3/MD interface, 4 the coiled-coil domain and 1 the non-helical tail.

Conclusions: Our results add knowledge to the characterization of MYH9-RD confirming its clinical and genetic heterogeneity and show that, in the presence of unclassified macrothrombocytopenia, MYH9-RD should be suspected.

PB 738 | Aberrant Gsalpha Signalling in Platelets from AHO Patients Linked to Impaired Platelet Function

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Background: Albright hereditary osteodystrophy (AHO) patients are diagnosed with pseudohypoparathyroidism, causing impaired (parathyroid) hormone signalling when inherited maternally. The syndrome is accompanied by mutations in the *GNAS* complex gene locus for Gsα, resulting in a variable degree of cellular signalling via Gsα, adenylate cyclase (AC) and cAMP-dependent protein kinase A (PKA).

Aims: To elucidate the altered platelet Gs-AC-PKA signalling and platelet function in AHO patients.

Methods: Platelet function (activation, aggregation, thrombus formation) was studied in blood from diagnosed AHO patients and control subjects. Platelets were treated with a dose range of Gs stimulating prostaglandins. Using mass spectrometry quantitative changes in the prostaglandin-induced phosphoproteome were assessed for patient and control platelets.

Results: In platelets from diagnosed AHO patients we measured normal to impaired responsiveness to prostaglandins, in terms of suppression of platelet activation, aggregation and thrombus formation. Concerning the target protein of PKA signalling, Gs stimulation caused either no (n=2), a moderately impaired (n=3) or a greatly impaired (n=3) phosphorylation of vasodilator-stimulated phosphoprotein. We identified 3,457 phosphopeptides (for 1,170 proteins), of which 239 peptides were Gs regulated. In platelets from an AHO patient, the global proteome was in essence unchanged, but 149 protein sites were altered in phosphorylation that: *(i)* agreed with prior iloprost-induced changes, *(ii)* contained a PKA target consensus sequence, *(iii)* could be classified to Signaling & adapter proteins (20×), Protein kinases & phosphatases (18×) and Small GTPases & regulators (12×).

Conclusions: Patients diagnosed with AHO to a variable degree have platelets with aberrant PKA-dependent signalling and phosphorylation patterns, along with altered function; pointing to a different disease penetrance. Quantitative platelet phosphoproteomics is a powerful tool to detect such altered phosphorylation.

PB 739 | Pseudo-Glanzmann Thrombasthenia due to Mutation in RasGRP2 Gene Encoding for CalDAG-GEFI

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Background: The nature of an inherited platelet disorder was investigated in three patients that exhibited massive mucocutaneous bleeding episodes, impaired platelet aggregation with ADP, epinephrine and collagen but normal aggregation with ristocetin, and normal platelet count and size, and were therefore misdiagnosed as Glanzmann Thrombasthenia.

Aims: To detect the basis for the bleeding disorder.

Methods: Flow cytometry and molecular sequencing.

Results: Using flow cytometry we observed normal surface expression of the integrins αIIbβ3 and αvβ3, as well as normal integrin αIIbβ3 activation following incubation with the activating antibody LIBS6. In contrast, platelet activation following ADP or epinephrine was impaired as measured by anti P-selectin or PAC1 antibodies. Direct sequencing of the exons encoding for the cytoplasmic tails of integrin αIIbβ3 did not detect any candidate mutation, therefore we performed whole exome sequencing (WES) followed by bioinformatic analysis of platelet expressed genes. WES detected a mutation in RasGRP2 gene in two patients:

1 - A 9 month old boy of Kavkazi Jewish origin was found to be homozygous for deletion G1279 in exon 11; his parents were found to be heterozygous for this deletion despite absence of consanguinity;

2 - A 20 year old girl of Christian-Arab origin was found to be homozygous for a single nucleotide insertion, 1480-7C in a CCCCCCC sequence in exon 13. Interestingly, the third patient, a 22 year old girl of Kavkazi Jewish origin was also homozygous for the deletion G1279 in exon 11, suggesting that this mutation might be common in the Kavkazi Jewish community.

Conclusions: RasGRP2 encodes the CalDAG-GEFI protein, a major signaling molecule functioning as Rap1 GTPase activator regulating integrin-mediated aggregation and granule secretion. Only 7 patients carrying 5 different mutations in RasGRP2 gene were previously published. All of them suffered from severe bleeding without any impact on leukocyte function, in contrast to the observations reported in mice.

PB 740 | GATA1 T296P Mutation Causes a Severe Bleeding Disorder due to Platelet A-Granule and Partial δ-Storage Pool Deficiency

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Background: The transcription factor GATA1 regulates megakaryopoiesis including megakaryocyte relevant genes, e.g. *GPIBA*, *NBEAL* and *SYK*. *GATA1* mutations in humans are predominantly located within the N-terminal zinc finger domain leading to complex disorders such as X-linked thrombocytopenia associated with gray-platelet like syndrome.

Aims: To elucidate the platelet phenotype and genetic diagnosis of a 35-year-old patient and his 4-year-old daughter who suffer from severe bleeding, e.g. frequent epistaxis and hematoma since infancy not caused by von Willebrand disease or subhemphilia.

Methods: Platelet phenotype and function was analyzed by aggregometry, flow cytometry, immunoblotting, ELISA and electron microscopy. Whole exome sequencing and pyrosequencing was used for genetic analyses.

Results: Agonist-induced aggregation of patients' platelets with increased mean volume was impaired but normal in response to high concentration of collagen or arachidonic acid which was associated with an ATP-secretion defect. Western analysis and electron microscopy revealed paucity of α- and δ-granules accompanied by big vacuoles containing granule/membrane fragments. Patients' platelets did not take up mepacrine and serotonin levels were reduced but still in the normal range. A not yet described *GATA1* mutation (c.886A>C, p.T296P) close to the C-terminal zinc finger domain was identified in the index patient (hemizygous), in his symptomatic daughter (heterozygous) and his asymptomatic mother (heterozygous).

Conclusions: We describe for the first time a *GATA1* T296P mutation as cause of severe platelet dysfunction due to paucity of α-granules and dense bodies which lack adenosine nucleotides but contain normal levels of serotonin. Recent platelet proteome analysis is expected to help the identification of downregulated proteins that explain the granule defects. Imbalanced X-chromosome inactivation might explain the different phenotypes of the *GATA1* mutation carriers.

PB 741 | Detection and Analysis of Gene Mutations in Patients with Glanzmann Thrombasthenia by Next Generation Sequencing

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Background: Glanzmann thrombasthenia (GT) is an inherited genetic disorder affecting platelets, which is characterized by spontaneous mucocutaneous bleeding and abnormally prolonged bleeding. The underlying defect is failure of platelet aggregation due to qualitative and/or quantitative deficiency of platelet integrin αIIbβ3 resulting from molecular genetic defects in either *ITGA2B* or *ITGB3*. When striving for applicability in routine clinical practice, it soon becomes clear that population Sanger sequencing is too labor-intensive and time-consuming.
Aims: In this study, we aimed to assess *ITGA2B* or *ITGB3* gene mutations through next generation sequencing (NGS) in diagnosed GT patients.
Methods: A cohort of 5 patients with clinical symptoms of GT were enrolled for molecular genetic analysis by NGS. Sample DNA was amplified using Ampliseq primer panel, and libraries were prepared following the manufacturer´s Ion Ampliseq Library Preparation protocol. Individual samples were barcoded, pooled, templated, and sequenced on the Ion Torrent Proton Sequencer.Raw sequencing reads were quality and adaptor trimmed by Ion Torrent Suite and then aligned to the hg19 reference sequence.
Results: We identified pathogenic mutations from all the 5 patients, including homozygous mutations in ITGA2B gene in 2 patients (p.Q778P; p.L1020L). Compound heterozygous mutations were identified in 3 patients, 2 patients with ITGA2B:p.Q778P and ITGA2B:p.R584X, and 1 patient with ITGA2B:p.R584X and ITGB3:p.R662H. The Q778P, R584X and L1020L mutations of ITGA2B gene were missense, nonsense and splicing mutation, respectively. Mutations in *ITGB3* p.R662H was novel missense mutation that had never been reported.
Conclusions: NGS technology may be an useful method to detect the missense, nonsense and splicing mutations in GT patients. NGS technology is systematic and efficient, and can provide a convenient method for clinical diagnosis and scientific research. Novel mutation of *ITGB3* gene found in GT patients need to be further studied.

PB 743 | Refined Detection and Characterization of Delta Storage Pool Disorders: A Cohort Study on Pediatric Patients

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Background: Delta storage pool disorders (SPD) remain frequently undetected due to moderate bleeding symptoms and insensitive detection methods. Since the cause of acquired and inherited delta-SPDs can be either a dense granule uptake or release defect it is of high interest to readily discriminate subgroups for precise diagnosis and therapy.
Aims: We aimed to classify a cohort of pediatric patients with unspecified bleeding disorder by delta-SPD characteristics and to prove reliability of our kinetic mepacrine assay.
Methods: We included 51 patients with defective ADP/ATP release as well as aggregation and a mild to moderate bleeding diathesis according to the ISTH-BAT bleeding score without differential diagnosis and prior to drug treatment. 46 patients declared a positive bleeding family history, indicating an inherited cause. We developed a whole-blood-based flow cytometric kinetic assay, measuring mepacrine uptake in platelet dense granules and agonist-induced mepacrine release over 5 minutes.
Results: We found a positive correlation between mepacrine release and ADP/ATP release, which was overall significantly lower in the patient cohort compared to healthy controls. 12 patients showed a significantly reduced CD63 exposure after stimulation, measured by flow cytometry. However, this could not be correlated to mepacrine release defects, excluding CD63 as a reliable dense granule marker. Patients could be classified into following groups: inconspicuous (16), uptake defect (7), release defect (10), or a combined defect (11). Noticeably, patients with a selective release defect showed a higher ISTH-BAT bleeding score compared to other subgroups.
Conclusions: Our data demonstrate that implementation of a kinetic mepacrine assay as sensitive and cost-effective diagnostic tool helps to detect unclassified SPD and allows discrimination of relevant subgroups. Since platelet activation measured by flow cytometry correlated well with aggregometry, the underlying defect can be confirmed as granule-based.

PB 744 | Multiplex PCR and Cycle Sequencing for Detection of Mutations in *ACTN1*, *TUBB1* and *ANKRD 26*

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Background: Genetic investigation of patients with inherited thrombocytopenia involves screening for mutations in multiple (>20) genes including *ACTN1*, *TUBB1* and *ANKRD 26*. This list of genes is likely to become even longer as the causative mutation remains unaccounted for in ~ 50% of patients. Conventional methods to screen for mutations in multiple genes are tedious and time-consuming.
Aims: To improve efficiency of analysis, we have developed a multiple gene screening method using multiplex PCR and universal primer tags coupled with cycle sequencing.
Methods: In an assay system for screening of three genes *ACTN1*, *TUBB1* and *ANKRD 26*, 18 amplicons were designed to cover 21 exons of *ACTN1*. These amplicons were divided into 2 sets of multiplex PCR, each containing 9 primer pairs. All the primers were tagged with a series of 9 pairs of different universal primers. To analyze *TUBB1* and *ANKRD 26*, the third set of multiplex PCR was designed to contain 3 amplicons for 4 exons of *TUBB1* and 1 amplicon for the 5' UTR of *ANKRD 26*, and these primers were also tagged with the universal primers used in the sets 1 and 2. Following multiplex PCR, cycle sequencing was performed using the universal primer tags to acquire DNA sequences.
Results: The developed method was evaluated in screening 8 unrelated patients with thrombocytopenia. 8 mutations were identified,

including 2 novel mutations in *ACTN1* (p.Ala135Ser and p.Gln533Leu), 2 novel mutations in *TUBB1* (p.Leu253Val and p.Gly235Alafs*2), and 4 mutation in the regulatory region of *ANKRD 26* (c.-128G>C in one patient, c.-134G>A in one patient, and c.-140 C >G in two patients).

Conclusions: Multiplex PCR simplifies the template preparation for DNA sequencing, and incorporation of universal primer tags to the specific primers further speeds up subsequent cycle sequencing processes. The developed method can easily be expanded to include more genes, and provides an efficient approach to analysis of the multiple genes involving in inherited thrombocytopenia.

PB 745 | Delta Storage Pool Deficiency in a Patient with a R216Q Mutation of GATA1

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Background: The transcription factor GATA1 is fundamental in the differentiation of hematopoietic progenitor cells to platelets and red blood cells. Several GATA1 mutations have been linked to human diseases , including X-linked thrombocytopenia with thalassemia (XLTT). XLTT is caused by a missense mutation (R216Q) and is characterized by thrombocytopenia, abnormal platelet structure and hemolytic anemia resembling beta-thalassemia. Delta storage pool deficiency (δ-SPD) has been reported in one of eight patients with XLTT, but it remains uncertain whether δ-SPD is a pathological feature of this specific GATA1 mutation.

Aims: To study dense granules in platelets of a patient with the R216Q mutation of GATA1.

Methods: -

Results: The patient is a 46 year old male with a lifelong history of bleeding (ISTH-BAT score 16), but normal aggregation tests. Informed consent was obtained for blood collection to perform several diagnostic tests to screen for δ-SPD. Platelet ADP content was measured in platelet lysates using bioluminescence and revealed a platelet ADP content of 0,8 µmol/10^{11} platelets (ref range 1,7-3,8 µmol/10^{11} platelets) on repeated measurements. Mepacrine, an acridine derivative that binds to adenine nucleotides in dense granules and emits green fluorescence, was used to determine dense granule content, by measuring mepacrine uptake in the FITC spectrum of the flow cytometer. Mepacrine uptake was decreased (< 2.5th percentile of normal) compared with healthy platelets. Whole mount electron microscopy (EM) was performed to visualize and quantify dense granule numbers and showed near absence of dense granules.

Conclusions: The decreased platelet ADP content, lowered mepacrine uptake and the virtual absence of dense granules on EM are consistent with the diagnosis of δ-SPD. Hence, this study confirms the presence of δ-SPD in another patient with the R216Q mutation of GATA1. Further studies are required to determine the precise role of the R216Q mutation in the defective formation of dense granules.

PB 746 | Genetic Heterogeneity in Familiar Macrothrombocytopenia with Decreased Expression of GpIIbIIIa

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Background: Congenital autosomal dominant macrothrombocytopenia (AD-MT) associated with decreased expression of GpIIbIIIa, although rare, represents the main congenital macrothrombocytopenia in our centre. Gain of function mutations in ITGA2B or ITGB3 with constitutive GpIIbIIIa activation and impaired platelet (PLT) maturation have been described in these cases.

Aims: Characterization of eight Portuguese families with macrothrombocytopenia and decreased expression of GpIIbIIIa.

Methods: Clinical and laboratory evaluation of 27 individuals from eight unrelated families with AD-MT and decreased GpIIbIIIa expression. Laboratory studies: PLT counts (impedance/flow cytometry), mean PLT volume (MPV) and morphology, PLT occlusion time, PLT aggregation and ATP release studies, antiPLT antibodies, PLT glycoprotein expression (CD41a/GpIIbIIIa, CD42b/GpIb and CD61) by flow cytometry, PLT electron microscopy and genetic analysis of ITGA2B and ITGB3 genes.

Results: AD inheritance pattern. Absent to moderate bleeding. Moderate thrombocytopenia with increased MPV. Normal to slightly increased PLT occlusion times. Decreased ADP and collagen-induced PLT aggregation, normal ristocetin-induced PLT agglutination. Decreased levels of CD41a/GpIIbIIIa (41-65%) and CD61/GpIIIa (48-77%) and increased CD42b/GpIb (113-225%) expression. Heterozygous variants in ITGA2B (n=3) and ITGB3 (n=2) were identified in six families. Two variants were previously reported in association with AD-MT. Three are novel and classified as pathogenic based on their absence in variant databases, bioinformatic and segregation analysis. The genetic study of two families is still being carried-out.

Conclusions: Macrothrombocytopenia with decreased expression of GpIIbIIIa presents with wide clinical spectrum and needs to be identified in order to avoid misdiagnosis. Our results reveal high genetic heterogeneity in our cohort and expand the mutational profile of AD-MT.

PB 747 | Pseudodominance in a Family with Bernard-Soulier Syndrome due to a Homozygous Variant in the Platelet Glycoprotein IX (GP9) Gene

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Background: Bernard-Soulier syndrome (BSS) is a rare platelet (PLT) disorder due to a defect of the GPIb/IX/V (which binds to von Willebrand factor), characterized by mucocutaneous bleeding, thrombocytopenia, giant platelets and impaired ristocetin-induced agglutination. Mutations in the GP1BA, GP1BB and GP9 genes have been described.

Aims: To describe the clinical and laboratory features of a family with BSS, with an apparent autosomal dominant (AD) pattern (Figure 1).

Methods: Platelet counts (impedance/flow cytometry), mean PLT volume (MPV), morphology, occlusion time, aggregometry (ADP/collagen/ristocetin) and flow cytometry (CD42a/GpIX, CD42b/GPIb, CD41a/GPIIbIIIa). Genetic study was done in patient I.2 using a panel for massive parallel sequencing (394 loci). Variant filtering was restricted to exonic regions and splice-sites and with minor allelic frequency below 1%. Confirmation and screening of the disease-causing variant was done by Sanger sequencing.

Results: Moderate bleeding, thrombocytopenia, giant PLT, increased MPV (>20fL) and PLT occlusion time, impaired ristocetin-induced agglutination, normal ADP/collagen-induced aggregation, and reduced expression of CD42b/GPIb (6.1-9.6%) and CD42a/GPIX (6.8-8.5%). Among the filtered-in variants a homozygous single nucleotide change (NM_000174.4:c.182A>G) was identified in exon 3 of GP9, in patient I.2. This variant causes a missense substitution (p.Asn61Ser) previously reported as pathogenic (Wright,1993 - PMID:8481514; ClinVar), associated with BSS type C. It was also found in homozygosity in the patient's affected daughters, whereas asymptomatic individuals I.1, III.1 and III.2 were found to be heterozygous for this variant.

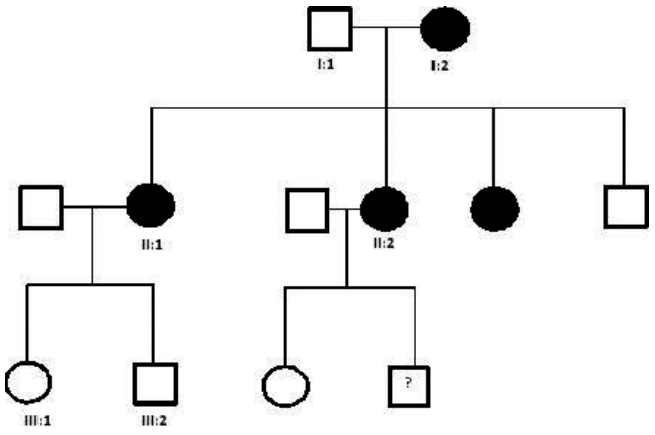

FIGURE 1

Conclusions: This is the first report of pseudodominant BSS in a family where transmission of a recessive disease mimics an AD inheritance. BSS is a rare entity (estimated prevalence of 1/1000000 live births), making this pedigree all the more interesting for lack of declared consanguinity.

PB 748 | Highly Significant Bleeding Diathesis in Patients with Platelet Dysfunction due to a Novel Mutation in *RASGRP2*, Encoding CalDAG-GEFI (p.Gly305Asp)

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Background: Mutations affecting RASGRP2, the gene encoding the Rap GTPase activator, CalDAG-GEFI, give rise to a novel, and rare, group of platelet signal transduction abnormalities. We here report platelet studies of a novel variant, CalDAG-GEFI(p.Gly305Asp), previously identified in an Argentinian pedigree.

Aims: To fully characterize the platelet function defect and to describe the therapeutic approach for the treatment of hemorrhage in the two siblings (P1, P2) affected in this consanguineous family.

Methods: Flow cytometry was used to quantify platelet adhesion receptor expression, and to measure integrin activation (fibrinogen binding) and granule secretion (surface expression of CD62P and CD63) in the patients' and control platelets activated or not with classic agonists and PMA. Platelet aggregation was measured by lumi-aggregometry. Thromboelastometry (ROTEM®) and Thromboelastogram [TEG®] were used to measure platelet procoagulant activity. Western blotting procedures were used to detect CalDAG-GEFI.

Results: Two brothers (P1 and P2) have a lifelong history of bleeding with severe epistaxis successfully treated with rFVIIa. Other bleedings include hemorrhage from minor wounds. Platelet counts were normal, as was αIIbβ3 and GPIb expression. Fibrinogen binding, granule release, and the platelet aggregation response were significantly impaired with ADP or collagen but not PMA-activated platelets. ROTEM and TEG were normal, suggesting intact plasma coagulation, residual αIIbβ3 outside-in signaling and platelet pro-coagulant activity. CalDAG-GEFI protein expression was markedly reduced but not absent. Homology modeling places the Gly305Asp substitution at the GEF-Rap1 interface, suggesting that the mutant protein has very limited catalytic activity.

Conclusions: We characterize a new loss-of-function mutation in RASGRP2 causing impaired platelet adhesive function and highly significant bleeding in humans.

PB 749 | Positive Impact of Social Media as a Support Group for Females with Glanzmann Thrombasthenia in Saudi Arabia - A Mother's Initiative

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Background: Glanzmann Thrombasthenia (GT) is a rare inherited platelet disorder with limited information available. Most common presentation of the disease is mucous membrane bleeding and menorrhagia a major bleeding manifestation in females, Hematology service at King Faisal Specialist Hospital and Research Centre, Riyadh, deals with several such female patients and their mother´s.

Aims: To assess use of social media as a support group providing medical and psychosocial support to families affected with GT.

Methods: WhatsApp group titled "Female GT Support Group" was created in January 2016 by a mother, with an intention to provide a portal for mothers like her to be able to discuss challenges unique to females with GT; for medical validation purposes a female hematologist was added. Mothers and patients learnt of the group from one another and started referring.

Results: Thirty participants comprising of patients and mothers were added within a year. Majority of discussions involved, heavy periods, nose and gingival bleeding, hormonal, oral contraception and iron supplement therapy, feasibility of transfusion. Mothers discussed post-marital concerns experienced particularly by women involving bleeding during antenatal period, pregnancy and methods of delivery. Women who bore children shared their experiences and information on how PGD can help in family planning. Information on Iron Nutrition, Dental care and first aid to stop bleeding was shared. Other concerns on how to cope with school absence were addressed.

Conclusions: They were psychologically relieved to learn that they are not alone and felt comfortable sharing their stories resulting in better understanding of disease spectrum from mild to severe; helping them remain calm avoiding immediate emergency room visits and had a positive perspective towards family planning. In our experience social media proved to be an effective support model, however, addition of a Gynecologist, Dentist and Geneticist will result in comprehensive information to families.

PB 750 | Five New Cases of Hermansky-Pudlak Syndrome: Identification of Novel Genetic Variants in *HPS4* and *HPS3* Associated to Relevant Clinical Complications

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Background: Hermansky-Pudlak syndrome (HPS) is an inherited platelet disorder characterized by bleeding diathesis, oculocutaneous (OC) albinism and sometimes serious clinical complications. Heterogeneous clinical symptoms and a large numbers of possible genetic culprits (9 *HPS* genes, >118 exons) complicate unequivocal HPS diagnosis.

Aims: To assess the clinical and platelet phenotype in 5 patients with HPS suspicion and to identify their genetic defect by high-throughput sequencing (HTS).

Methods: We studied 5 patients from 3 families (2 Spanish, 1 Turkish) presenting with OC albinism. Clinical records were reviewed and bleeding scored with ISTH-BAT. Platelet phenotyping (only Spanish patients) included: platelet aggregation, GPs expression and granule secretion,^{14}C-serotonin uptake and whole mount electron microscopy. Patients DNAs were analyzed by HTS using a 71 gene panel (Lozano ML et al 2016).

Results: Clinical and laboratory findings in these patients are shown in Table 1. The Spanish patients (P1,P2,P5) showed impaired platelet aggregation to mild agonists and reduced platelet dense granules. In family 1 (F1), HTS identified a homozygous, potentially harmful, c.2054delC (p.Pro685Leu fs*17) variant in *HPS4*. One sister (P1) had Crohn´s disease and severe gastrointestinal (GI) bleeding. This variant had been reported in a 46yr Asian patient with pulmonary fibrosis. *A* novel missense homozygous *HPS4* variant, c.272T>C (p.Leu91Pro), was found in two Turkish siblings (F2). One had severe GI bleeding requiring colectomy (P4) and the other developed pulmonary fibrosis. Patient 5, suffering from mild GI bleeding, bears a heterozygous novel variant in *HPS3* (c.2464C>T, p.Arg822X) and, most likely, an additional unrevealed mutation.

Conclusions: HTS facilitates genetic confirmation of HPS diagnosis, and may help investigating phenotype-genotype relationships in HPS. The novel p.Leu91Pro variant in *HPS4* associates with severe clinical phenotype.

Funding: GRS 1370/A/16; ISCIII & Feder (PI14/01956), CIberer CB15/00055, SETH

TABLE 1 Clinical, laboratory and molecular findings in five HPS patients

FAMILY	PATIENTS	BLEEDING SYMPTOMS	OTHER CLINICAL FEATURES	ISTH-BAT	LIGHT TRANSMISSION AGGREGOMETRY	FLOW CYTOMETRY	14C-SEROTONIN UPTAKE	ELECTRON MICROCOPY	MOLECULAR VARIANTS
1	P1: 13y, female P2:16y, female No Familial consanguinity	P1+P2: Epistaxis, ecchymosis, menorrhagia P1: GI, and post-surgery	P1+P2: oculocutaneous (OC) albinism P1: Crohn's disease	P1: 11 P2: 3	Impaired aggregation with ADP (5µM), epinephrine (5µM) and ristocetin (1.2 mg/mL)	P1+P2: TRAP-induced CD63 release: 10% Platelets+ vs. 65% in control	25% reduction in 14C-Serotonin uptake	Absence of dense granules	HPS4 c.2054del (p.Pro685Leu fs*17)
2	P3: 40y, female P4:48y, female Familial consanguinity	P3+P4: easy bruising and epistaxis. P3: GI bleeding	P1+P2: OC albinism and nistagmus. P3: Colectomy P4. Bilateral pulmonary fibrosis	P3:7 P4:6	---	---	---	---	HPS4 c.272T>C p.Leu91Pro
3	P5:25y, male No Familial consanguinity	Epistaxis, ecchymosis and GI	OC albinism and strabismus Angiodysplasias	P5: 5	Mildly impaired aggregation epinephrine (5µM) and ristocetin (1.2 mg/mL)	TRAP-induced CD63 release: 1% Platelets+ vs. 60% in control	75% reduction in 14C-Serotonin uptake	Reduced number of dense granule	HPS3 c.2464C>T (p.Arg822X)

PB 751 | Quality of Life Is Reduced in Patients with (Suspected) Congenital Platelet Function Disorders

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Background: Platelet function disorders (PFDs) are rare bleeding disorders, characterized by mucocutaneous bleeding and prolonged bleeding after surgery or childbirth. Repeated bleeds throughout life can have a significant effect on health related quality of life (HR-QoL).

Aims: To assess how HR-QoL is affected by PFDs as compared to healthy controls.

Methods: HR-QoL is determined within a nationwide cross-sectional study in the Netherlands (‚Thrombocytopathy in the Netherlands' (TiN)), where adult patients with an increased bleeding tendency, suspected for having a PFD, are prospectively included. HR-QoL is assessed using the Short Form (SF)-36 survey, including 8 domain scores ranging from 0 to 100 (optimal). Bleeding severity is evaluated using the ISTH Bleeding Assessment Tool (BAT), resulting in a bleeding score (BS, range 0-48 for men, 0-56 for women; 0 optimal). For this interim analysis we used data of all patients included in the TiN-study in 2016. T-tests are used to compare SF36 domain scores to the general Dutch population scores and univariable linear regression analysis to assess the association between BS and HR-QoL.

Results: In this interim analysis, 83 patients were included, of whom 23% male, median age 43 (range 18-76) years. The median BS was 11 (IQR 7-15). Compared to the general population, patients with a (suspected) PFD showed significantly lower (p < 0,001) scores in the domains of role limitations due to physical functioning, bodily pain, general health perception, vitality and social functioning.

Patients with more severe bleedings had a lower HR-QoL in several domains as compared to patients with less severe bleedings.

TABLE 1 Univariable regression analysis for the association between bleeding score and SF36 domains. * p-value < 0,05]

	β	95% CI	p-value
Physical functioning (PF)	-1,852	-2,636 to -1,068	<0,001 *
Role limitations due to physical functioning (RP)	-2,456	-4,130 to -0,782	0,005 *
Bodily pain (BP)	-1,465	-2,465 to -0,466	0,005 *
General health perception (GH)	-1,243	-2,224 to -0,261	0,014 *
Vitality (VT)	0,064	-0,782 to 0,910	0,880
Social functioning (SF)	-2,322	-2,322 to -0,316	0,010 *
Role limitations due to emotional problems (RE)	-1,893	-1,893 to 0,855	0,454
Mental health (MH)	-0,307	-1,009 to 0,396	0,387

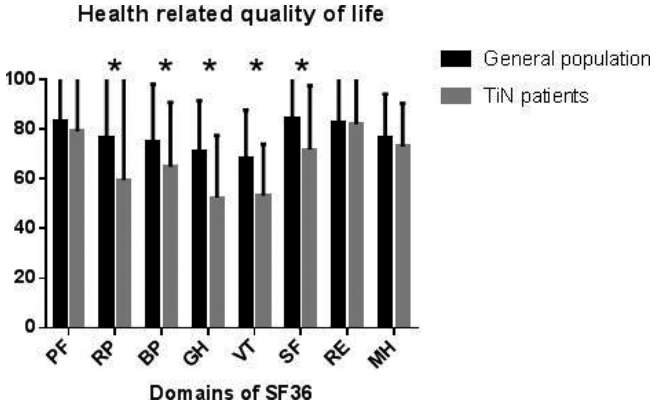

FIGURE 1 Health related quality of life for the domains of SF36. *: p-value < 0,001

Conclusions: We demonstrate a lower HR-QoL in patients with a (suspected) PFD compared with the general population and an inversed association between HR-QoL and bleeding phenotype, mainly in the physical domains. Final analyses will be performed in the full TiN study population (estimated 150 patients), including multivariable analyses adjusted for other determinants of HR-QoL.

PB 752 | The Diagnostic Performance of Impedance Aggregometry and Platelet Function Analyzer Regarding Platelet Function Disorders as Detected by Light Transmission Aggregometry

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Background: Light transmission aggregation (LTA) is the gold standard for the diagnosis of platelet function disorders (PFDs). Due to its time-consuming and variable (pre)analytical methods, the LTA is subjected to specialized laboratories. Whole blood impedance aggregometry (Multiplate) and platelet function analyzer (PFA) may be used as rapid screening tools to exclude PFDs.

Aims: To assess the diagnostic performance of Multiplate and PFA regarding the detection of PFDs, using the LTA as reference test.

Methods: Adults from three observational studies investigating bleeding symptoms were included in this analysis: preoperative patients with and without bleeding symptoms; patients referred to the hematologist for bleeding evaluation and patients with a known bleeding disorder of any kind. Patients using medication interfering with hemostasis, with platelet count < $100x10^9$/L and hematocrit < 0.25 L/L were excluded. A PFD was defined as ≥2 abnormal LTA curves. Abnormal test results on Multiplate and PFA were compared to the gold standard LTA for platelet function disorder. Informed consent was obtained and the medical ethical committee approved the studies.

Results: A total of 415 patients were included. In preoperative patients with and without bleeding symptoms and in patients referred to the hematologist for bleeding evaluation, the sensitivity for the detection of a PFD was low (0-36%) and specificity was high (84-98%) for both PFA and Multiplate. In patients with a known bleeding disorder, sensitivity for the detection of a platelet function disorder was 100%, with a specificity of 73-100% (see table 1 and 2).

Conclusions: In preoperative patients with and without bleeding symptoms, and in patients referred for bleeding disorders, the Multiplate and PFA cannot be used as a screening test to exclude PFDs. However, in patients with a known bleeding disorder, the Multiplate and PFA performed reasonably well, which might indicate that in more severe bleeders these assays could be used to screen for PFDs.

TABLE 1 Diagnostic performance of the Multiplate (AA, TRAP, ADP, COL) for the detection of PFDs diagnosed by the LTA (AA, TRAP, ADP, COL, EPI, RIST)

Patient population	Multiplate ≥1 agonists abnormal	LTA ≥2 agonists abnormal	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
preoperative patients without bleeding symptoms n = 91	2	6	0 (0-26)	98 (98-99.5)	0 (0-79)	93 (93-95)
preoperative patients with bleeding symptoms n = 226	8	10	10 (0.5-39)	97 (96-98)	13 (0.7-49)	96 (95-97)
patients referred for bleeding evaluation n = 50	5	11	36 (15-45)	97 (91-99.9)	80 (32 -99)	84 (79-87)
patients with a bleeding disorder n = 24	3	3 (Glanzmann´s thrombasthenia)	100 (37-100)	100 (91-100)	100 (37-100)	100 (91-100)

TABLE 2 Diagnostic performance of the PFA (ADP, EPI) for the detection of PFDs diagnosed by the LTA (AA, TRAP, ADP, COL, EPI, RIST)

Patient population	PFA ≥1 cartridge prolonged	LTA ≥2 agonists abnormal	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
preoperative patients without bleeding symptoms n = 93	6	6	0 (0-41)	93 (93-96)	0 (0-0.41)	93 (93-96)
preoperative patients with bleeding symptoms n = 222	27	10	0 (0-33)	87 (87-89)	0 (0-12)	95 (95-97)
patients referred for bleeding evaluation n = 48	9	10	30 (8.6-58)	84 (79-92)	33 (9.6-64)	82 (77-89)
patients with a bleeding disorder n = 18	7	3 (Glanzmann´s thrombasthenia)	100 (34-100)	73 (60-73)	43 (15-43)	100 (82-100)

PB 753 | Evaluation of the Platelet Function in Different Inherited Thrombocytopenias. Correlation of Platelet Response Measured by Flow Cytometry with Bleeding Scores and Clinical Phenotypes

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Background: Inherited thrombocytopenias (IT) are a heterogeneous group of exceedingly rare conditions characterized by low platelet counts. IT patients differ in their tendency to bleed despite similarly low platelet counts, therefore abnormal platelet function is likely to contribute to the bleeding. Whether platelet dysfunction coexists in ITs, worsening the bleeding risk, is unknown in most of them.

Aims: Our aim was to investigate platelet function in patients with different ITs using the flow cytometry, i.e. the sole platelet function assay that can distinguish between the effects of thrombocytopenia and the effects of abnormal platelet function. The platelet function was correlated with platelet count and a bleeding score.

Methods: Flow cytometric analysis of platelet activation was performed using the PE-conjugated P-selectin antibody and the FITC-conjugated PAC-1 antibody recognizing the activated integrin αIIbβ3 complex, together with Pe-Cy5-conjugated anti-CD42b to identify platelet population in whole blood samples. Platelets were stimulated with ADP 4-10 mM or PAR1-AP 10-25 mM and the binding of antibodies was analyzed by flow cytometry. ISTH-BAT and WHO bleeding scores were used to assess the bleeding risk of the patients.

Results: Thirteen patients with different inherited thrombocytopenias, having both great/giant platelets (Gray Platelet Syndrome, MYH9-RD disorders, Bernard-Soulier biallelic and mono allelic) and small platelets (Wiskott Aldrich syndrome) and different degrees of thrombocytopenia were studied in our center (Fig.1). Interestingly, those patients with higher bleeding scores had also a worse platelet activation pattern, independently of platelet count.

ID PATIENT	DIAGNOSIS	PLT count (x 10^3/ µL)/ MPV (fl)	WHO/ISTH-BAT Score	P-Selectin (MFI % ctrls)		PAC-1 (MFI % ctrls)	
				ADP (10 uM)	PAR1-AP (25 uM)	ADP (10 uM)	PAR1-AP (25 uM)
# 1	Gray Platelet Syndrome (*NBEAL2* biallelic mutations)	70 /16	2/12	49,4	20,0	77,1	21,4
# 2	Gray Platelet Syndrome (*NBEAL2* biallelic mutations)	43 /15.8	2/8	57,2	19,7	42,6	11,6
# 3	MYH9-related disorders	20 /14.2	0/0	101,9	136,3	161,0	99,1
# 4	MYH9-related disorders	97 /14.3	0/0	197,5	222,0	99,0	100,9
# 5	MYH9-related disorders	115 /14.4	0/0	48,8	114,3	66,5	77,7
# 6	MYH9-related disorders	15 /15	2/6	148,8	193,4	69,4	121,6
# 7	MYH9-related disorders	39 /15.8	1/6	109,4	164,8	83,9	60,5
# 8	MYH9-related disorders	18 /19.3	0/4	108,3	237,9	182,1	76,7
# 9	Bernard Soulier (homozygous *GP9* mutations)	64 /21.4	0/0	415,6	225,3	273,9	120,9
# 10	Bernard Soulier (homozygous *GP9* mutations)	44 /15.1	1/4	275,0	307,7	291,3	118,8
# 11	Monoallelic Bernard Soulier (heterozigous *GP1BA* mutation)	91/15.1	0/2	77,2	124,2	103,9	100,0
# 12	Wiskott Aldrich Syndrome	13 /7.7	4/16	70,3	42,5	29,4	14,2
# 13	Wiskott Aldrich Syndrome	11 /6.8	2/11	55,0	31,1	61,9	32,3

FIGURE 1 Clinical and laboratory characteristics of IT patients

Conclusions: ITs are very rare diseases and studies on platelet function have scantly been performed. Although preliminary results are shown here, the correlation between the clinical phenotype and the platelet activation pattern suggests interesting application of this method for the identification of patients at higher risk of bleeding.

PB 754 | Management of a Patient with Glanzmann Thrombasthenia during a Coronary Artery Bypass Graft Surgery: A Case Study

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Background: Glanzmann thrombasthenia (GT) due to mutations that affect the αIIbβ3 integrin. Platelets (PLT) have been suggested to play a role in the early development of atherosclerosis. As one test of this hypothesis that GT may have a protective effect from atherothrombosis.

Aims: We report a Coronary Artery Bypass Graft Surgery (CABG) and right Carotid endarterectomy (CEA) in a 57-year-old Tunisian man with GT type I disease (no platelet αIIbβ3 expression).

Methods: The patient showed typical signs of GT with no PLT aggregation in response to physiological agonists. He presented a low PLT count (80 G/l). GT was diagnosed at 5 years old; He suffered from repeated epistaxis and gingivorrhagia but never required transfusion. He received blood transfusions only for his circumcision at 11 years old. He presented recently unstable angina, atrial fibrillation and right carotid atherosclerosis which first treated by Aspirin. These lesions were deemed inaccessible to percutaneous therapy. Therefore, CABG and CEA were recommended.

Results: A multidisciplinary team was established to optimize perioperative management. The research of anti-HLA and anti-integrin αIIbβ3 allo-antibodies was negative.

Prophylactic HLA-matched PLT administration was continued before and through the immediate postoperative period and no bleeding complications occurred. PLT transfusion recovery was confirmed by the percentage of αIIbβ3 expression on PLT surface (expression of αIIbβ3 increased from 0 to 55%). The PLT function analysis by aggregation and function tests was not normalized.

The patient was discharged under a treatment of VKA in the reason of his atrial fibrillation chads2.

His screening tests for risk factors for thrombosis revealed presence of FII G20210A mutation.

Conclusions: This observation suggests that atherosclerosis can develop despite the lack of αIIbβ3 integrin and that cautious administration of aspirin or VKA is possible in patients with congenital hemostatic disorder in event of severe thrombotic complication.

PB 755 | Compound Heterozygosity for Mutations in ITGA2B Including a Novel p.Cys198Ser in Glanzmann Thrombasthenia

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Background: Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder of platelet aggregation caused by quantitative or qualitative deficiencies of the αIIbβ3. The proband was a 2-year-old Japanese boy, whose platelet count was 380,000/μL and Ivy bleeding time was prolonged for more than 20 min. The expression level of αIIbβ3 on his platelets was 5.2% of normal.

Aims: To elucidate the genetic basis of the type II GT, we analyzed *ITGA2B* and *ITGB3* for genomic DNAs from the patient and his parents.

Methods: We obtained genomic DNAs from peripheral blood leukocytes and analyzed *ITGA2B* and *ITGB3* by PCR-based direct sequencing for all exons including exon/intron boundaries. Transient expression of *ITGA2B* and *ITGB3* constructs in 293T cells followed by flow cytometry and Western blotting (WB) were also performed. The research was approved by the Institutional Review Boards.

Results: We identified a novel missense mutation in *ITGA2B* exon 5 (c.593G>C; p.C198S) and a previously reported mutation in exon 23 (c.2333A>C; p.Q778P). Since his farther had the former mutation and his mother had the latter one, the patient was expected to be a compound heterozygote for both mutations. No mutation was found in his *ITGB3*. In the expression experiment using 293T cells, the surface αIIbβ3 expression determined by flow cytometry was greatly reduced in the p.C198S compared to the wild type, and moderately reduced in the p.Q778P. This suggested that the p.C198S was a type I mutation, while the p.Q778P was a type II mutation. On the other hand, WB analysis showed that both β3s were reduced as compared with the wild type, and αIIb of the p.Q778P was reduced, but not of the p.C198S. Both *ITGA2B* mutants may affect assembly and stability of αIIbβ3 and lead to decreased surface expression, but further study is needed to clarify the precise molecular mechanisms disturbed surface expression of type II GT.

Conclusions: Genetic analysis of a type II GT patient revealed compound heterozygosity for novel and previously reported *ITGA2B* mutations.

PB 756 | Quantitative Evaluation of Bleeding due to Platelet Disorders Associated with Dense Granule Deficiency and/or Impaired Aggregation Responses

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Background: Inherited platelet disorders (IPD) that cause dense granule deficiency (DGD) and/or impaired platelet aggregation (IPA) responses are a heterogenous group of uncharacterized bleeding disorders whose bleeding problems have rarely been quantified, except by bleeding scores.

Aims: Our goal was to quantify bleeding risks for these IPD.

Methods: Bleeding histories were evaluated for subjects recruited to a study on IPD (n=32 with IPD from 8 families and 10 sporadic cases; 17 unaffected relatives from IPD families; 60 general population controls) using: (i) the International Society for Thrombosis and Haemostasis bleeding assessment tool (ISTH-BAT) to determine bleeding scores; and (ii) CHAT-P, a clinical history assessment tool (Haemophilia, in press) to gather information on bleeding and treatment responses and to estimate bleeding risks as odds ratio (OR) with 95% confidence intervals (CI) for IPD and control subjects.

Results: Subjects with IPD (n=28 IPA; n=6 DGD) had higher ISTH-BAT scores (median: 9, range: 0-18) than unaffected relatives (median: 1,

range: 0-6) and controls (median: 0, range: 0-6) (p< 0.01). Subjects with IPD had increased risks (OR, 95% CI, p value) for experiencing bleeding including: abnormal bruising (63, 16-241, p< 0.0001); prolonged nosebleeds (27, 7-108, p< 0.0001); excessive bleeding from injuries (14, 3-57, p< 0.0001) or surgery (17, 4-72, p< 0.0001); and menorrhagia (12, 3-53, p=0.0004). Subjects with IPD that had exposures to hemostatic challenges such as operations (27/32, 84.3%) had significantly higher bleeding scores (median: 11.5, range: 4-18) than those without (median: 8, range: 0-15) (p= 0.01) and a number of patients with IPD (6/14, 42%) had received treatments after diagnosis that were successful in preventing bleeding with surgeries and dental procedures.

Conclusions: IPD associated with DGD and/or IPA responses are mild bleeding disorders that significantly increase bleeding risks. ISTH-BAT scores for IPD are influenced by exposures to hemostatic challenges.

PB 757 | MYH9 Disorders Are the Most Common Cause of Macrothrombocytopenia in Australia: Importance of Mean Platelet Diameter Measurement and Döhle Body Detection for Improved Diagnosis

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Background: Inherited macrothrombocytopenias (IMT) are rare and often misdiagnosed as immune thrombocytopenia (ITP) leading to unnecessary treatments including splenectomy. Giant platelets are suggestive of IMT rather than ITP, but are not detected by automated cell counters, which can underestimate mean platelet volume (MPV).

Aims: MYH9 disorders are characterised by macrothrombocytopenia and leukocyte inclusions which are often missed on blood films. They may also cause nephropathy, sensorineural hearing loss or cataract, with a strong correlation between genotype and phenotype, hence the importance of correct diagnosis.

Methods: We used Next-Generation Sequencing (NGS) to investigate a large cohort (N=121) of patients with lifelong thrombocytopenia not previously diagnosed with IMT.

Results: Variants of the *MYH9* gene, which encodes the non-muscle myosin heavy chain IIA (NMMIIA) were by far the most common with 10 rare benign variants (N=14), 3 variants of uncertain significance (VUS) (N=4) and 7 pathogenic variants (N=15) identified. NMMIIA immunofluorescent (IF) staining on blood film is the current diagnostic gold standard but not widely available. MPV values for subjects with MYH9 variants were not consistently elevated. In contrast, mean platelet diameter (MPD) derived from scanned blood films was clearly increased in IMT compared to ITP. When available, the blood films of patients with pathogenic mutations were evaluated. All demonstrated macrothrombocytopenia; however, inclusion bodies were not easily appreciated. NMMIIA-IF was performed in 4 cases; all demonstrated abnormal NMMIIA clustering, including 2 cases with a VUS.

Conclusions: From our cohort, MYH9 disorders are the commonest cause of IMT. MPDs are markedly and consistently increased in MYH9 disorders and useful for differential diagnosis. As neutrophil inclusions are often missed on stained blood films, NMMIIA-IF is critical for diagnosis which may also include MYH9 genotyping.

PB 758 | Hemorrhagic Diathesis in Four Patients with the Heterozygous Pro258Thr Mutation of the P2RY12 Gene

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Background: The platelet ADP-receptor P2Y12 plays a crucial role in platelet activation and aggregation. Inherited defects of this receptor, caused by mutations in the P2RY12 gene, are classified as autosomal recessive disorders, implying that only homozygous mutations will affect the function of the P2Y12 receptor. However, a few patients with bleeding symptoms and defective aggregation in response to ADP have been reported to have heterozygous mutations.

Aims: To describe the phenotype and functional assays in four additional patients with a heterozygous mutation of P2RY12.

Methods: -

Results: Patients 1 and 2 are unrelated women, 22 and 32 years of age. Patients 3 and 4 are a father and daughter, age 62 and 30. Bleeding symptoms mainly include epistaxis, menorrhagia and prolonged bleeding after minor wounds. The platelet count and standard coagulation tests showed no abnormalities. All patients had an abnormal aggregation in response to ADP; patients 1,2 and 3 with light transmission aggregometry and patient 4 with flow cytometry. Platelet aggregation in response to collagen and arachidonic acid was normal. Genetic analysis identified a heterozygous missense mutation (Pro258Thr) in the P2RY12 gene in all patients.

Conclusions: The heterozygous Pro258Thr mutation in the P2RY12 gene results in bleeding symptoms and defects in ADP-activating signaling pathway by affecting the function of the ADP-receptor. It is

suggested that the Pro258 region of the P2Y12 molecule is important for receptor function and that the Pro258Thr mutation results in a dysfunctional variant of P2Y12 by altering the protein hydrophobicity, size and rotational mobility.

PB 759 | Nine-year Effective Management of an LAD III Patient with Conventional Pro-hemostatic and Immunological Treatment

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Background: Leukocyte adhesion deficiency type III (LAD III) is a severe recessive autosomal condition characterized by a leukocyte adhesion deficiency and a Glanzmann-like bleeding tendency caused by *FERMT3* variants. The outcome is typically poor, due to life threatening infections and bleedings. Hematopoietic stem cell transplantation (HSCT) is a pivotal treatment, albeit associated with high treatment-related mortality. In 2011, our team reported a 33-month-old LAD III boy carrying a novel c.310-2A>C *FERMT3* variant.

Aims: The purpose of the study was to discuss the therapeutic management in LAD III.

Methods: A retrospective analysis of the patient's medical record was performed (follow-up time 9 years). A systematic analysis of all other reported cases of LAD III was performed, focusing on complications and treatments.

Results: Regarding infections, the patient had probable Pneumocystis jiroveci pneumonia at 5 months of age, treated with wide-spectrum antibiotic therapy and cotrimoxazole. An immunoglobulin (Ig) G deficiency was diagnosed and supplemented every 3 weeks. He has received prophylactic cotrimoxazole and itraconazole until 3.5 years of age and then cotrimoxazole only. Several major bleedings including hematuria and post-traumatic tongue bleed, were treated with a combination of recombinant factor VIIa (off-label use), tranexamic acid and platelet transfusion. In the literature, platelet transfusion was the main reported treatment. Overall, the patient was treated with conventional pro-hemostatic and immunological treatment and has not undergone HSCT, which was performed in 59% of LAD III patients reported in the literature.

Conclusions: The therapeutic management of this LAD III patient, excluding HSCT, led to favorable outcome at 9 years of age. Such discrepancy with the literature as regards therapeutic and prognosis issues highlights the need for international registries and cohort studies to identify prognostic factors that could guide the therapeutic strategy.

PB 760 | Assessment of the Severity of the Epistaxis by Epistaxis Severity Score in the Inherited Platelet Disorders

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Background: A nosebleed is bleeding from the nasal cavity, it can be the revealing symptom of inherited platelet disorders.

Aims: We found during our clinical practice difficulties in the assessment and quantification of an episode of epistaxis, in order to adapt the therapeutic strategy.

Methods: We applied the ESS score validated for patients with hereditary hemorrhagic telangiectasia to the inherited platelet disorders.

Results: 60 patients with inherited platelet disorders (TG 34, 18JBS, 8MH).The average age of our population is (21, 33 ± 12,61) years, the youngest had 11 months old and the oldest was 52 years. The results of the .The average severity score of epistaxis is in the moderate zone for the GT (5,34 ± 2,33) and Jean Bernard Soulier disease (4,93 ± 2,58) and in the minor area for May-Hégglin disease (3,22 ± 3,27).15 patients or 44,1% in the GT have moderate epistaxis and 11 patients or 32,3% have severe epistaxis. In JBS disease 7 patients or 38,8% have minor epistaxis and 7 patients 38,8% had severe epistaxis. In May-Hegglin disease 3 (37,50%) patients did not have epistaxis, 2 (25%) patients had a minor epistaxis and 2 patients with moderate epistaxis. The comparison test did not find any significant differences in the 3 platelet disorders on the ESS score (p = 0,540).

Conclusions: The ESS score is a clinical tool that quantifies epistaxis during the inherited platelet defects and thus makes the objective medical information. We await further studies of inherited platelet disorders with the introduction of ESS score in order to better assess the severity of the disease.

PB 762 | A Study of Comparison of Platelet Aggregation Response Obtained on Sysmex CS-2000i with Chrono-log Light Transmission Aggregometer

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Background: Light transmission aggregometry (LTA) is considered as the gold standard for testing platelet function. However LTA is time consuming, technically challenging and affected by many pre analytical variables related to sample preparation and performed usually in

specialized laboratories only by experienced personnel who needs to be present till the end of testing. These challenges can be overcome by performing platelet aggregometry in an automated coagulometer that detects photo optical changes as the endpoint. We performed platelet aggregometry studies on carefully prepared platelet rich plasma (PRP).

Aims: To compare and correlate results of aggregometry obtained on automated coagulation analyser Sysmex CS-2000i (wavelength: 660 nm) with the current reference instrument Chrono-log light transmission aggregometer (wavelength: Infrared)

Methods: We performed platelet aggregometry studies on platelet rich plasma (PRP) of 5 patients which included 2 Glanzmann thrombasthenia (GT), 1 Glanzmann Thrombasthenia variant, 1 Bernard Soulier syndrome (BSS) and 1 patient with platelet function defect alongside 6 normal controls on both CS 2000i (Sysmex Corporation, Japan) and on chrono-log (Chrono-Log Corporation, USA). Concentrations of various agonists used in both platforms is shown (Table 1). Stirrer speed was 1200 rpm for Chrono-log and 800 rpm for Sysmex CS-2000i.

TABLE 1 Concentrations of various agonists on Sysmex and chrono-log

Agonist	Concentration in sysmex	Concentration in chrono-log
Ristocetin	1.2 mg/ml	1.5 mg/ml
ADP	4 µmol/L	10 µmol/ml
Epinephrine	5 µmol/L	10 µ mol/ml
Collagen	2 µgram/ml	2 µ gram/ml
Arachidonic acid	1 mmol/L	10 µ mol/ml

Results: The reference range for various agonists on Sysmex were as follows: Ristocetin (66.7% - 87.5), ADP (58.2%- 86.03%), Epinephrine (59.96%-86.04%), Collagen (67.3%-93.73%) and AA (62.86-93.46%). Platelet aggregometry results done on both platforms shown in Table 2.

Conclusions: This data suggests that automated platelet aggregometry in CS 2000i is reproducible and offers the advantage of walkaway automation saving on personnel.

PB 763 | Minor Dental Surgery in Patients with Glanzmann Thrombasthenia Patients

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Background: Glanzmann's Thrombasthenia (GT) is a rare inherited autosomal recessive disorder where bleeds are variable and can be treated with platelets transfusion, rVIIa and antifibrinolytics,

Aims: Report on efficacy of rVIIa use in three GT patients undergoing invasive dental procedures.

Methods: Platelet aggregation was measured by light transmission aggregometry (LTA). Platelet GP expression level was detected by flow cytometry using monoclonal antibodies anti GP IIb/IIIa (Beckman Counter); antiGPIb/IX/V (Alexis BioBiochemicals); anti GP IIIa (Dako).

Results: Case 1: A patient (44yrs) with GT recognized in childhood following prolonged bleeding. Tooth 36 extraction under cover of rFVIIa at 90µg/kg every 3 hrs, . 4 days bleeding was continuing despite of rFVIIa, acidum tranexamicum (At) and Spongostan anal seams (Sa). Teeth extraction within 2 yrs : rFVIIa was administered 2 h before procedure and then at two doses every 2 hrs, followed by 2 doses every 6 h. and 3 doses every 8 hrs. Complete hemostasis was restored. Case Case 2:GT patient (29yrs) with recurrent vitreous hemorrage, epistaxis and menorrhagia required RBC and PC transfusions. Seven multi-stage teeth extractions were performed. Before extraction (teeth 36 and 37) 2 units of PC were transfused and Sa applied. Bleeding subsided on day 2 after transfusion of 1 unit of PC. Before extraction of teeth 14 and 16 rFVIIa, At and Sa were administered. Two units of PC, 1 unit of RBC and rFVIIa were administered every 12 hours for 2 days because of following night bleeding from the socket. Before removal of teeth 46, 47 and 21 rFVIIa (every 12hrs,3 days), At and 2 units of PC were given. Bleeding subsided on the 3rd day.Case report 3: GT patient (40yrs) with bleeding history. Tooth 36 and 18

TABLE 2 Platelet aggregometry results on Sysmex and chrono-log (AA- Arachidonic acid, PFD- Platelet function defect)

Patients	Ristocetin (%)	ADP (%)	Epinephrine (%)	Collagen (%)	Arachidonic acid (%)
GT (Average of 2 patients)	Sysmex: 36 Chrono-log: 89	Sysmex: -19.8 Chrono-log: 1	Sysmex: -12 Chrono-log: 8	Sysmex: -13 Chrono-log: 5	Sysmex: -23 Chrono-log:5.5
BSS	Sysmex: 0.7 Chrono-log: 14	Sysmex: 24.2 Chrono-log: 20	Sysmex: 28.5 Chrono-log: 20	Sysmex: 31.8 Chrono-log: 15	Sysmex: 30.5 Chrono-log: 16
GT variant	Sysmex: 0.9 Chrono-log: 73	Sysmex: -11.2 Chrono-log:1	Sysmex: -3.8 Chrono-log: 3	Sysmex: 1.3 Chrono-log: 1	Sysmex: 1.4 Chrono-log: 37
PFD	Sysmex: 42.6 Chrono-log:115	Sysmex: 9.1 Chrono-log: 39	Sysmex: 9.2 Chrono-log: 18	Sysmex: 34.3 Chrono-log: 73	Sysmex: 62.5 Chrono-log: 76

extractions were made under cover of rFVIIa and Sa. rFVIIa was given 8-11 times every 2-3 hours and then every 4-6 hrs. for 3 days.

Conclusions: rFVIIa is an effective drug in the management of bleedings in patients with GT following teeth extractions.

PB 764 | Flow Cytometric Mepacrine Uptake Related to Platelet Size in Patients with Moderate Macrothrombocytopenia

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Background: Flow cytometric mepacrine uptake (MU) is used in diagnosis of dense granule (DG) disorders. MU has disadvantages as variable background due to retention of excess of mepacrine in platelet cytoplasm which will also depend on mean platelet volume (MPV). This makes it difficult to apply MU to measure DG content in macrothrombocytopenias (MTP).

Aims: To evaluate MU in patients with moderate MTP compared to a normal range related to platelet size.

Fig.1

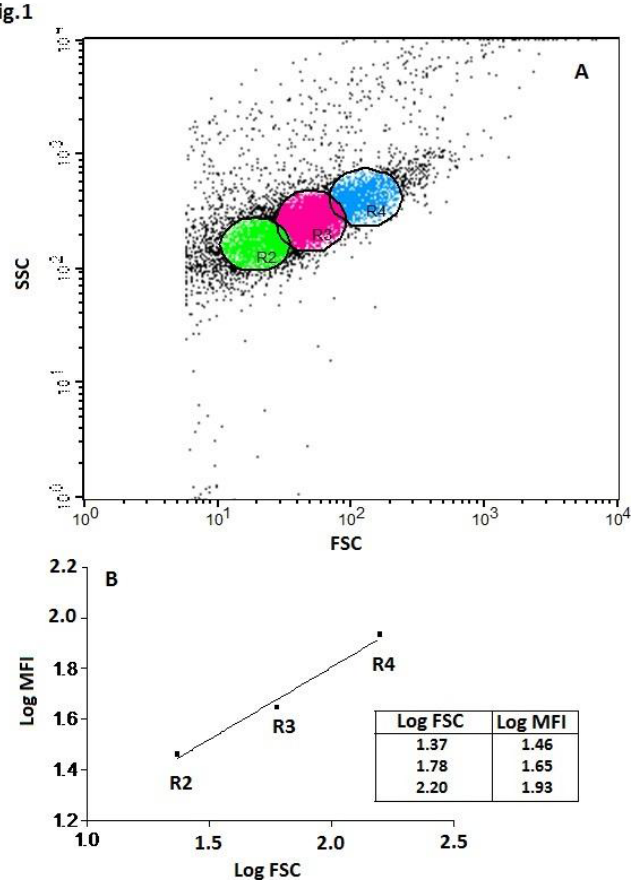

FIGURE 1 A: Dot plot SSC vs FSC and regions to obtain pairs of values MFI-FSH. B: linear regression MFI (FL1) vs FSH in one N.

Fig.2

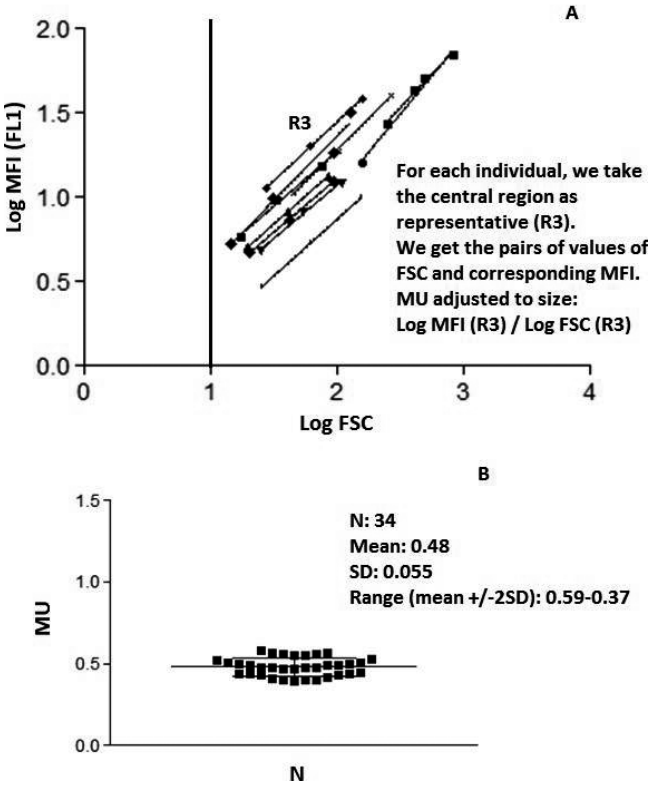

FIGURE 2 A: linear regressions . B: normal range

Methods: We evaluated three groups: subjects with normal ATP release (N,n=34) patients with decreased ATP release (SPD,n=4) and patients with MTP (n=5).Platelet-rich plasma (PRP) was adjusted to $300x10^9$ / L. The PRP range of the MTP patients was: $88\text{-}137x10^9$ /L. MPV was measured in all PRPs. ATP release with collagen 8.0 ug/uL by lumiaggregometry was taken as complete release(3.9-8.0 uM ATP). For MU, 25uL of PRP were incubated with 500uL of 5uM mepacrine in Tyrodes' buffer or Tyrodes' buffer as negative control,30 minutes at 37°C and diluted 1/80 in Isoflow.Means of frontal dispersion (FSC) and fluorescence intensity (MFI) were obtained and analyzed as shown in Fig.1-2.

Results: MPV of MTP vs N was significantly higher (13.5+/-0.41fL vs 8.6+/-1.5fL p=0.0027). Analyzed together the three groups data,VPM vs FSC correlated positively (r = 0.89 p < 0.0001). Each group member presented a positive linear relationship between MFI vs FSC on a logarithmic scale with similar slopes (Fig.2A).The normal range of MU was calculated dividing the logarithm of MFI with the logarithm of FSC in the N group (Fig.2B) (mean+/-2SD =0.48+/- 0.11). All SPD and only one MTP had decreased values (range 0.25-0.28 and 0.32 respectively).

Conclusions: MU in N, SPD and MTP showed a positive relationship between size and DG content with a similar rate of increased. This allowed us to calculate a normal range fitted to platelet size. This range was validated with SPD group. One of MTP had a decreased MU suggesting a concomitant reduction of DG content. More studies are necessary to confirm our results.

PB 765 | Circumcision in Children with Inherited Bleeding Disorders - A Tertiary Care Centre Experience in Saudi Arabia

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Background: Circumcision is one of the most common surgical procedure performed amongst male neonates. Apart from religious obligations, it is considered a vital ritual for a young adult to become a member of the society. It could pose serious hemorrhagic side effects amongst bleeders, which could be controlled with appropriate coagulation factor infusion, blood products, and antifibrinolytic agents ;pre and post circumcision.

Aims: To analyze outcomes of circumcision amongst children with inherited bleeding disorders , to determine extent of complications and provide guidelines for circumcision management.

Methods: We retrospectively reviewed medical records of pediatric patients (< 14 years) with inherited bleeding disorders, who underwent circumcision at our institute. IRB approved case report form was used to collect data and SPSS Version 20.0 was used for statistical analysis.

Results: A total of 33 patients with inherited bleeding disorders were identified, 49% (16) with Hemophilia, 30% (10) Glanzman Thrombasthenia,15% (5) Von Wille Brand disease and 6% (2) rare bleeding disorder, with a median age of 1 year (1.99 - 13.0 yrs) at the time of procedure. Twenty-two (67%) out of the 33 patients received appropriate factor replacement therapy (FRT) before and after the procedure,3 of whom had bleeding complications and of the eleven (33%) patients who did not receive FRT, 2 experienced bleeding. Overall incidence of bleeding complication was 15% (5/33).

Conclusions: Post-circumcision complication rate of 15% in our patients is comparable to published rates of 0.1 to 35% ,in patients without a bleeding disorder. In our experience circumcision for bleeders could be practiced under supervision of hematologist with adequate FRT, blood product support and local control by fibrin glue. However, parents and patients must be informed of bleeding risks despite taking adequate measures. National guidelines on use of haemostatic agents amongst bleeders for circumcision is required.

PB 766 | Patient Experiences with Hereditary Thrombotic Thrombocytopenic Purpura (hTTP): A Conceptual Framework of Patient-reported Outcomes (PROs)

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Background: Hereditary thrombotic thrombocytopenic purpura (hTTP) (severe ADAMTS13 deficiency) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and diverse clinical signs and symptoms. Existing patient-reported outcome (PRO) tools are limited in assessing the burden of hTTP. A better understanding of hTTP symptoms and impacts will help identify outcomes meaningful to patients for the purpose of developing a disease-specific tool.

Aims: The purpose of this study was to gain an in-depth understanding of the most salient symptoms and impacts of hTTP experienced by patients, in order to formulate a conceptual model of the burden of illness.

Methods: A literature review was conducted to construct a preliminary conceptual model of symptoms and impacts of hTTP. Interviews were conducted with 5 hematologists treating hTTP in the United States, United Kingdom, and Austria to revise the model. Concept elicitation interviews were then conducted with 11 patients in the United States (mean age, 38.2 years; range, 21-52 years). The clinician and patient interviews were used to refine the preliminary model, resulting in a final conceptual model of the most relevant symptoms and impacts that reflect the patient experience with hTTP.

Results: The most salient hTTP symptoms in the model were fatigue, pain (muscular and joints), cognitive impairment (forgetfulness, difficulty communicating), vision problems (temporary blindness, blind spots, blurred vision, worsening vision), headache, and bruising. The most salient effects of the disease on patients' lives were identified as ability to work/study, and emotional consequences (depression, anxiety, mood swings).

Conclusions: The final conceptual model indicates high disease burden and highlights areas of need within the current treatment paradigm. This conceptual model will inform the development of a PRO instrument that can be used to assess the outcomes of current and future therapies.

PB 767 | Application of ISTH - Bleeding Assessment Tools to Inherited Platelet Disorders

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Background: The inherited platelet disorders are a heterogeneous group of diseases responsible for bleeding manifestations at widely

TABLE 1 Comparison of bleeding ISTH -Bleeding Assessment Tools score (BAT), severity class

	T.G(34)	J.B.S.S(18)	M.H(8)	Total(60)	P
Average score	11,70±3,98	12,11±5,96	6,75±4,30		0,02
< 5	1 (2,9%)	0 (0,0%)	2 (25,0%)	3 (5,0%)	0,001
5-10	15 (44,1%)	8 (44,4%)	5 (62,5%)	28 (46,7%)	0,19
> 10	18 (52,9%)	10 (55,5%)	1 (12,5%)	29 (48,3%)	0,09

varying degrees. Despite the development of diagnostics means, we observed during our clinical practice deficiencies in assessment tools bleeding risk and quantification of bleeding symptoms.

Aims: Our goal is to provide a clinical scoring at targeted screening and prognosis.

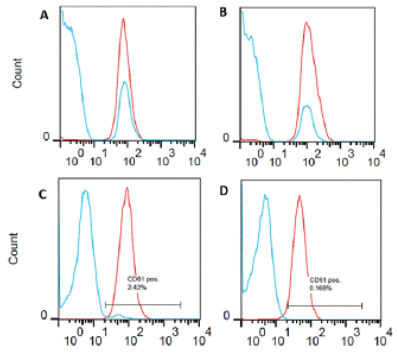
Methods: Application of clinical score ISTH-BAT for all adult patients and children with inherited platelet disorders (34 thrombasthenia glanzmann, 18 Jean Bernard Soulier diseases, 8 diseases of May-Hégglin).

Results: 60 patients with inherited platelet disorders (TG 34, 18JBS, 8MH), the average age of our population is (21,33 ± 12,61) years, the youngest was 11 months old and the oldest was 52 years.

In the TG, the average ISTH-BAT 11,70 ± 3,98, 12,11 ± 5,96 in the JBS, higher against the disease MH 6 , 75 ± 4.30, the comparison test found a significant difference (p = 0,02).

The sensitivity of the ISTH-BAT in the detection of thrombopathy is 97% in the Glanzmann thrombasthenia and 100% in the Jean-Bernard Soulier disease.

Conclusions: The ISTH-BAT bleeding score is tool for the screening of new patients and prognosis for patients with severe hemorrhagic symptoms, were expected to generalize in the coming years for all patients of the Algerian territory for its validation for inherited platelet disorders.



Flow cytometry post platelet transfusion, using antibodies directed against CD61-APC. Blue – patient. Red - normal control. A. Day of transfusion B. Two days post transfusion C. four days post transfusion D. A week post transfusion

FIGURE 1 Consecutive post transfusion flow cytometry

Results: A total of 3 female GT patients (ages 1, 19, 39 years) undergoing 4 surgeries were included. Surgical procedures were: diaphragmatic hernia repair with CVL insertion and extraction, cholecystectomy and salphingo-oophorectomy. Consecutive blood counts and FC analysis following platelet transfusions showed gradual decrease of donor platelets from 21%, 15%, 1%, correlating with 82,740, 49,200, 3,280 X 10^9/L active platelets respectively in one of the patients (figure 1), whereas in another patient one hour post transfusion FC demonstrated 3% donor platelets equivalent to 6,060 active platelets, both patients did not experience any bleeding. The third patient experienced post-surgical bleeding at day 8 after salphingo-oophorectomy, when FC demonstrated 1% of donor platelets correlating with 2600 active platelets.

Conclusions: Results suggest that very low number of active donor platelets (as revealed by FC) may suffice to achieve proper hemostasis. Our study points to the potential role of consecutive FC examinations to demonstrate the number of active platelets as an ancillary tool for decision making in GT patients undergoing surgery.

PB 768 | Post Platelet Transfusion Flow Cytometry Analysis as an Ancillary Test Guiding Treatment of Glanzmann Thrombastenia Patients Undergoing Surgical Procedures

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Background: Glanzmann thrombasthenia (GT) is a disorder of platelet function caused by mutations in the αIIbβ3 genes. Standard therapy includes platelet transfusions, yet GT patients may form antibodies against αIIbβ3 antigen or against MHC-class I, hampering efficacy of treatment.

Aims: We aimed to assess potential correlation between bleeding and number of active platelets in GT patients undergoing surgical procedures.

Methods: GT patients undergoing surgery interventions during study period were included. Sequential blood counts and flow cytometry analysis (FC) were performed as an ancillary test to estimate the effectiveness of platelet transfusions, given at investigator discretion.

PB 769 | Successful Management of Severe Bleeding with Combined Platelet Transfusion, rFVIIa and Tranexamic Acid Therapy in Glanzmann Thrombasthenia Patient

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Background: Glanzmann Thrombasthenia (GT) is a rare autosomal recessive bleeding disorder .The bleeding tendency in GT is variable. Standard treatments for GT comprise platelet concentrate transfusions (PCT), rFVIIa and antifibrinolytic agents.

Aims: Report of successful therapy with rVIIa in a 48 year old female with GT diagnosed at the age of 2.

Methods: Case report: The patient experienced 10 severe bleeding episodes (menorrhoea, epistaxis, gingivorrhoea, gastrorrhagia) and was treated with PCT and rVIIa. She was admitted to IHTM in a severe condition with abdominal pain, nausea, vomiting blood and stomach contents. Lab tests revealed anaemia and hyperbilirubinaemia. No alloimmunization to human leukocyte antigens (HLA) and/or platelet membrane GPIIb/IIIa was determined. Gastroduodenoscopy and laparoscopic cholangiography by duodenal route were performed under haemostatic cover of platelet transfusions. The diagnosis: reflux oesophagitis, haemorrhagic gastritis, severe gastric ulcer and Vater's papilla bleeding, major duodenal papillis and cholecystolithiasis.

Results: Treatment consisted in platelet (6 units) and RBC (18 units) transfusions as well as rFVIIa (total dose of 42mg) and tranexamic acid administrations and several gastric coagulations. Applied therapy allowed to control the bleeding. The patient recovered and cholecystectomy was successfully performed 10 months later. Isoantibodies against GP IIb-IIIa were then detected and 9 severe bleeding episodes followed at 2-3 month intervals (gastrorrhagia and Vater's papilla bleeding, Vater's papilla bleeding, transverse colon hemorrhage). Successful therapy was administered with argon coagulation-9 procedures, HLA compatible platelet transfusions (47units), transfusions of RBC (38 units) and tranexemic acid.

Conclusions: Management of severe bleeding in GT proved successful in our case owing to combined use of rFVIIa, tranexamic acid and HLA-compatible platelet transfusion as well as and RBC transfusions supported by argon coagulations and cholecystectomy.

PB 770 | Immature Platelets Fraction and its Application in the Differential Diagnosis of Congenital and Acquired Trombocytopenia

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Background: The immature platelets fraction (IPF) quantifies the platelet population with higher RNA contente, reflecting medullary response.

Congenital thrombocytopenia is a heterogeneous group of entities that are difficult to recognize. Late recognition and absence of family history may lead to misdiagnosis of acquired thrombocytopenia, in particular immune thrombocytopenic purpura (ITP). Differential diagnosis between this condition (ITP) and congenital trombocytopenia may be clinically difficult, requiring experience in the evaluation of platelet morphology and further studies with specific tests, such as aggregometry, flow cytometry and genetic studies. These tests are unavailable in most clinical laboratories. In this context, determination of IPF may offer an advantageous and reproducible alternative.

Aims: IPF clinical application in differencial diagnosis of thrombocytopenia

Methods: Prospective study included 27 patients diagnosed with ITP, 11 patients with MYH9-associated macrothrombocytopenia (MYH9MT), 5 patients with Bernard Soulier´s syndrome, 22 patients with other congenital macrotrombocytopenia, and 50 healthy individuals. Whole-blood automated platelet parameters were measured on the Sysmex XE-5000 hematological analyzer.

Results: IPF was significantly increased in all groups compared to the control group. The highest values were observed in the MYH9MT group, followed by Bernard Soulier syndrome, other congenital macrotrombocytopenias, and ITP groups, respectively. All differences observed among groups were statistically significant ($p < 0.001$).

Conclusions: The fact that pacientes with congenital trombocytopenia had higher IPF values than patients with ITP may provide the evidence to pursue further evaluation for congenital thrombocytopenia. However IPF parameter is not completely discriminative of this condition.

In conclusion, our results support IPF as an automated, fast-performing and low cost parameter, useful for the differential diagnosis of congenital and acquired thrombocytopenia.

PB 771 | A Case of Acquired, Transient Bleeding Diathesis Associated with Platelet Dysfunction

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Background: Delta-storage pool deficiency (dSPD) is a congenital platelet function disorder that is associated with decreased number and/or contents of platelet delta-granules. Cases of acquired dSPD have been described in association with some conditions.

Aims: We report a case of a 14-year old girl, referred to us in October 2014 for moderate mucocutaneous bleedings, which manifested for the first time 6 months before, in the absence of any clinically overt disorders. At the time of her referral, her bleeding score (ISTH/SSC) was 6 and physical inspection revealed no abnormalities. Two weeks before the appearance of bleeding manifestations, a mycoplasma pneumonia infection was diagnosed, which was successfully treated with Clarithromycin.

Methods: We measured: platelet aggregation and secretion (lumiaggregometry); the platelet content of serotonin (spectrofluorometry), ATP and ADP (luminometry); platelet membrane glycoproteins (GP) and phosphorylation of vasodilator-stimulated phosphoprotein (VASP-P) (flow cytometry); serum thromboxane B2 (TXB2) (ELISA).

Results: Blood cells count and morphology, von Willebrand factor antigen and ristocetin cofactor activity, GP and VASP-P were normal. Platelet aggregation and ATP release induced by ADP, epinephrine, thrombin receptor activating peptide, and collagen were impaired. Serotonin and ADP content, and serum TxB2 were reduced. Similar results were obtained 4 months later (February 2015). On February 2016 the bleeding manifestations had disappeared spontaneously, and platelet function were normal.

	Oct 2014	Feb 2015	Feb 2016	normal range
Platelet 5HT (nmol/10^8plts)	0,14	0,15	0,68	0,21-0,67
Platelet ADP (nmol/10^8plts)	1,1	1,76	3,29	1,99-4,67
Platelet ATP (nmol/10^8plts)	6,5	7,62	8,02	5,29-11,34
ATP/ADP	6	4,32	2,43	1,30-3,29
serum TXB2 (pmol/10^8plts)	38	38	127	63-472

FIGURE

Conclusions: To the best of our knowledge, this is the first case of acquired, transient platelet dysfunction associated with delta-granules defect and defective thromboxane production, with apparently spontaneous onset and offset. Whether the mycoplasma pneumonia infection of the patient at the time of first bleeding manifestations had any pathogenic role is uncertain, and deserves further investigation.

PB 1557 | Twice-daily Low-dose Aspirin Improves Platelet Inhibition in Patients with Essential Thrombocytosis

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Background: Insufficient platelet inhibition has been reported in up to 40% of aspirin-treated patients, including patients with essential thrombocytosis. In order to maintain sufficient platelet inhibition, a shorter dosing interval with aspirin has been suggested.

Aims: To investigate the antiplatelet effect of low-dose aspirin given twice-daily compared to standard once-daily dosing in patients with essential thrombocytosis.

Methods: We included 22 patients with essential thrombocytosis. Patients were treated for 7 days with standard once-daily aspirin (75 mg once-daily) followed by 7 days treatment of twice-daily aspirin (37.5 mg twice-daily). The two regimens were separated by 14 days aspirin washout. Blood samples were obtained 1h and 24h after the last pill intake in each regimen. The effect of aspirin was evaluated by platelet aggregation and serum thromboxane B_2 levels. Platelet aggregation was measured by whole blood impedance aggregometry (Multiplate® analyzer, agonist: arachidonic acid (ASPItest 0.5 mM)). Serum thromboxane B_2 levels were determined using an enzyme-linked immunosorbent assay.

Written informed consent was obtained from all participants. The Dansih Ethics Committee and Medical Agency approved the study.

Results: Platelet aggregation at the end of the dosing interval (24h) was significantly higher than at 1h during both regimens. The difference in platelet aggregation from 1h to 24h was used to compare the two regimens. We demonstrated a significantly lower difference in platelet aggregation in the twice-daily regimen compared to the once-daily: mean of difference = 227 AU*min (95% CI: 92 to 363, p< 0.01).

In addition, a significantly lower difference in thromboxane B_2 was demonstrated in the twice-daily regimen compared to the once-daily regimen: mean of difference = 10.3 ng/mL (95% CI: 6.3 to 14.3, p< 0.01).

Conclusions: Twice-daily dosing with low-dose aspirin provides a more consistent platelet inhibition compared with standard once-daily dosing in patients with essential thrombocytosis.

PB 1558 | Long-term Aspirin (ASA) Use Significantly Attenuates Platelet Interactions with von Willebrand Factor (VWF) in Acute Coronary Syndromes (ACS)

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Background: In ACS platelets tether to VWF via the glycoprotein (GP)Ib receptor and initiate a complex signalling cascade, ultimately activating the integrin $\alpha_{IIb}\beta_3$ receptor that crosslinks fibrinogen and causes platelet arrest. Platelet adhesion via the GPIb receptor is rapidly reversible, shear dependent, and includes characteristic start-stop translocation of platelets. Long-term ASA use (≥ 5 years) is associated with a reduction in the incidence of cancer and cardiovascular events.

Aims: It is not clear if prior use of ASA is beneficial in patients with ACS. We hypothesise that platelet function will differ in ACS patients on prior long-term aspirin compared to those recently started on ASA.

Methods: We have developed a microfluidic assay utilizing video microscopy to accurately measure dynamic platelet behaviour in microliters of blood perfused across VWF at arterial shear rates (1500 s^{-1}), termed the DPFA. Tracking multiple individual platelets from frame to frame with unique motion-analysis software, the assay measures the total number of platelets 1) interacting with VWF, 2) translocating across VWF, and 3) stably adhering to the surface, as well as 4) the speed and distance platelets travel across VWF.

Results: We recruited 67 patients with ACS. Standard light transmission aggregometry demonstrated that all patients had a uniform response to aspirin. The duration of ASA therapy ranged from 1 day to 395 months. Platelets from patients on ASA therapy for greater than 5 years travelled a greater distance at higher velocities (P≤0.04 and 0.03 respectively, 1-way ANOVA) compared to those of patients newly prescribed ASA therapy. Quintile analysis based on length of treatment with ASA demonstrated that long-term ASA use reduced the number of platelets adhered to the surface, with a significant decrease in platelets that translocate and then form stable bonds to VWF (ANOVA, p≤0.01).

Conclusions: We demonstrate for the first time that prior long-term use of ASA significantly attenuates platelet interaction with VWF.

PB 1559 | Patients with Coronary Artery Disease Have a Pathological P2Y12 Platelet Response with Heightened Procoagulant Platelet Potential

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Background: Procoagulant platelets (PP) play a critical role in thrombin generation. We previously demonstrated that patients with coronary artery disease (CAD) have a heightened PP response to thrombin and thrombin/collagen, the mechanism of which is unknown.

Aims: To determine if the $P2Y_{12}$ pathway is involved in the heightened PP response to thrombin in patients with CAD.

Methods: We adapted a flow cytometry assay based on cell death marker, GSAO (PMID 26474813), to assay PP formation ex vivo in whole blood. Blood from healthy controls and patients undergoing elective coronary angiography was treated with thrombin ± collagen and assayed by FACS. PP subset was defined as GSAO+/CD62P+, and expressed as % all platelets.

Results: The enhanced PP response associated with CAD was not correlated with changes in platelet aggregation, dense granule release and activation of α2bβ3 integrin, suggesting independence of activation and procoagulant function. CAD patients demonstrated a significantly increased PP response to 5μM ADP compared with healthy controls (7.5±1.0% vs 2.7± 0.9%, P< 0.05), which was attenuated in patients on DAPT (7.5 ± 1.0% vs 4.1 ± 0.5%, P < 0.01) (Fig. 1). The heightened response to thrombin or combination of thrombin + collagen was not supressed by aspirin but showed partial attenuation in patients on DAPT. Patients on the weaker $P2Y_{12}$ inhibitor clopidogrel, showed no difference in response to thrombin + collagen, compared with patients on no $P2Y_{12}$ inhibitors ($P = 0.99$). In contrast, patients on prasugrel or ticagrelor demonstrated marked reduction in thrombin + collagen response relative to clopidogrel treated patients (27.2 ± 4.5% vs 12.0 ± 1.8%, n = 8-22, $P < 0.05$) or those on no $P2Y_{12}$ inhibitors (27.6 ± 3.1 vs 12.0 ± 1.8%, $P < 0.01$) (Fig. 2).

Conclusions: Our data suggests activation of the $P2Y_{12}$ pathway leads to pathological PP response in patients with CAD in response to either ADP, or thrombin + collagen, with a suppression of the thrombin + collagen response seen with strong $P2Y_{12}$ antagonists.

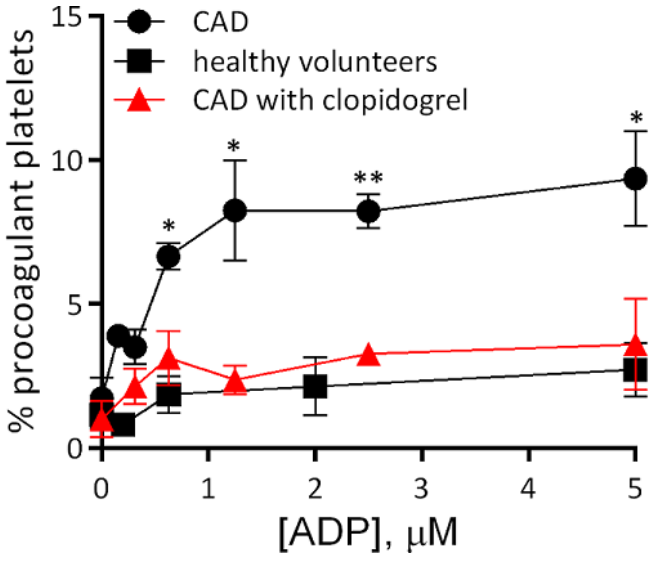

FIGURE 1 Clopidogrel partially attenuates a heightened procoagulant platelet response to ADP observed in CAD

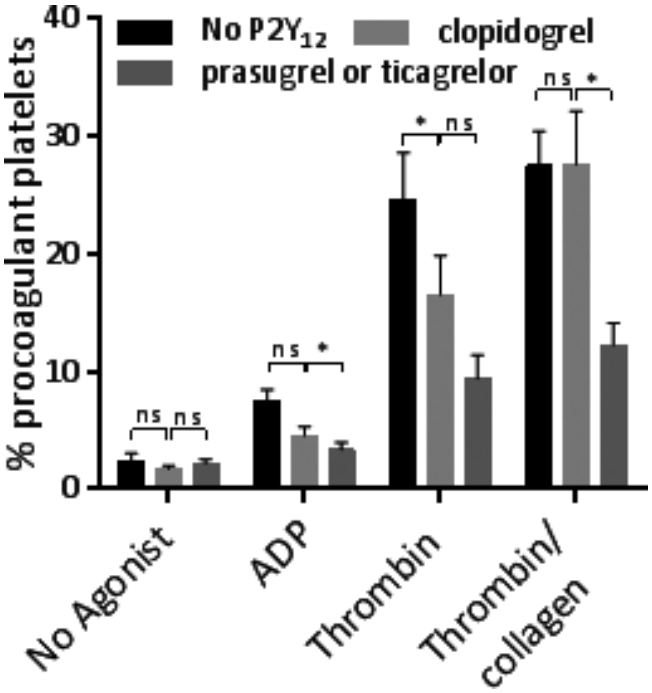

FIGURE 2 Differential suppression of agonist-induced procoagulant platelets by P2Y12 inhibitors

PB 1560 | Platelet Inhibition with Ticagrelor Versus Clopidogrel in Patients with Peripheral Artery Disease: The EUCLID Platelet Substudy

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Background: Patients with peripheral artery disease (PAD) are at increased risk of atherothrombotic events and benefit from platelet inhibiting therapies. The EUCLID trial demonstrated no significant benefit from ticagrelor versus clopidogrel for the reduction of cardiovascular or limb events in patients with PAD.

Aims: The EUCLID platelet substudy aimed to compare the antiplatelet effects of ticagrelor and clopidogrel in patients with PAD.

Methods: Patients were randomized to receive either clopidogrel (75 mg daily) or ticagrelor (90 mg twice daily). The effect of study drug was studied in 75 patients during maintenance therapy (≥6-weeks post randomization). In 42 patients, platelet inhibition was measured at baseline and after 2- and 6-weeks of study drug (≈12 hrs after last dose). In all patients, pharmacodynamic assessments were made using light transmission aggregometry (in response to ADP 5 and 20uM, collagen 1ug/ml and arachidonic acid [AA] 150uM), VerifyNow P2Y12, and VASP phosphorylation.

Results: During maintenance therapy, ticagrelor achieved lower platelet activity of ADP-induced platelet aggregation than clopidogrel (maximum platelet aggregation to ADP 5uM, 39% vs. 60.5%, P< 0.001; ADP 20uM 51% vs. 67.5%, P=0.004; VerifyNow, 74 vs. 165 PRU, P< 0.001; VASP 16.9 vs. 45.8 PRI, P< 0.001). High on-treatment platelet reactivity was observed more frequently in the clopidogrel treated group (P< 0.05 for each ADP-mediated assay). The effect at 14-days and 6-weeks was consistent with a greater platelet inhibition with ticagrelor compared with clopidogrel. The inhibition of collagen- or AA-induced platelet aggregation was not different between groups.

Conclusions: In patients with PAD, ticagrelor achieved greater inhibition of ADP mediated platelet activity versus clopidogrel. The inhibition of non-ADP mediated platelet activity was not different between groups. Despite greater platelet inhibition, ticagrelor was not superior to clopidogrel for the reduction of cardiovascular events in patients with PAD.

PB 1561 | Antiplatelet Agents are the Key Drugs for the Prevention of Recurrent Arterial Thrombosis in Patients with Antiphospholipid Syndrome

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Background: Antiphospholipid syndrome (APS), one of the most common acquired thrombophilia, is a systemic autoimmune disease characterized by the presence of arterial and/or venous thrombosis. However, the optimal prophylaxis of recurrent arterial thrombosis for APS patients is controversial.

Aims: The objective of this study is to evaluate the efficacy of several prophylactic treatments for recurrent arterial thrombosis in patients with APS.

Methods: This study involved a cohort of 206 patients with APS who visited Hokkaido University Hospital rheumatology clinic from April 1990 to March 2016. Patients who were followed-up for less than 2 years and those without arterial thrombosis were excluded. A total of 90 APS patients (female 73, age 43 years (9-79)) were subsequently analysed. We retrospectively assessed the efficacy of warfarin monotherapy (wf, n=13), antiplatelet monotherapy (AP, n=41), combination therapy of warfarin and antiplatelet agent (wf+AP, n=21), and dual antiplatelet therapy (DAPT, n=15) in the secondary prevention of arterial thrombosis in patients with APS.

Results: The median follow-up period was 12 years (2-27). Thrombotic events recurred in 40 (44.4%), patients (wf, AP, wf+AP, DAPT: 11, 18, 8, 3). A total of 14 (15.6%) patients died, (wf, AP, wf+AP, DAPT: 1, 5, 4, 4), and 9 (10.0%) patients had serious bleeding events, (wf, AP, wf+AP, DAPT: 0, 5, 2, 2). In Kaplan-Meier analysis, 10-years recurrence-free survival rate was 62%, and the treatment with warfarin monotherapy was less effective than other treatment options (Log-rank p=0.001). There were no statistically significant differences in bleeding events and mortality among the groups.

Conclusions: Antiplatelet agents are the key drugs for the prevention of recurrent arterial thrombosis in patients with APS.

PB 1562 | Cyclic Nucleotide Modulators and P2Y12 Antagonists as Novel Anti-platelet Combination Therapy

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Background: Cyclic nucleotides are intrinsic to maintaining platelet quiescence and a synergistic relationship exists between cyclic nucleotides and $P2Y_{12}$ inhibition. Drugs that modulate soluble guanylate cyclase or those that inhibit phosphodiesterase (PDE), targeting cyclic nucleotide generation and degradation respectively, are approved treatments for pulmonary hypertension.

Aims: Determine anti-thrombotic efficacy of cyclic nucleotide modulators in combination with $P2Y_{12}$ inhibitors.

Methods: Mice were administered (i.v) sub-optimal doses of prasugrel (0.3mg/kg), cinaciguat (0.3mg/kg) + dipyridamole (2.0mg/kg), or a combination of all three (PCD). Whole blood aggregometery was performed using as agonists arachidonic acid, collagen, PAR-4 amide, or thromboxane mimetic U46619. Alternatively mice underwent FeCl-induced thrombosis and carotid artery blood flow was monitored.

Results: Blood from mice treated with PCD demonstrated the lowest aggregation response to each agonist. *In vivo* thrombotic response was not altered in mice treated with either prasugrel or cinaciguat+dipyridamole. Complete and stable occlusion occurred within 8 minutes in all groups, including vehicle. In contrast, PCD strongly inhibited the thrombotic response with time to occlusion >24 minutes.

Conclusions: Our studies demonstrate that cinaciguat and dipyridamole when combined with prasugrel produce strong anti-platelet effects. We therefore provide proof of concept that modulation of cyclic nucleotides in combination with $P2Y_{12}$ inhibition powerfully combine as a potentially novel therapeutic regimen. This combination, achievable using approved drugs, should allow for timely translation to clinical investigation. Moreover, by taking advantage of intrinsic physiological synergism, such an approach may be effective using lower doses of individual drugs than are currently prescribed.

PB 1563 | Prasugrel vs. Ticagrelor after STEMI: Results from the Düsseldorf STEMI Registry

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Background: P2Y12 inhibition is indispensable after coronary intervention. By now prasugrel and ticagrelor are recommended in patients with ST-elevation myocardial infarction (STEMI) in secondary prevention of ischemic events. Prasugrel is a thienopyridine. It is more potent in platelet inhibition than clopidogrel, as its metabolization to the active substance is less complex. Ticagrelor is a cyclopentyltriazolpyrimidine. As active metabolite, it does not require metabolization. In contrast to thienopyridines, it inhibits the P2Y12 receptor reversibly. Both agents are superior to clopidogrel in secondary prevention.

Aims: In this registry analysis we compared ticagrelor with prasugrel P2Y12 inhibition in STEMI patients.

Methods: We included 318 patients during hospitalization and in a 12 months follow-up during ambulatory care at our department. Patients received dual antiplatelet therapy with aspirin and ticagrelor (Ti) or prasugrel (Pr) during follow-up period. Major adverse cardiac and cerebrovascular events (MACCE) (death, myocardial infarction, stroke, unplanned reintervention) and thrombolysis in myocardial infarction (TIMI) bleeding events (major/minor) were registered.

Results: Ti 35 vs. Pr: 24 MACCE (Hazard Ratio 1.24, 95% confidence interval [CI] 0.79-2.09; log-rank p-value = 0.41). TIMI major bleedings: Ti-group: 6 vs. Pr-group: 1 (HR 3.87, 95%CI 0.87-17.3). TIMI minor bleedings: Ti: 6 vs. Pr: 1 (HR 3.75, 95%CI 0.85-16.7). Weight (Ti: 84±17 kg vs. Pr: 84±20; p=0.8), peak-troponin (Ti: 4383±6191 ng/l vs. Pr: 5615±10748; p=0.2), peak-creatine kinase (Ti: 1740±2317 U/l vs. Pr: 1928±2251; p=0.5), Age: (Ti: 63±12 vs. Pr: 57±10 years; p< 0.0001).

Conclusions: In this register analysis no difference in 12 months follow-up existed between ticagrelor and prasugrel treated patients. Interestingly, prasugrel treated patients were significantly younger. Meta-analysis and larger registry analyses are needed to assess the optimal P2Y12 inhibition in STEMI patients.

PB 1564 | Acetazolamide and Methazolamide Suppress Platelet Procoagulant Responses and Thrombosis in vivo

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Background: The carbonic anhydrase (CA) inhibitors acetazolamide (ACZ) and methazolamide (MTZ) have been used extensively clinically as weak diuretic agents, with applications in the treatment of glaucoma and altitude sickness in particular. Recently, we showed acetazolamide to be potently antithrombotic in a mouse model of arterial thrombosis.

Aims: To investigate the effects of ACZ and MTZ on *in vitro* and *in vivo* procoagulant responses of activated human platelets.

Methods: Biochemical assays and immunocytochemistry, super-resolution microscopy and 4D live-platelet imaging, thromboelastometry and $FeCl_3$ model of arterial thrombosis.

Results: We confirmed the expression of CA1, 2 and 13 isoenzymes in human platelets and determined that while CA1 and 13 were essentially cytosolic, CA2 co-localised significantly with P-selectin expressing granules. Furthermore, CA activity was blocked by 100 μM ACZ or MTZ. However, ATP secretion or aggregation in response to thrombin 0.2 U/ml or collagen related peptide 5 μg/ml were comparable in ACZ- or MTZ-treated to untreated platelets. In contrast, pre-incubation of platelets with 100 μM ACZ or MTZ attenuated intracellular chloride ion entry and membrane phosphatidylserine exposure. This was associated with diminished membrane ballooning, procoagulant-spreading and microvesiculation in platelets adherent to fibrillar collagen. Bolus pre-treatment of whole human blood with 50 μM ACZ or MTZ, in the presence of 30 μM aspirin, significantly attenuated clot formation, as assessed by thromboelastometry. Finally, *in vivo* thrombus formation

was suppressed after $FeCl_3$ injury in mice administered 7 or 10mg/kg single dose ACZ or MTZ, respectively.

Conclusions: Acetazolamide and methazolamide suppress platelet procoagulant responses and *in vivo* thrombosis. These CA inhibitors may now be evaluated for a new indication as antiprocoagulant antithrombotics in translational research.

PB 1565 | Effect of Aspirin Therapy on Abacavir-associated Platelet Hyperreactivity in HIV-infected Patients: A Randomized, Double-blind, Placebo-controlled, Cross-over Study

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Background: Ischemic cardiovascular events are a relevant cause of morbidity and mortality in HIV-infected patients. Use of abacavir (ABC), a nucleoside analog reverse transcriptase inhibitor, has been associated with increased risk of myocardial infarction (MI). ABC induces platelet hyperreactivity that may contribute to enhance MI risk.

Aims: To explore whether low-dose aspirin reduces in vivo platelet activation and platelet hyperreactivity associated with ABC use in HIV-infected subjects.

Methods: In a randomized, placebo-controlled, double-blind cross-over study forty ABC-treated HIV-infected patients with platelet hyperreactivity were randomized to aspirin 100 mg daily for 15 days with subsequent cross-over to placebo for additional 15 days or placebo for 15 days with subsequent cross-over to aspirin for further 15 days. In vivo platelet activation and ex vivo platelet hyperreactivity were measured at day 15 and 30. One group of healthy subjects was studied concomitantly.

Platelet hyperreactivity was defined by a score based on laboratory variables reflecting in vivo platelet activation and ex vivo platelet hyperresponsiveness.

Results: Aspirin therapy reduced significantly platelet hyperreactivity (from 9.3±0.3 to 7.5±0.2, $p< 0.05$), however without bringing it back to the levels of healthy controls (4.2±0.4). Circulating platelet microparticles were reduced by aspirin treatment, although they remained higher than in healthy subjects while s-Pselectin and sCD40L remained elevated, showing persistence of in vivo platelet activation. Suppression of serum TxB_2 and urinary 11-dehydro-TxB_2 excretion confirmed compliance to therapy.

Conclusions: Aspirin reduces in vivo platelet activation and platelet hyperreactivity associated with ABC-treatment in HIV-infected patients, however without normalizing them. Whether the observed reduction of platelet activation is sufficient to prevent cardiovascular events requires a prospective large scale trial.

PB 1566 | Investigation of the Physiological Mechanisms Leading to the Release of BDNF by Platelets and their Susceptibility to Antiplatelet Agents

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Background: A large extra-cerebral pool of Brain-derived neurotrophic factor (BDNF) is found in platelets. With concentrations reaching 100-1000 fold those of neurons, platelet-stored BDNF is hypothesized to play an important role in the vasculature.

Aims: To investigate the platelet mechanisms that regulate circulating levels of BDNF in healthy volunteers and patients suffering from coronary artery disease (CAD) on dual antiplatelet therapy.

Methods: Platelet aggregation was studied in response to arachidonic acid, ADP, collagen, epinephrine and TRAP. Antiplatelet agents were added in vitro for samples obtained from healthy volunteers (aspirin, P2Y12 inhibitor AR-C66096, and GPIIbIIIa inhibitor abciximab) and in vivo for samples obtained from CAD patients.

Results: BDNF was abundant in platelets within cytoplasmic granules. Exogenous BDNF did not induce platelet aggregation, either on its own or in synergy with other agonists. All agonists induced the release of platelet BDNF. The ADP signalling pathway was an important regulator as in vitro inhibition of the P2Y12 receptor systematically reduced the release of BDNF. Inhibition of the GPIIbIIIa receptor abolished the release of platelet BDNF, thereby confirming the role of platelet aggregation in the regulation of peripheral BDNF. Baseline BDNF levels in CAD patients on dual antiplatelet therapy were similar to that of healthy volunteers. The increase in the release of platelet BDNF in response to all agonists was less important in CAD patients in comparison with healthy volunteers (Fig).

Conclusions: Platelet aggregation is required for BDNF release by platelets. The use of antiplatelet drugs, especially a P2Y12 receptor inhibitor, decreases the quantity of BDNF released by platelets. Further studies are needed to assess the clinical importance of modulating BDNF release in CAD patients, especially on cognitive function in this high-risk population for vascular cognitive impairment.

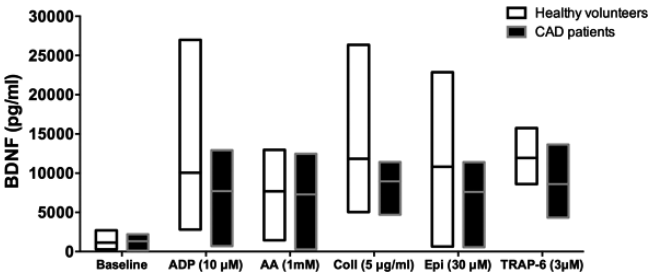

FIGURE 1 BDNF release in response to agonists in healthy volunteers and patients suffering from coronary artery disease (CAD)

PB 1567 | Clopidogrel Efficiency in Cerebral Aneurysm Stenting: Are Biological Tests Useful?

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Background: Pretreatment by clopidogrel and aspirin is accepted as a standard step in cerebral aneurysm stenting in order to decrease thrombotic complications. Current pre-procedure anti-aggregant therapy protocols have been adopted from interventional cardiology. However no guidelines are clearly defined in this setting. Correlation between clopidogrel biological resistance and thrombotic risk in intracranial stent implantation is not clearly established.

Aims: To determine if the threshold of different tests evaluating biological pre-procedure clopidogrel resistance used in cardiology could be applied to cerebral aneurysm stenting to detect thrombotic risk.

Methods: We prospectively studied 111 patients scheduled for aneurysm stenting by flow diverter because of lateral aneurysm (n=78) or bifurcation (n=33). All of them were treated by clopidogrel 75 mg/day at least 5 days before the procedure. Clopidogrel resistance was evaluated by ADP (5 mM)-induced light transmitted aggregation (maximal aggregation >50% and late aggregation after 6 min >14%), VASP-Phosphorylation by flow cytometry (VASP-P >50%), VerifyNow^{å} P2Y12 (PRU>240) and shear-induced platelet aggregation (SIPA) at 4000 sec-1 >40%.

Results: A thrombotic event was reported in 12 patients (10.8%). 2 of them occurred during the procedure and the other 10 post-procedure. Biological resistance to at least one the 5 parameters tested was observed in 59.8% of the patients. Performance of the tests was evaluated. Poor sensitivity was observed: 8.3% for ADP late aggregation and SIPA and 66.7% for VASP-P. Specificity of the tests varied between 48% for VASP-P and 86.7% for VerifyNowP2Y12.

Conclusions: Biological tests evaluating pre-procedure clopidogrel efficiency in intracranial flow-diverting stent implantation are not able to predict thrombotic complications. The location and the size of the aneurysm together with procedure complexity are probably more relevant to predict thrombotic complications.

PB 1568 | Comparison of Platelet Function Tests in a Trial of Different Aspirin Dose Regimens in Type 2 Diabetes

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Background: Aspirin (ASA) efficacy is reduced in type 2 diabetes (T2D) but the best method of assessment is uncertain.

Aims: We compared six platelet function tests for the assessment of ASA responsiveness.

Methods: We examined 3 ASA dosing regimens in 24 T2D patients randomized to 2-week treatment periods in a crossover design with ASA 100mg/day, 200mg/day or 100mg twice daily. Platelet function after each regimen was compared using kappa statistics when measured with: Light transmission aggregometry (LTA-0.5mg/ml of arachidonic acid (AA) and 10µM ADP); Multiplate whole blood aggregometry (WBA-0.5mM AA and 6.5µM ADP); PFA-100-CADP and CEPI; VerifyNow ASA; Urinary 11-dehydro-thromboxane B_2 (TxB_2); Serum TxB_2. All COX-1 dependent tests and some COX-1 independent tests (PFA-CEPI, LTA-ADP) demonstrated significant reductions in platelet reactivity with all ASA doses. Two COX-1 independent tests (WBA-ADP and PFA-CADP) showed no overall reduction in platelet reactivity.

Results: Overall classifications for detecting all ASA doses, compared to baseline, were: Very good-LTA-AA (k=0.95) & VerifyNow ASA (k=0.85); Good-Serum TxB_2 (k=0.79); Moderate-LTA-ADP (k=0.59), PFA-100-CEPI (k=0.56), Urinary TxB_2 (k=0.55), WBA-AA (k=0.47); Poor-PFA-100-CADP (k=-0.02) and WBA-ADP (k=-0.07). No significant kappa statistic differences were seen for each test for each ASA dose. Correlations for each test with serum TxB_2 measurements were: Very good-VerifyNow ASA (k=0.81, R^2=0.56), LTA-AA (k=0.85, R^2=0.65); Good-PFA-100 CEPI (k=0.62, R^2=0.30); Moderate-Urinary TxB_2 (k=0.57, R^2=0.51), LTA-ADP (k=0.47, R^2=0.56); Fair-WBA-AA (k=0.31,R^2=0.31); Poor-PFA-100-CADP (k=0.04,R^2=0.003), WBA-ADP (k=-0.04, R^2=0.0005).

Conclusions: Platelet function tests are not equally effective in measuring the antiplatelet effect of ASA and correlate poorly amongst themselves. The clinical usefulness of the different assays to measure ASA responsiveness remains undetermined.

PB 1569 | The Offset of Ticagrelor Prior to Coronary Artery Bypass Graft (CABG) Surgery

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Background: The optimal timing of discontinuation of ticagrelor prior to coronary artery bypass graft (CABG) surgery is unknown. In the ONSET/OFFSET study of patients with stable coronary artery disease, ticagrelor´s effects dissipated within 48-120 hours of discontinuation. However, there is evidence that the pharmacodynamics of antiplatelet therapy are altered during an acute coronary syndrome (ACS). Regulatory authorities recommend that ticagrelor be discontinued 5 days prior to CABG. The PLATO study showed that ticagrelor was associated with fewer deaths following pulmonary adverse events and sepsis compared to clopidogrel. It is unknown if ticagrelor‘s effect of inhibiting cellular adenosine uptake might underlie this observation.

Aims: To characterise the effect of discontinuation of ticagrelor on platelet activity and adenosine uptake inhibition.

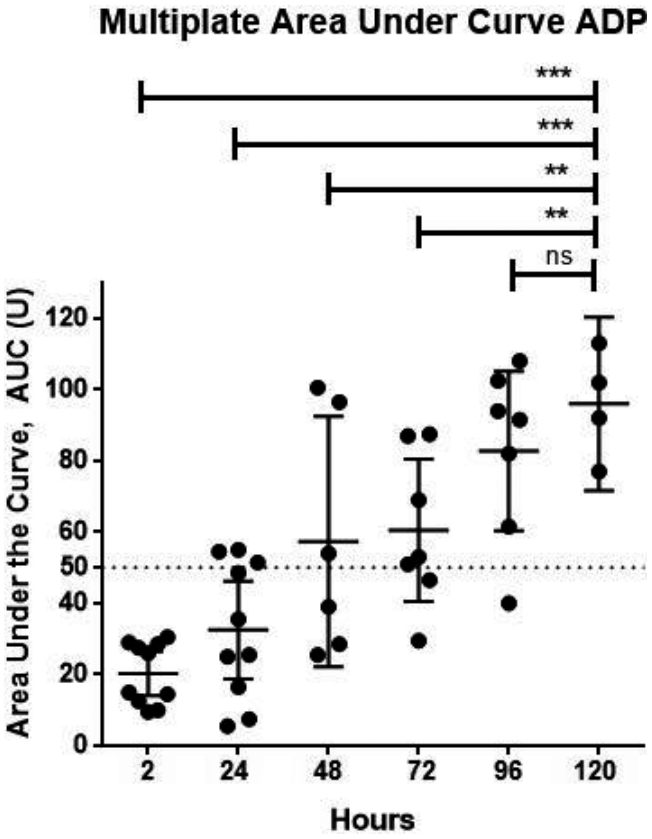

FIGURE 1 Whole-blood aggregometry data points of each patient, obtained from the Multiplate analyser and also the mean of each respective timepoint

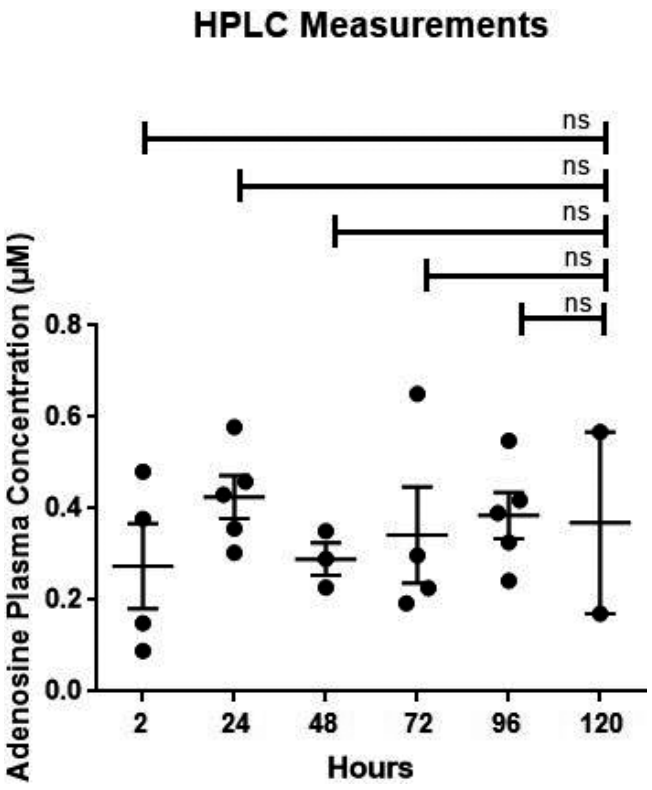

FIGURE 2 Adenosine plasma concentration data points of each patient, measured using HPLC and also the mean values of each respective timepoint

Methods: ACS patients, treated with ticagrelor and referred for CABG were recruited. Venous blood was drawn at 6 timepoints:2 hours(h), 24h, 48h, 72h, 96h, and 120h after the last dose of ticagrelor. Whole-blood aggregometry was carried out using the Multiplate analyser and the value of area under the curve (AUC)(units, U) was recorded. A protocol was referred to, where AUC of >50U supports safe progression to surgery, while values ≤50U indicate re-testing 24h later. Inhibition of adenosine uptake was determined by measuring the adenosine plasma concentration (APC).

Results: 13 patients recruited, received ticagrelor prior to CABG. At 96h post ticagrelor, the mean AUC was 82.8U, (95% confidence interval, CI, 60.4, 105.2). Only at 96h was the lower limit of the CI >50U. The comparison between 72h and 120h showed a significant difference (P=0.007) while 96h and 120h showed no significant difference. APC was measured in 7 patients and showed no significant difference across all timepoints.

Conclusions: ACS patients might be safe to undergo CABG surgery 96h after the cessation of ticagrelor. Ticagrelor might have no measurable effect on adenosine transport in ACS patients.

PB 1570 | Nanoparticle Inhibition of Shear Induced Platelet Aggregation

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Background: Thrombosis in stenoses requires vWF unfolding under pathologically high shear rates with binding of platelets through A-1 (SIPA).

Aims: We hypothesize that vWF refolding and/or separation of A-1 from GPIb on platelets will prevent SIPA.

Methods: We test for SIPA in a microfluidics chamber with a stenosis shape of an atherosclerotic plaque. Whole blood is mixed with charged nP, ~ 60 nm. Occlusion time (OT) is measured in normal controls, then with + and - charged nP.

We then studied vWF -platelet interaction under high shear rates through a simulation using Lattice-Boltzman computational fluid dynamics. This multiscale study simulates the interaction of vWF polymers with nanoparticles (nP) under high shear conditions and vary the charge density.

Results: Experimental SIPA was rapid under high shear for normal controls and positively-charged nP (OT=200 +/- 10 s). Negatively-charged nP exhibited a significant lengthening of OT (1100 +/- 300 s, n=4, p< 0.05). The effect was dose-dependent and charge-dependent. Computational modeling showed that negatively-charged nP can cause shear-elongated vWF molecules to roll back to a globular form provided the charge is high enough. This configuration change was dependent on nP charge and charge density.

Figure 1. nP (red) and vWF (blue) in shear flow (6500 1/s) with arrows showing flow direction. vWF fully elongates in shear flow; then - nP adhere to positive A1 patch to create a fold (middle) and roll up the elongated vWF back into globular state (bottom).

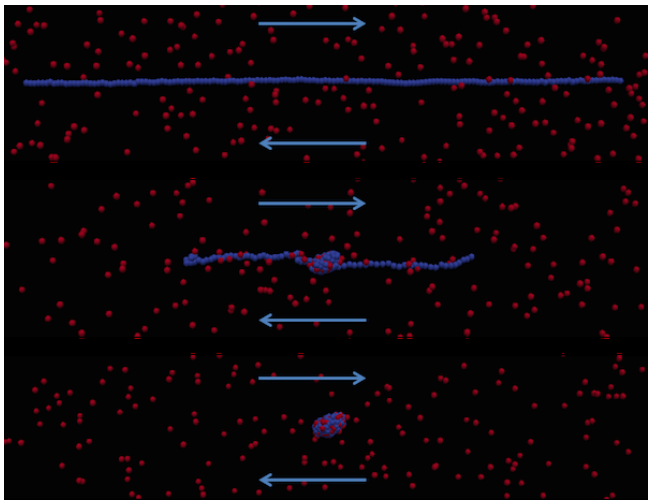

FIGURE 1 nP (red) and vWF (blue) in shear flow (6500 1/s). vWF elongates in shear; then - nP adhere to + A1 to fold and roll vWF into globular state

Conclusions: Negative nP can significantly inhibit the formation of arterial-type thrombus under pathologically high shear rates. The particles alter the shear threshold of vWF unfolding by physical interactions between domains under hemodynamic conditions. The particles may further inhibit vWF-platelet binding by physical steric separation. The demonstration of reduction in SIPA by nanoparticles opens up a new class of possible anti-platelet therapeutics based on physics instead of chemical mechanisms.

PB 1571 | Plasma Fibrinogen Level is an Important Determinant of Platelet Reactivity in the VerifyNow Assay

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Background: The VerifyNow assay is based upon the ability of activated platelets to cross-link beads coated with fibrinogen (Fg). However, Fg is abundant protein of blood, and therefore it may affect test results by competing with Fg of beads for binding to activated platelets.

Aims: The objective of this study was to assess the influence of artificial alteration of Fg level in blood on platelet reactivity measured by the VerifyNow $P2Y_{12}$ assay.

Methods: A 10 ml samples of citrate blood were obtained from donors (n=9) and patients on clopidogrel therapy (n=8), who provided informed consent to participate in the study. Then each blood sample was divided into 3 portions: baseline and two aliquots in which Fg level was altered by adding either 1/10 volume of purified Fg solution (10.6 g/l, clotting ≥95%) or corresponding buffer. In these samples we measured indices of platelet reactivity (Base and PRU) using the VerifyNow $P2Y_{12}$ cartridges and concentration of Fg by the clotting assay of Clauss. For each studied individual we then calculated magnitudes of change in platelet reactivity values (dBase and dPRU) and Fg concentration (dFg) by subtracting from baseline value those, which were measured after addition of Fg or buffer.

Results: Relative to baseline, addition of buffer significantly increased platelet reactivity, whereas addition of fibrinogen decreased it. Analysis of the relationship between change of platelet reactivity values and change in fibrinogen concentration revealed strong negative correlations: dBase = -63.3xdFg-27.1 (r=-0.924, p< 0.0005) and dPRU= -54.4xdFg-21.8 (r= -0.764, p< 0.0005).

Conclusions: Thus, our experiments demonstrated that: (1) the VerifyNow $P2Y_{12}$ assay is significantly influenced by Fg level in analyzing samples; (2) this effect of Fg is an *in vitro* phenomenon; (3) correcting for fibrinogen effect may be needed to improve the accuracy of the test in the measuring of antiplatelet effect of clopidogrel therapy.

PB 1572 | Effects of Clopidogrel with or without Aspirin on the Generation of Extracellular Vesicles in the Microcirculation and in Venous Blood in Healthy Young Males

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Background: Dual anti-platelet therapy with clopidogrel (C) and aspirin (A) protects from arterial thrombosis but confers a considerable bleeding risk. Extracellular vesicles (EV) are prothrombotic. We tested the hypothesis that C+A has a stronger effect on EV generation than C alone.

Aims: To compare the effects of C+A and C alone on EV generation in blood obtained from the microvasculature and in venous blood.

Methods: In a prospective, randomized, double-blind, placebo-controlled trial 44 healthy males (mean age: 26 years) received either C (600mg loading dose, 150mg maintenance dose daily) + A (100mg daily) or C alone over 7 days. Blood was obtained from a microvascular injury (bleeding time incision) and by venipuncture at baseline (BL), 2h, 24h and 8 days. The number of EV was assessed by FACS (FACSCalibur®, Becton Dickinson). EV were defined by size (forward scatter, < 1 µm) and annexin V binding and labeled using antibodies (anti-CD41a: platelet positive EV [PLT+EV]; anti-CD142: TF positive EV [TF+EV]). Data are given as median (quartiles). The Wilcoxon signed rank test was used for short-term treatment effect (BL vs 2h), the Wilcoxon rank sum test for differences between groups (A vs placebo). AUC was used to discriminate the difference in effects over time (Wilcoxon rank sum test).

Results: EV generation was similar in subjects treated with C+A or C (both in shed blood and in venous blood) when levels at BL and

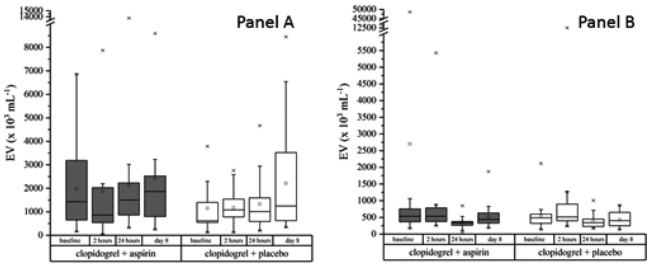

FIGURE 1 Effects of clopidogrel (C) + aspirin (A) and of C on the generation of microvascular EV (Panel A) and on venous EV (Panel B).

2h were compared [shed blood: C+A: 1433 (653; 3184) vs 862 (545; 2026), p=0.4; C: 614 (552; 1402) vs 1079 (781; 1538), p=0.7; venous blood: C+A: 532 (369; 754) vs 534 (375; 782), p=0.91; C: 486 (322; 597) vs 514 (399; 897), p=0.17]. There was also no difference in the effects of C+A and C alone on EV generation in shed- or venous blood over 24 hours and 8 days (Fig. 1).

Conclusions: Neither C+A, nor C alone affected EV generation in the microvasculature or in venous blood.

PB 1573 | Assessment of Low Platelet Reactivity (LPR) with Multiple Electrode Impedance Aggregometry (MEA) and Risk of Bleeding in Patients Receiving P2Y12 Receptor Inhibitors

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Background: Antiplatelet therapy with P2Y12 inhibitors recommended in patients undergoing percutaneous coronary intervention (PCI) is associated with an increased risk for bleeding. Patients with LPR show a 1,7-fold higher risk for major bleeding complications.

Aims: To evaluate the proportion of patients with LPR and bleeding receiving P2Y12 receptor inhibitors - clopidogrel, prasugrel and ticagrelor and to determine the cut-off value of LPR for risk of bleeding.

Methods: The platelet function was assessed with MEA on Multiplate analyzer by ADP-test in 3760 patients who underwent PCI with stent implantation in for period of 4 years. Patients were treated with 75 mg/d clopidogrel, or 10 mg/d prasugrel, or 2 x 90 mg/d ticagrelor.

Results: We found TIMI minor bleeding complications like cutaneos haematoma, epistaxis, haemoroidal bleeding and hemoptoe in 0.6% of patients as maintenance therapy per year. The mean value of ADP-induced platelet aggregation in the subgroup of patients on clopidogrel therapy with bleeding was 12 ± 6,9 U vs. 22 ±10 U in the group of patients with good response without bleeding. In the subgroups of patients on prasugrel and ticagrelor therapy with bleeding ADP -induced platelet aggregation was 9,3 ± 6,4 U and 10,7 ± 6,4 U, respectively. Antiplatelet therapy in patients with bleeding was adjusted with decreasing of the daily dose of P2Y12 inhibitors or switched to another P2Y12 inhibitor. Tailored treatment leads to optimal level of ADP inhibition and decreasing of the bleeding.

Conclusions: ADP-test < 15 U has been associated with increasing risk of minor bleeding; LPR predicts a higher risk for bleeding complications. There were no differences in MEA values in patients with bleeding complications between used P2Y12 inhibitors. Tailored antiplatelet therapy into the optimal range of platelet reactivity, based on standardized platelet functional assay reduces the risk of secondary bleeding events.

PB 1574 | Concomitant Use of Antiplatelet and Direct Oral Anticogulants Increase but Not Significantly the Bleeding Risk in Patients with Atrial Fibrilation

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Background: Direct oral anticoagulants (DOACs) are a very useful drugs in prevention of thromboembolic events for patients with atrial fibrillation due to the excellent safety profile has presented in clinical trials and real life studies. However these patients sometimes need to take antiplatelet in combination with these drugs increasing the bleeding risk.

Aims: The objective of this study was analyze if the antiplatelet use in combination with DOACs is associated with a significantly elevation of the bleeding risk.

Methods: A retrospective study was conducted of patients who were treated with DOACS in our unit from 2011-2016. Data were obtained from our hospital register that has local committee approval. Patients were followed until discontinuation drug or to the last follow. Dose of DOACS was chosen to clinical judgment. The main variable investigated were the percentage of mayor bleedings. Major bleeding was defined according to the criteria of the ISTH 2005. Statistical analyzes were performed using SPSS 21.0 (SPSS Inc.,Chicago, IL). All the confidence interval was at 95%.

Results: A total of 514 patients were finally included with 74(73-75) years old. Gender was female in 301(58.6%) patients. Mean of CHA2DS2-VASc and HAS-BLED was 3,9(3.8-4.1) and 2.5(2.4-2.6) points respectively. Patients used antiplatelets and DOACs were 59(11,5%). Mean follow-up was 1,2(1.0-1.3) years. The number of patients with a mayor bleeding in the concomitant group was 6(10%) compare with control group 22(4.9%). In univariate analyze this different was not significant, p value equal to 0.101, despite the concomitant group had an odds ratio 2.1(0.8-5.6) times superior to suffer a mayor bleeding.

Conclusions: The use of antiplatelet therapy with DOACs has been described like a factor to increase bleedings in some studies. In our case we showed a double risk of bleeding in this kind of patients

however without a significant outcome, may be due to we need more patients in the case group.

PB 1575 | Arginine-containing Oligopeptides as Antiplatelet Agents Under Experimental Type-2 Diabetes Mellitus

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Background: The correlation between high blood glucose level and dysfunction of the anticoagulation system has been identified. In addition to hyperglycemia, increase in platelet aggregation (PA) was observed under diabetes mellitus. It has previously been shown that oligopeptides have anticoagulant and antiplatelet effects in organism. Also it is known that arginine improves rheological properties of blood and inhibits platelet aggregation. Special attention is drawn to regulatory oligopeptides with the addition of arginine to their molecules.

Aims: The study of arginine-containing oligopeptides Arg-Pro-Gly (RPG), Pro-Arg-Gly (PRG) and Pro-Gly-Pro-Arg (PGPR) influence on ADP-induced PA (ADP-PA) under development of experimental type-2 diabetes mellitus (T2DM) in rats.

Methods: All experiments were carried out on male Wistar rats and were conformed with the Declaration of Helsinki. Experimental T2DM was induced by intragastrically administration of 40% glucose solution (2g/kg) for 10 days. After that the rats intranasally administrated the peptides (1000 μg/kg) for 6 days with continued glucose administration. Control T2DM rats was administrated saline. Blood for measuring of ADP-PA was collected 1h after the last peptides injection and 7 days after peptides withdrawal.

Results: The studied peptides decreased ADP-PA during all time of experiments. PRG, RPG and PGPR lead to ADP-PA decrease 1h after the last peptide injection on 55, 38, 30% accordingly. These effects were observed after peptides withdrawal: ADP-PA decreased on 57, 42, 44% vs. control. PRG had maximal antiplatelet effect.

Conclusions: Thus arginine-containing oligopeptides have protective antiplatelet effects against enhanced rat PA induced by a continuous glucose load. These effects were preserved after peptides withdrawal, that indicate prolonged effects of peptides. We conclude that arginine-containing oligopeptides RPG, PRG and PGPR could potentially used as antiplatalet agents against hypercoagulation due to the development of experimental T2DM.

PB 1576 | Combined Effect of *CYP2C19* G681A Polymorphism and Plateletcrit on Clopidogrel Low-responsiveness in Thai Patients with Acute Coronary Syndrome

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Background: The *CYP2C19* G681A (rs4244285) polymorphism, platelet indices, high platelet reactivity, and clopidogrel low-responsiveness have been shown to predict the major adverse cardiovascular events (MACEs) in patients with acute coronary syndrome (ACS). However, the relationship between platelet indices and clopidogrel responsiveness is unclear.

Aims: To investigate the combined effect of *CYP2C19* G681A polymorphism and platelet indices on clopidogrel responsiveness in Thai ACS subjects.

Methods: A total of 56 ACS subjects treated with clopidogrel were recruited. Polymorphism of *CYP2C19* G681A was determined by polymerase chain reaction-restriction fragment length polymorphism techniques. Platelet reactivity in term of closure time (CT) was measured by using platelet function analyzer-200 (PFA-200). The CT ≤ 106 and exceeding 106 seconds were classified as clopidogrel low-responder and responder, respectively. Platelet indices were examined by automated analyzer XE-2100 (Sysmex, Japan).

Results: Individual with 681AA revealed significantly lower CT compared to 681GA and 681GG (p=0.002 and 0.001, respectively). However, no significant influence of the carrier of G681A on clopidogrel low-responder when compared to 681GG (p=0.051). Among platelet indices, only plateletcrit (PCT) revealed a significant increase in clopidogrel low-responder compared to clopidogrel responder group (p=0.034). Based on ROC analysis, PCT at the cutoff point of 0.2 could predict clopidogrel low-responder with a sensitivity of 57.5% and a specificity of 66%. However, no significant influence of PCT > 0.2 on clopidogrel low-responder when compared to PCT ≤ 0.2 (p=0.080). Interestingly, when combined 681GA or AA with PCT > 0.2, the result revealed an increased risk of clopidogrel low-responsiveness compared to the group with 681GG + PCT ≤ 0.2 [OR (95% CI) = 4.3 (1.1, 16.5)].

Conclusions: These findings suggested that the combination of *CYP2C19* G681A polymorphism with PCT enhance risk of clopidogrel low-responsiveness in Thai ACS subjects.

PB 1577 | Inhibition of Platelet Aggregation by Fibrinogenase from the Antarctic Scallop *Adamussium colbecki*

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Background: Thrombolytics have been extensively used in the therapeutic treatment of thrombosis. Fibrinogenases because of their role

in dissolving of blood clots as well as prevention their formation have attracted special medical and scientific attention. Enzymes that affect hemostasis have been isolated from different sources. Now special attention is paid to the hydrobionts from the Antarctic region which are poorly explored and potentially can be a valuable source of new bioactive compounds.

Aims: We test the effect of fibrinogenase from *A. colbecki* on platelet aggregation.

Methods: For estimating fibrinogenolytic activity fibrinogen was incubated with enzyme at 100:1 ratio at different time intervals. The fibrinogen digestion was analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Platelet aggregation was determined using aggregometer.

Results: Fibrinogenase was isolated using affinity and size exclusion chromatography which provided a high level of homogeneity as confirmed by SDS-PAGE. Purified fibrinogenase directly degraded fibrinogen. Susceptibility of fibrinogen chains to proteolysis was different - the enzyme cleaved preferentially Aα-chain and more slowly Bβ-chain. Furthermore, fibrinogen hydrolysis occurred in a concentration-dependent manner. Taking into account that fibrinogen is an essential for platelet aggregation we tested whether fibrinogenase from *A. colbecki* could affect platelet aggregation. According to our result investigated enzyme inhibited ADP-induced platelet aggregation and the inhibition increased with increasing concentration of the enzyme. Data analysis revealed inhibition of platelet aggregation by 80% for 12.5 µg/mL fibrinogenase and by 31% for 6.25 µg/mL fibrinogenase.

Conclusions: We hypothesized that fibrinogenase may inhibit platelet aggregation by hydrolyzing the Aα-chain of fibrinogen to prevent fibrinogen from combining with fibrinogen receptor on platelet membrane.

PB 1578 | Platelet (plt) reactivity in patients with essential thrombocythemia (ET) receiving antiplatelets therapy with aspirin (ASA)

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Background: Antithrombotic prophylaxis with ASA (100 mg/o.d.), alone or in combination with hydroxyurea (HU) cytoreductive therapy, is widely used in ET to prevent thrombosis and control microcirculatory symptoms. A recent study suggested that in these patients twice-daily 50 mg ASA may be more effective than 100 mg/o.d.. The effect of daily versus twice-daily ASA on platelet reactivity is unknown in ET.

Aims: To evaluate by multiple electrode aggregometry (MEA) the influence of ASA therapy ±HU on platelet aggregation in ET patients.

Methods: We compared platelet response to Arachidonic acid (ASPI test) and thrombin receptor activating peptide (TRAP test) in 65 ET patients. Control groups were: 72 non-ET patients on chronic ASA prophylaxis, and 111 healthy subjects (CTR). A cutoff value of < 30U in ASPI test was used to identify the response or not to the antiplatelet effect of ASA. A normalized AA-induced aggregation (r-AA-agg), defined as ASPI/TRAP ratio, was calculated to reflect individual variation to ASA.

Results: In ET patient group TRAP values, but not ASPI values, were significantly higher ($p < 0.001$) compared to CTR. Analysis of ET patients according to treatment, showed that ASPI values in ASA±HU-treated ET patients (n=45) were significantly lower ($p < 0.001$) compared to non-ASA-treated ET patients (n=20), while were significantly higher ($p < 0.001$) compared to ASA-treated non-ET patients. Only 11 (5 on ASA alone and 6 on ASA+HU) out of 45 ET patients (24.4%) responded to ASA treatment, showing ASPI values < 30U. Differently, 94 % of non ET-patients on ASA responded (i.e. ASPI values < 30U). Same results were obtained with the r-AA-agg, with ASA±HU-treated ET patients being less responsive than non-ET ASA-treated group.

Conclusions: MEA is a rapid method to evaluate the response to antiaggregating treatment. The results show a greater sensitivity of ET platelet to aggregating stimuli compared to non-ET patients on ASA. This may account for failure of the antithrombotic prophylaxis observed in these patients.

PB 1579 | The Influence of Prostaglandin E1 on the Correlation between the Multiple Electrode Aggregometry ADP and the VerifyNow P2Y12 Test in Patients Using P2Y12 Inhibitors

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Background: Platelet function tests, such as the Multiplate (MADP) and the VerifyNow P2Y12 (VNP2Y12), can be used to determine platelet reactivity in patients on P2Y12-inhibitors, which has been shown to be related to the risk of adverse events. However, correlation and agreement between therapeutic windows of these tests are poor. Both assays stimulate platelets with ADP. However, in the VNP2Y12, prostaglandin E1 (PGE1) is added to block the activation of the P2Y1 receptor by ADP, but PGE1 is not present in MADP.

Aims: We assessed if addition of PGE1 to MADP could improve the correlation between MADP and VNP2Y12.

Methods: Sixty patients with coronary artery disease on dual antiplatelet therapy (P2Y12-inhibitor, ascal) were included. Platelet function testing was performed 30-60 days after percutaneous intervention to help guide antiplatelet therapy. Forty patients used clopidogrel, 16 prasugrel and 4 ticagrelor. The MADP (6.5 µM ADP) and the VNP2Y12 (20 µM ADP/22 nM PGE1) were performed for clinical care purposes, addition of the PGE1 (9.4 nM) to the MADP was performed for research purpose only, in accordance with local medical ethical guidelines.

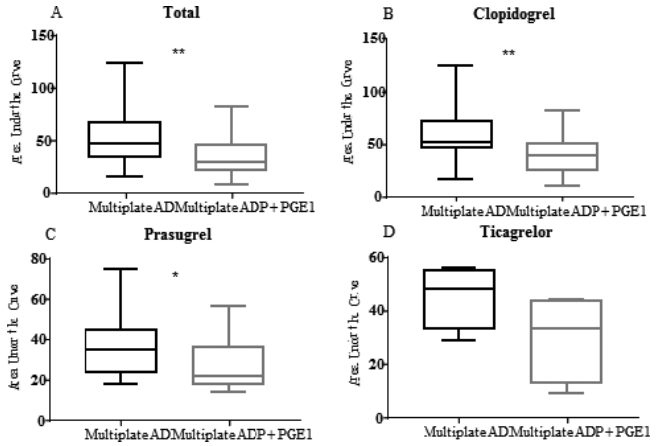

FIGURE 1 (A-D): Comparison of median area under the curve (AUC) MADP without PGE1 versus median AUC MADP with addition of PGE1

Results: In the total-, the clopidogrel-, and prasugrel group, the median of MADP with addition of PGE1 was significantly lower than the median of MADP without PGE1 (29.5 vs 47.0; 38.0 vs 51.0; and 22.0 vs 35.0 ($P < 0.05$), respectively. (Figure 1 A-C). The ticagrelor group showed the same trend (with PGE1 (33.5), without PGE1 (48.0)), however this was not significantly different (P=0.125) (Figure 1 D). Addition of PGE1 to MADP did not increase the correlation between MADP and VNP2Y12 (Table 1).

TABLE 1 Spearman correlation of MADP (without and with PGE1) versus VNP2Y12.

	Total Group (n=60)	Clopidogrel group (n=40)	Prasugrel group (n=16)
Correlation (r) MADP without PGE1 vs VNP2Y12	0.646	0.579	0.642
Correlation (r) MADP with PGE1 vs VNP2Y12	0.530	0.558	0.200

Conclusions: In patients on P2Y12-inhibitors, addition of PGE1 to MADP lowered the platelet reactivity, but did not improve the correlation between MADP and VNP2Y12. Therefore, the presence of PGE1 in the VNP2Y12, but not in the usual MADP assay, cannot explain the poor correlation and agreement between therapeutic windows of these tests.

PB 1580 | Could Aspirin Play a Role in Diminishing ADP Induced Platelet Aggregation in Clopidogrel Naïve Patients?

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Background: Platelet aggregation by ADP, collagen, l-epinephrine, and thrombin suggests a physiological process in blood coagulation. Excessive platelet aggregation is acknowledged to be responsible for the formation of thrombus on the coronary artery wall leading to acute coronary syndrome (ACS) or acute myocardial infarction. Antiplatelet treatment with aspirin and/or clopidogrel inhibits platelet aggregation protecting from possible thrombotic events. Aspirin blocks cyclooxygenase 1 (COX-1) and therefore thromboxane A2 (TXA2) generation while clopidogrel blocks the ADP-P2Y12-dependent platelet activation pathway.

Aims: To evaluate the platelet function of high risk patients for an acute coronary syndrome, or patients undergoing percutaneous coronary interventions (PTCI), that receive anti-platelet treatment at the Onassis Cardiac Surgery Center over a six-month period.

Methods: Laboratory assessment of platelet function was based on platelet aggregation tests performed by the in vitro addition of ADP, Collagen, Ristocetin and Arachidonic acid as aggregating agents on a BIODATA platelet aggregometer.

Results: A total of 500 platelet aggregation assays were performed at the Laboratory of Coagulation and Haemostasis of the Onassis Cardiac Surgery Center over a six-month period. In 35 cases of aspirin-only treated patients, we observed a diminished ADP-induced platelet aggregation ranging from 33% to 50% of normal aggregation for each patient.It has been reported that blocking of lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) inhibits ADP-induced aggregation. Platelet treatment with aspirin has been shown to reduce LOX-1 expression and to inhibit ADP-induced aggregation.

Conclusions: We can only speculate that antiplatelet treatment with aspirin in our patients may have affected ADP-induced aggregation through down regulation of LOX-1 expression; however further in depth studies are required to elucidate the possible role of aspirin in the diminished ADP-induced aggregation observed.

PB 1581 | Obesity Is Associated with Reduced Platelets SERCA3 Isoform Expression and Subsequent Platelet Inhibition, Reversible after Weight Loss

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Background: In obese subjects, platelet activity is described as increased and their sensitivity to antiplatelet agents as reduced. We previously observed that platelet Sarco/Endoplasmic Reticulum Ca2+ ATPase (SERCA3) expression was decreased in patients with morbid obesity when compared to non-obese subjects, in conjunction with reduced sensitivity to agonists and lower calcium response of platelets.

Aims: To explore these dysfunctions after weight loss.

Methods: Monocentric case/control study (2011-2014). Cases (women with body mass index (BMI) ≥35 kg/m^2, without hypertension, diabetes, dyslipidemia, cancer, sepsis or inflammation) and Controls (women with BMI >18.5 and < 25 kg/m^2, without same exclusion criteria) were matched for age ± 3 years. Each pair of subjects

Time	1 Controls n=40	1 Cases n=40	p	2 Controls n=40	2 Cases n=40	p
Age (years)	39.3 ± 9.6	36.4 ± 9.5	0.9			
BMI (kg/m²)	21.4 ± 1.9	44.1 ± 6.5	<10⁻³	21.4 ± 2.1	30.7 ± 7.0	<10⁻³
Platelets (Giga/L)	243 ± 49	284 ± 42	0.002	244 ± 56	269 ± 69	0.1
Fibrinogen (g/L)	2.8 ± 0.4	4.0 ± 0.9	<10⁻³	2.8 ± 0.6	3.3 ± 0.9	0.01
CRP (mg/L)	2.2 ± 1.3	13.2 ± 8.7	<10⁻³	2.4 ± 1.4	5.1 ± 10.7	0.2

FIGURE 1 Clinical and laboratory data of study population at inclusion (time 1) and one year after bariatric surgery in cases (time 2). p with Studen

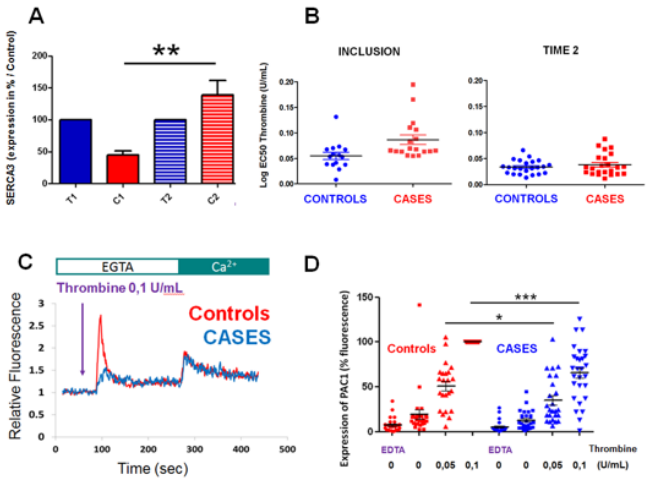

FIGURE 2 (A) SERCA3 expression in cases at inclusion (C1) is decreased when compared to controls (T1) and is increased after weight loss (C2). (B) At

has been seen twice: at inclusion and 1 year after gastric bypass surgery in case. SERCA levels were measured by immunoblotting. Aggregation was examined under magnetic agitation conditions in washed platelets stimulated with various agonists. Activity of thrombin-stimulated platelet was measured by flow cytometry using PAC-1. BAPTA Oregon-green (calcium fluorescent marker) loaded platelets were analyzed for calcium mobilization and influx in response to different agonists by flow cytometry and video microscopy.

Results: We included 40 cases and 40 controls, with one-year follow-up after bariatric surgery in cases (Figure 1).

After weight loss, platelet SERCA3 expression, calcium mobilization in response to agonists (Thrombin, collagen, PAR4-AP) and platelet aggregation was normalized when compared to control subjects (Figure 2).

Conclusions: This is the first report of a human pathophysiological condition, associated with a platelet SERCA3 deficiency. In obese subjects, these dysfunctions are reversible and normalized after weight loss. We suggest that SERCA3 decrease might be a physiological response to reduce the rate of spontaneous activation of platelets.

PB 1582 | Patterns and Levels of Platelet Glycosylation in Patients with Coronary Heart Disease and Type 2 Diabetes Mellitus

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Background: Coronary heart disease(CHD) and diabetes mellitus(DM) have high reactivity of platelets and an increased risk of thrombosis. Platelets play an important role in the pathogenesis of the underlying atherosclerotic process. Platelet glycosylation is closely related to platelet function and survival. However, the alteration of platelet glycosylation in CHD and T2DM still remains unknown.

Aims: The aim is to evaluate the patterns and levels of platelet glycosylation in patients with CHD or T2DM and to investigate the relationship between them.

Methods: Platelet samples were obtained from 55 healthy controls and 102 patients (including 33 CHD, 30 T2DM, 39 CHD complicated with T2DM (CHD+T2DM). Platelet glycosylation was detected using eight-lectin based assay by flow cytometry. Platelet activation (CD62P and PAC-1) was measured on resting and stimulated conditions by flow cytometry. Platelet aggregation was measured by light transmission aggregometry.

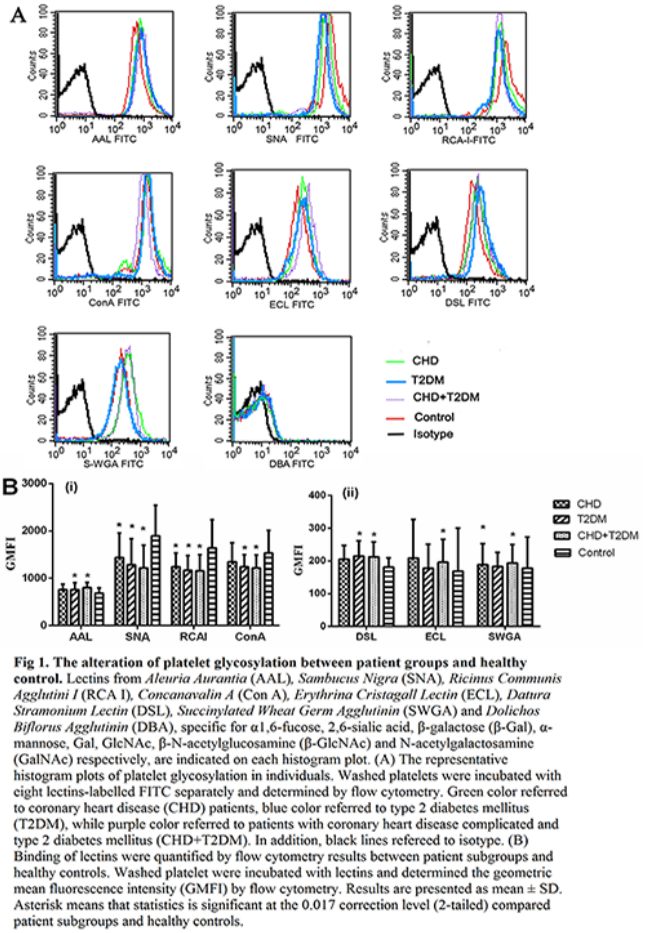

Fig 1. The alteration of platelet glycosylation between patient groups and healthy control. Lectins from *Aleuria Aurantia* (AAL), *Sambucus Nigra* (SNA), *Ricinus Communis Agglutini I* (RCA I), *Concanavalin A* (Con A), *Erythrina Cristagall Lectin* (ECL), *Datura Stramonium Lectin* (DSL), *Succinylated Wheat Germ Agglutinin* (SWGA) and *Dolichos Biflorus Agglutinin* (DBA), specific for α1,6-fucose, 2,6-sialic acid, β-galactose (β-Gal), α-mannose, Gal, GlcNAc, β-N-acetylglucosamine (β-GlcNAc) and N-acetylgalactosamine (GalNAc) respectively, are indicated on each histogram plot. (A) The representative histogram plots of platelet glycosylation in individuals. Washed platelets were incubated with eight lectins-labelled FITC separately and determined by flow cytometry. Green color referred to coronary heart disease (CHD) patients, blue color referred to type 2 diabetes mellitus (T2DM), while purple color referred to patients with coronary heart disease complicated and type 2 diabetes mellitus (CHD+T2DM). In addition, black lines refereed to isotype. (B) Binding of lectins were quantified by flow cytometry results between patient subgroups and healthy controls. Washed platelet were incubated with lectins and determined the geometric mean fluorescence intensity (GMFI) by flow cytometry. Results are presented as mean ± SD. Asterisk means that statistics is significant at the 0.017 correction level (2-tailed) compared patient subgroups and healthy controls.

FIGURE 1 The alteration of platelet glycosylation between patient groups and healthy control

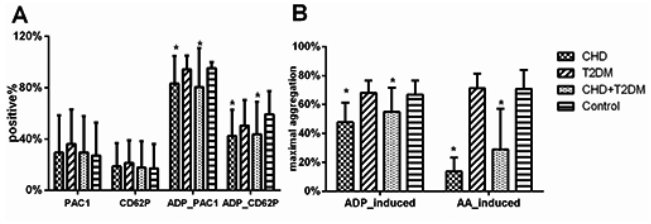

Fig 2. Platelet activation and aggregation between patient groups and healthy controls. (A) The activation was detected in resting and ADP-stimulated platelets. The levels of platelets activation analyzed on flow cytometry and were expressed as the percentage of CD62P or PAC-1 positive platelets. Activation of platelet on resting and stimulated in vitro by flow cytometry. (B) ADP- and AA-induced platelet aggregation by LAT. The maximal amplitude (%) was as equal as maximal aggregation. Results are presented as mean ± SD. Asterisk means that statistics is significant at the 0.017 correction level (2-tailed) compared patient subgroups and healthy controls.

FIGURE 2 Platelet activation and aggregation between patient groups and healthy controls

Results: In CHD group, platelet surface weakly expressed β-Gal and 2,6-sialic acid and strongly expressed β-GlcNAc. In T2DM group, lectins binding to platelet of β-Gal, 2,6-sialic acid and α-mannose were decreased, while α1,6-fucose and GlcNAc were increased. There was positive correlation between ConA (specific for α-mannose) and PAC-1 in T2DM patients, while negative correlation in healthy controls. Patterns and levels of platelet glycosylation in CHD + T2DM group are a combination of CHD group and T2DM group, in addition to the level of ECL highly elevated (specific for β-Gal). The level of ConA was significantly correlated with glucose in T2DM group, also correlated with HbA1c in CHD+T2DM group.

Conclusions: Our findings suggested that platelets decreased in sialylation, galactosylation and mannosylation, and increased in fucosylation and GlcNAcylation in CHD and T2DM patients. The changes of platelet glycosylation may be associated with high platelet reactivity, leading to the increased risk of thrombosis in CHD and T2DM.

PB 1583 | Hemostatic Abnormalities in Patients with Ehlers-Danlos Syndrome

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Background: The Ehlers-Danlos Syndrome (EDS) represents a heterogeneous group of disorders of the connective tissue. EDS is often associated with an increased bleeding risk, but a comprehensive study of hemostasis in patients with EDS is lacking.

Aims: To evaluate the bleeding severity in a cohort of patients with Ehlers-Danlos syndrome and to correlate the results of coagulation tests with a quantitative method to assess bleeding tendency.

Methods: 100 EDS patients (M/F:23/77, median age:34y) were included in the study. ISTH Bleeding Assessment Tool (ISTH-BAT) was used to evaluate the bleeding history of patients. Primary and secondary hemostasis tests were performed: whole blood count, PT ratio, aPTT ratio, fibrinogen, von Willebrand factor ristocetin co-factor (RICOF), endogenous thrombin potential (ETP), platelet aggregation and platelet secretion (studied by lumi-aggregometry). If PT or aPTT were prolonged, coagulation factors were tested.

Results: ISTH-BAT, ranged from 0 to 14, was above 5 in 49 patients (49%). PT and/or aPTT were slightly prolonged in 10 patients (10%), due to mild coagulation factors deficiencies. RICOF was above 45% in all patients. ETP was normal in all patients. Among patients with ISTH-BAT>5, 60% had a reduction of platelet aggregation and 40% had a secretion defect in response to ADP as aggregating agent. At least one platelet function abnormality is present in 74% of patients with a ISTH-BAT>5 and also in 67% of patients with a ISTH-BAT≤5. The impairment of platelet aggregation was correlated with ISTH-BAT. The risk of an abnormal response to ADP was 2-fold increase in patients with ISTH-BAT>5, for an odds ratio of 2.4 (95% CI 1.0-5.5).

Conclusions: In our cohort half patients with EDS had an ISTH-BAT>5 and platelet function abnormalities.

PB 1584 | Acquired Glanzmann Thrombasthenia due to Marked Reduction of Surface αIIbβ3 Expression with Non-function Blocking Anti-αIIbβ3 Antibodies

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Background: Acquired Glanzmann thrombasthenia (aGT) is a bleeding disorder generally caused by function-blocking autoantibodies against integrin αIIbβ3.

Aims: We analyzed an unusual case of aGT caused by marked reduction of surface αIIbβ3 expression with non-function blocking anti-αIIbβ3 antibodies (Abs).

Methods: A 72 year-old Japanese man suffering from ITP since his 50's showed exacerbation of bleeding symptom despite of mild thrombocytopenia (80-90 x 10^9/L). His bleeding time was prolonged and platelet aggregation was absent with all agonists but ristocetin. Analysis of αIIbβ3 expression in platelets and genetic analysis of *ITGA2B* and *ITGB3* was performed. We also analyzed effects of anti-αIIbβ3 Abs of this patient on platelet function and αIIbβ3 expression.

Results: Surface αIIbβ3 expression was markedly reduced to around 5% of normal. By sharp contrast, Western blotting of platelet lysates showed that the patient's platelets contain 40-95% of normal-sized αIIbβ3. Substantial amount of fibrinogen was also detected in his platelets. These results suggest that low surface expression of αIIbβ3 by internalization may cause GT-like phenotype of this patient. There were no abnormalities in whole coding region of *ITGA2B* and *ITGB3* cDNA. Anti-αIIbβ3 IgG Abs were detected in platelet eluates and plasma, and epitopes of these Abs are located in β-propeller domain of αIIb. However, these Abs did not interfere with aggregation and

PAC1 binding of normal platelets, indicating that the Abs were non-function blocking. Surface αIIbβ3 and GPIb expression of megakaryocytes (MgK) derived from cord blood cells was impaired by incubation with IgG purified from the patient's plasma, as observed by ITP autoantibodies. After 2 years of aGT diagnosis, his bleeding symptom has improved and surface αIIbβ3 expression was recovered to 20% of normal with reduction of anti-αIIbβ3 Abs.

Conclusions: Anti-αIIbβ3 Abs of this patient may cause internalization of αIIbβ3 leading to severe platelet dysfunction and impairment of MgK maturation.

PB 1585 | Platelet Function and Ibrutinib Treatment in Chronic Lymphocytic Leukaemia and Mantle Cell Lymphoma: Effects of Drug and of Disease Itself

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Background: Ibrutinib treatment in chronic lymphoblastic leukemia (CLL) and mantle cell lymphoma (MCL) is associated with bleeding. It can be due to a combination of inhibition of platelet glycoprotein VI signalling by ibrutinib and ADP degradation by CD39 on lymphocytes.

Aims: To investigate platelet function in CLL/MCL patients before and on ibrutinib treatment.

Methods: A total of 14 MCL patients and 20 CLL patients were included; controls were 70 healthy donors. All gave their written informed consent, and the study was approved by the hospital´s ethics committee. Blood was collected before ibrutinib treatment (point 0), then at 2, 4, 8 weeks (points 1-3) and 3-6 months (point 4) on treatment. Cell count, light transmission and whole blood aggregometry, APTT, and PT assays were performed. Platelets were phenotyped by flow cytometry before and after activation. Levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and mepacrine release were determined. Thrombin generation in platelet-rich plasma of healthy donors supplemented with ibrutinib (with or without collagen-related peptide) was monitored using the calibrated automated thrombogram method.

Results: Granule release, integrin activation and procoagulant activity in patients before treatment were significantly impaired in CLL versus MCL versus healthy controls. Further profound decrease of PAC1 binding and procoagulant PLT formation were observed on ibrutinib therapy. Aggregation in points 1 and 2, but not in points 0, 3, 4 was impaired in patients with bleeding. Platelet count was decreased in most patients before and on therapy; clotting assays remained within the normal range. Thrombin generation was dose-dependently inhibited by ibrutinib *in vitro*.

Conclusions: Platelet function is initially impaired in CLL and MCL patients, additionally inhibited by ibrutinib, and then restored on treatment. However, our data suggest that initial impairment involves other mechanisms than CD39, and that effect of ibrutinib is not only glycoprotein VI-dependent.

PB 1586 | Chronic Immune Thrombocytopenic Purpura (ITP) in Egyptian Children: A 10 Year Single Center Retrospective Study

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Background: 20 -25% of children with newly diagnosed ITP will develop chronic disease. Treatment guidelines are determined by many factors; bleeding risk, likelihood of response, impact on quality of life, side effects of drug(s) given, patient/parent anxiety and resource constraint.

Aims: To study the demographic and clinical data as well as response to treatment of chronic ITP in Egyptian children.

Methods: After the ethical committee approved the study, all records of children diagnosed with chronic ITP and following in the Paediatric haematology outpatient clinic in the past 10 years were reviewed. First line treatments included steroid therapy, intravenous immunoglobulin, anti-D and azathioprine. Second line treatments included splenectomy, triple therapy (0.8 mg/kg/d prednisolone, 2mg/kg/d, Azathioprine and 2.5mg/kg/d Cyclosporine), other as Mycophenolate Mofetil and Rituximab. Demographic, clinical, and treatment data was collected and analysed with descriptive statistics.

Results: 222/854 (30%) of our studied ITP cohort developed a chronic course. Their mean current age is 9.4±4.1 with equal gender prevalence, 24.8% are of consanguineous marriage and 5.4% have a similarly affected sibling .Patients' mean initial platelet count is 18x109/L. 46.4% developed epistaxis, 9.9%hematuria and menorrhagia was reported in 25/34 (73.5%) of teenage girls whilst 1.8% developed intracranial hemorrhage. 45.5% of the Egyptian cohort received short pulse methylprednisolone, 12.2% intravenous immunoglobulin, 7.7% anti-D, 24.3% Azathioprine,8.6% triple therapy, 14% underwent splenectomy and 3.6% received other treatment regimens. 39% achieved complete remission, 49% partial remission and 12% no response.

Conclusions: Our studied Paediatric cohort is more liable to develop a chronic course, require second line treatments due to the frequency and / or severity of mucous membrane bleeds and they are more prone to develop intracranial haemorrhage.

PB 1587 | Acute Episodes of Atrial Fibrillation Cause Altered Platelet Function and Procoagulant Microparticle Consumption

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Background: Increased coagulation activity has been described during atrial fibrillation (AF) requiring antithrombotic treatment. Platelets and microparticles (MP) have been shown to be involved this process.
Aims: To determine the consequences of induced-AF on platelet function and MP levels in the left atrium of patients with non-valvular AF.
Methods: Paroxysmal and persistent AF patients (n=51) referred for catheter ablation were recruited. Patients in sinus rhythm (n=12) were induced in AF and left atrium blood samples were taken before and after acute episode of AF (20min). Measures of platelet aggregation, P-selectin, αIIbβ3, GPIb, PAR-1, receptor expression by flow cytometry and platelet morphology by transmission electron microscopy were performed. Levels of procoagulant and fibrinolytic MP were determined by functional assays.
Results: A significant reduction in platelet aggregation to TRAP (66.0±2.9% vs 84.9±3.0%) was found under basal conditions in AF patients, but improved after acute induced-AF suggesting a process of desensitization partially reversed by the acute AF episode. Platelet morphology revealed the presence of pseudopods and an increased number of vacuoles indicating some platelet reactivity. No differences in surface platelet receptor levels of P-selectin, αIIbβ3, GPIb, or PAR1 were found by flow cytometry. Finally, while the left atrium levels of fibrinolytic MP remained stable, a significant decrease of tissue factor-dependent procoagulant activity of MP was observed after acute induced-AF (15.9±3.7fM vs 5.3±2.0fM) suggesting that the MP were captured either by the surrounding tissues and/or incorporated into micro-clots.
Conclusions: During acute AF episodes an increased platelet response to thrombin receptor through PAR1 and associated with tissue factor-dependent procoagulant MP consumption is in favor of local increased thrombotic events. Therefore, AF pathophysiology involves in addition to plasma coagulation, a cellular participation which acts in synergy to promote thrombus formation.

PB 1588 | Impaired Platelet Responsiveness in Thrombocytopenic Cancer Patients after Chemotherapy as a Consequence of Mitochondrial Dysfunction

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Background: Severe thrombocytopenia (≤50x10^9 platelets/L) in hematological malignancies after intensive chemotherapy is associated with an increased risk of bleeding. However, platelet count only weakly correlates with bleeding risk, suggesting involvement of other hemostatic factors.
Aims: To investigate possible changes in platelet and coagulation activities accompanying thrombocytopenia in cancer patients receiving chemotherapy.
Methods: After ethical approval and written informed consent, blood was obtained from 89 patients with hematological malignancies before and after (1h) platelet transfusion. Coagulation factors were determined. Platelet functions were assessed by flow cytometry. Mitochondrial morphology and activity were determined by electron microscopy and high-resolution respirometry. Thrombus and fibrin formation were assessed on a collagen-tissue factor surface under flow.
Results: Patients showed normal coagulation factor activity levels, except for high VWF and fibrinogen and low FVII levels. The patients´ platelets were reduced in Ca^{2+} responses, integrin $\alpha_{IIb}\beta_3$ activation and secretion upon agonist stimulation, compared to control platelets ($p< 0.01$). Patients´ platelets showed a time-dependent increase in phosphatidylserine exposure independent of agonist stimulation or caspase activity, which was enhanced by the mitochondrial apoptosis inducer ABT-737. Mitochondrial function in patients´ platelets was markedly impaired, as evidenced by a reduced mitochondrial membrane potential
($p< 0.001$) and lower maximal O_2 consumption ($p< 0.05$), in spite of normal mitochondrial content. After platelet transfusion, the diminished thrombus and fibrin formation normalized, underscoring the loss-of-function of autologous patients´ platelets.
Conclusions: Impaired platelet responsiveness in chemotherapy treated patients is the consequence of mitochondrial dysfunction. Transfusion partly recovers platelet functionality, improving thrombus and fibrin formation.

PB 1589 | Coated-platelet Formation is a Sensitive Indicator of Dasatinib Elicited Side-effects

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Background: Tyrosine kinase inhibitors (TKI) are a very effective group of drugs that considerably prolong survival in patients with chronic myeloid leukemia. Several lines of evidence suggest that dasatinib (Sprycel) may induce bleeding due to its effect on platelets.
Aims: We hypothesized that dual agonist activated platelet formation (coated platelets) may be a useful marker to monitor TKI side-effects in addition to classical platelet function tests.
Methods: Citrate-anticoagulated blood samples from healthy volunteers were used in PFA-100 assay, lumi-aggregometry and during coated platelet formation. This latter test was executed by dual activation of gel-filtered platelets by the snake venom convulxin and thrombin to simultaneously activate collagen and thrombin receptors.
Results: We could confirm that dasatinib at 400 nM prolongs PFA-100 closure times by the collagen/epinephrine cartridge but not by collagen/ADP cartridge. This supra-therapeutic dasatinib concentration also prevented platelet aggregation and ATP release after 1 µg/ml collagen and 500 µg/ml arachidonic acid challange and similar inhibitory effect could be observed down to 150 nM dasatinib concentration. Coated-platelet formation was significantly inhibited by preincubation with 400 nM dasatinib (p=0.001) resulting in 9±8.5% of coated platelet formation versus controls with no TKI (41±10%). The effect of dasatinib was dose-dependent and the inhibitory effect could be observed already at 10 nM dasatinib that is the low therapeutic concentration of the drug (coated platelet = 34±4.2%, p=0.02), however this low dasatnib concentration had no inhibitory effect on platelet aggregation and release results. Contrary to dasatinib, another second generation TKI, nilotinib (Tasigna) did not exert any effect on coated platelets.
Conclusions: We suggest that the measurement of coated platelets is a more sensitive marker than lumi-aggregometry to detect TKI associated side-effects in platelets.

PB 1590 | Thrombocytopenia and Altered Coagulation as Independent Predictors of the Etiology of "Tropical Jaundice" in Adults Presenting to Emergency in a Tertiary Care Hospital in North India

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Background: Fever with jaundice or tropical jaundice is common presenting feature of the patients visiting for emergency.The knowledge of region specific prevalence is needed to improve the management of such patients.
Aims: To study the region specific aetiology of Fever and Jaundice in patients presenting to medical emergency.
Methods: 106 adult patients with a diagnosis of "Tropical Jaundice" with fever(body temperature > 101°F) of 14 days without any localisation but causing jaundice (hyperbilirubinemia ≥ 1.5 mg/dl or elevated AST/ALT more than three times) were enrolled.All these patients were evaluated for malaria(peripheral smears/rapid diagnostic kits), scrub typhus(PCR /IgM ELISA),leptospirosis(IgM ELISA), enteric fever by blood cultures,dengue(NS1 antigen/IgM ELISA),Hepatitis(IgM ELISA of EBV/HSV, IgM ELISA of HAV/HEV and HBsAg with IgM HBc ELISA if HBsAg positive).
Results: 63 were males and 43 were females. The mean duration of fever before presentation was 7.75 ± 3.58 days. 7 patients (6.6%) died. 92 patients (86.8%) improved with treatment,7 patients (6.6%) left treatment against medical advice. The various etiologies included scrub typhus24 (22.6%), Hepatitis E 14 (13.2%),malaria 9 (8.5%),dengue fever, enteric fever, hepatitis A and leptospirosis in 4 (3.8%), 2 (1.9%), 2 (1.9%) patients and 1 (0.9%).Probable sepsis accounted for 31 patients. Conjunctival suffusion (OR=22.17), severe anemia (OR=5.5), respiratory crepitations (OR=5.27), thrombocytopenia (OR=1.14), hepatomegaly (OR=1.04), normal INR (OR=0.29) and altered mentation (OR=0.25) were significant predictors of a diagnosis of scrub typhus. Severe anemia(Hb< 8),Hypoalbuminemia,severe thrombocytopenia (Platelet count < 50,000)and a near normal INR at admission were predictors of a malarial vs a viral etiology of Tropical jaundice.
Conclusions: Scrub infection has emerged as common etiology of tropical jaundice in recent times. Simple tests like a baseline platelet count and coagulogram(INR/PTTK) can help in early diagnosis and thus treatment.

PB 1591 | Plasma microRNAs Characterizingpatients with Immune Thrombocytopenia

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Background: Dysregulation of microRNA (miRNA) expression is associated with many diseases. The expression profile of miRNAs in plasma of immune thrombocytopenia (ITP) patients have not been fully understood.
Aims: The aims of this study were to determine whether plasma from ITP exhibit an altered miRNAs expression profile and evaluate their diagnostic value for ITP prediction.
Methods: Plasma samples were obtained and allocated into a discovery set composed of 10 ITP patients and 6 healthy controls, a validation set with 52 ITP patients and 52 healthy controls. miRNA

microarray assay and quantitative real time PCR (qPCR) verification were performed in the discovery set and validation set, respectively. Receiver operator characteristic (ROC) curves were constructed to evaluate their diagnostic value for ITP prediction.

Results: Microarray data showed a alteredplasma miRNA profile and a total of 23 plasma miRNAs were identified in ITP patients compared to healthy controls(fold change>1.5, $P< 0.01$). The qPCR results verified eight upregulated miRNAs (miR-320c,miR-642b-3p, miR-1275, miR-3141, miR-4270, miR-4499, miR-4739 and miR-6126) and three downregulated miRNAs (miR-144-3p, miR-1281 and miR-3162-3p) in ITP patients. The plasma levels of miRNAs varied in different phases of ITP disease. ROCanalysis showed a good diagnostic value could be achieved by a panel of four miRNAs (miR-144-3p, miR-1275, miR-3141 and miR-3162-3p).A significant positive correlation was found between platelet count and plasma levels of miR-3162-3p(r=0.338, P=0.01). Bioinformatic analysis indicated that the highest scoring functional pathways associated with ITP wereproteoglycans, thyroid hormone signaling pathwayand estrogen signaling pathway.

Conclusions: Our results indicate a differential plasma miRNA profile exists in patients with ITP disease. The plasma miRNA signature could be served as an independent biomarker in the diagnosis of ITP.

PB 1592 | Evaluation of in vitro Effects of Tyrosine-kinase Inhibitors on Primary Haemostasis

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Background: Tyrosine-kinase inhibitors (TKIs) are established for the treatment of chronic myeloid leukaemia (CML). An increased bleeding risk in some patients on dasatinib is described but the underlying mechanism is not yet clear.

Aims: We investigated platelet function and haemostasis screening testing in TKI-incubated blood samples collected from healthy young adult volunteers.

Methods: Each specimen (n=15) of citrated whole blood (WB) was incubated for 30 min with TKI at different final concentrations (imatinib 1.5 µmol/l, dasatinib 0.4 and 0.2 µmol/l, bosutinib 0.4 µmol/l, nilotinib 4 µmol/l). Platelet function (aggregation and ATP release) was investigated by the Chronolog lumi-aggregometer type 560 using collagen (1 µg/ml), arachidonic acid (0.5 mmol/l) and thrombin (0.5 U/ml). Function of primary haemostasis was screened by the PFA-100® test using buffered citrated WB.

Results: Dasatinib at 0.4 µmol/l almost completly inhibited collagen-induced aggregation and ATP-release and caused a marked prolongation of the PFA-100® closure times (CT) with the collagen/epinephrine cartridge. Contrastingly, platelet activation by the other agonists and the CT with the collagen/ADP cartridge was not influenced. The concentration dependency of this effect was demonstrated using dasatinib at 0.2 µmol/l.

In imatinib-incubated samples the collagen-induced aggregation was significantly increased compared to controls while for the other parameters no significant differences were found. The PFA-100® CT was not influenced by imatinib. Comparable results were obtained for bosutinib and nilotinib.

Conclusions: Our data clearly demonstrate that dasatinib but not imatinib, bosutinib and nilotinib impairs primary haemostasis by influencing the collagen-mediated platelet activation.

As CML is mostly diagnosed in elderly people, clinical relevance of these findings must be judged on in the context of underlying vascular endothelial diseases.

PB 1593 | What Do we Know about Post Transfusion Purpura? A Novel Insight into the Correlation between HPA Antibodies and Clinical Picture

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Background: A 72 year old patient was admitted with purpura and severe thrombocytopenia (PLT 6000/mm3), 10 days after receiving a red blood cell transfusion. Laboratory investigations showed Human Platelet Antigen (HPA) HPA-1aa and HPA-3bb genotype, in the presence of anti HPA-3a, HPA-1b and HLA antibodies. Post Transfusion Purpura (PTP) was diagnosed, and the patient was treated with intravenous immunoglobulin (IVIG), achieving a full PLT recovery within 14 days. This abstract is the first systemic review summarizing the current literature on this rare phenomenon, focusing on the correlation between HPA antibodies and PTP.

Aims: The primary outcome was to examine the differences in anti HPA distribution before and after the employment of the Monoclonal Antibody Immobilization of Platelet (MAIPA) test since 1995, and correlate the results with the demographic and clinical characteristics.

Methods: A review of PubMed and Medline was performed, looking for all articles since 1965 , that included the keywords ´´PTP" and anti HPA.

Results: A total of 69 articles were examined . 173 cases were reviewed ; 157 in women and 15 (including our case) in men. Data are summarized in the table below.

TABLE 1 Anti HPA distribution by gender

Population (n)	HPA 1a	HPA 1b	HPA others	Combination*
All Women (157)	131(83%)	6(4%)	23(15%)	3(2%)
**Women 1995 (28)	21(75%)	1(3.5%)	6(21%)	0(0%)
All Men (16) combination(4)	8 3(42%)	3 ***1(14%)	9 4(43%)	4(25%)
**Men 1995 (12) combination(4)	7 3(44%)	1 1(6.25%)	8 4(50%)	4(33%)

* combination - at least 2 antibodies not included HLA

**sub analysis after MAIPA standardization

*** we add our patients to analysis

Patients with HPA -1a had a lower platelet nadir(1-26,000, median 6), with only 6 out of 38 patients had a platelet count > 10,000/mm3. In patients expressing other anti HPA groups, 6/13 had platelet counts > 10,000/mm3 (2 -26,000, median 11.6). There was no correlation between the incidence of bleeding events and PLT level.

Conclusions: Despite the small number of cases and the bias associated with retrospective analysis the current data suggest that in women, HPA-1a antibody is the predominant culprit for PTP, whereas in men, the distribution is wider. Furthermore, a combination of anti HPA is more common in men than in women.

PB 1594 | Further Characterization of Platelets Bearing Mutations in Calreticulin or JAK2 Genes in Essential Thrombocythemia Patients

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Background: Essential Thrombocythemia (ET) is a chronic blood disorder characterized by overproduction of platelets by megakaryocytes and is associated with an increased risk of thrombo-hemorrhagic complications. Acquired mutations in the genes of *JAK2* or calreticulin (*CALR*) are associated with the disorder in the majority of the cases. CALR-mutant patients have a very high platelet count but relatively low risk of thrombosis compared to JAK2-mutant patients.

Aims: To examine whether CALR mutations have a different effect on platelets activation and intracellular Ca^{+2} concentration compare to JAK2 mutation and wild type.

Methods: Two platelet activation markers, CD62P and PAC1, were measured following ADP activation of patients' platelets carrying the JAK2 mutation (JAK-mt) (11 patients), the *CALR* mutation (CALR-mt) (8) and normal subjects (13). In addition, immature platelets fraction (IPF) and absolute number of immature platelets were counted. Intracellular calcium concentration was measured using a fluorescent indicator by flow cytometry. CALR was stained along with actin in adherent platelets, using confocal microscopy.

Results: Expression of activation markers following ADP stimulation decreased significantly in CALR-mt patients compare to JAK-mt patients or normal subjects. Interestingly, intracellular calcium concentration was significantly higher in resting platelets of CALR-mt patients. CALR in adherent platelets was dispersed in the cytoplasm in ET patients of both mutations compare to normal subjects, where it seemed in the center of the platelet. No significant difference between the three groups was detected in the IPF. However, since the CALR-mt patients had higher platelet count compare to JAK-mt patient, and by far more than normal subjects, the absolute number of immature platelets was significantly higher in those patients.

Conclusions: Platelets of CALR-mt patients are less reactive compared to JAK2 patients and this compensates the higher platelet count and hence they are less prone to thrombosis.

PB 1595 | Clinical Evaluation of the Revised International Prognostic Score of Thrombosis for Essential Thrombocythemia (IPSET-Thrombosis) in a Cohort of 746 Chinese Adult Patients

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Background: For predicting thrombotic events in patients with essential thrombocythemia (ET), the revised International Prognostic Score of Thrombosis model (IPSET-thrombosis) was reported to be better than the original IPSET-thrombosis.

Aims: The study aimed at evaluating Whether the revised IPSET-thrombosis model was applicable to Chinese patients ET.

Methods: Medical records of 746 patients with a diagnosis of ET were retrospectively analyzed.

Results: According to the revised IPSET-thrombosis model, the number of very low-, low-, intermediate-, and high-risk patients were 271 (36.3%), 223 (29.9%), 63 (8.4%) and 189 (25.3%), respectively. The four groups exhibited significantly different thrombosis-free survival ($P< 0.001$). Thirty-six patients were reclassified as intermediate-risk according to the revised IPSET-thrombosis instead of low-risk per the original IPSET-thrombosis. Nineteen intermediate-risk patients per the original IPSET-thrombosis were upgraded to high-risk according to the revised IPSET-thrombosis. Fifty-one high-risk patients per the original IPSET-thrombosis were reclassified as low-risk in the revised IPSET-thrombosis. It suggests that the revised IPSET-thrombosis potentially avoids over- or under-treatment. In low-risk patients per the revised IPSET-thrombosis, the rate of thrombosis in patients with cardiovascular risk factors (CVF) was higher than in those without (16.3% *vs.* 5.2%, $P=0.022$), and comparable with intermediate-risk patients per the revised IPSET-thrombosis (16.3% *vs.* 14.3%, $P=0.765$). As

a result, a new revised IPSET-thrombosis model more applicable to Chinese ET patients was developed in which patients with CVF in the low-risk group per the revised IPSET-thrombosis were reclassified as intermediate-risk group.

Conclusions: For predicting thrombotic events, the revised IPSET-thrombosis model was better than the original IPSET-thrombosis model. The revised IPSET-thrombosis was optimized and a new revised IPSET-thrombosis model more applicable to Chinese ET patients was developed.

PB 1596 | Variations of Mean Platelet Volume with Time Since Blood Sampling in Different Anticoagulants

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Background: The mean platelet volume (MPV) is useful for the classification of congenital thrombocytopenias and to distinguish between congenital and acquired thrombocytopenias. It has also been suggested that high MPV is associated with increased risk for cardiovascular events. It is known that MPV increases in EDTA samples with time. Anticoagulants different from EDTA have been tested and proposed as potentially useful for assessing platelet indices.

Aims: Aim of our study was to identify the ideal pre-analytical variables for measuring MPV.

Methods: MPV was measured in blood samples from of 100 thrombocytopenic patients and 100 healthy blood donors collected in K2EDTA, Citrate-Theophylline-Adenosine-Dipyridamole (CTAD), Citrate-Tris-Pyridossalphosphate (CPT) and magnesium-anticoagulant (Mg-AC). Blood cell count was performed (COULTER® LH 750 Hematology Analyzer) in each sample immediately after sampling (t0) and at 20 (t20), 40 (t40), 60 (t60), 120 (t120) and 180 (t180) minutes.

Results: MPV steadily increased with time both in blood donors and thrombocytopenic patients, in all anticoagulants, with the exception of CTAD, and stabilized at t120 (Figure 1 and 2). As a consequence, the percentage of subjects with high MPV, defined as greater than the 95th percentile of values obtained in blood donors at t0, increased with time both in blood donors and thrombocytopenic patients: for instance the prevalence of thrombocytopenic patients with high MPV in K2EDTA blood samples increased from 49% at t0 to 82% at t180.

Conclusions: Our study shows that: 1) MPV varies with time of measurement after blood sampling and with the anticoagulant used to collect blood samples; 2) the increase in MPV remained stable between 120 and t180. Thus, MPV should be measured either immediately after blood sampling or between 120 and 180 min. Variations of MPV with time were lowest in CTAD anticoagulant.

Figure 1. Variation over time of MPV in blood samples in different anticoagulants from 100 **blood donors.**

	Anticoagulant			
Time	K2EDTA	CTAD	CPT	Mg-AC
t 0	7.9 (7.2-8.6)	7.5 (6.9-8.3)°	7.3 (6.6-7.9)°	7.0 (6.4-7.4°
t20	8.6 (7.8-9.3)*#	7.7 (7.0-8.4)*#	7.6 (6.9-8.3)*#	7.2 (6.5-7.9)
t40	8.8 (8.0-9.5)*#	7.7 (6.9-8.3)*#	7.7 (7.1-8.5)*#	7.4 (6.7-8.1)*#
t60	8.9 (8.1-9.8)*#	7.7 (6.9-8.4)*	7.9 (7.1-8.5)*	7.4 (6.8-8.1)*#
t120	9.2 (8.4-9.9)*#	7.7 (6.9-8.4)*	7.7 (7.0-8.6)*	7.6 (6.8-8.3)*#
t180	9.2 (8.4-10)*	7.6 (6.8-8.3)#	7.9 (7.1-8.6)*	7.6 (6.9-8.4)*

Median values in fL (IQR). Friedman's analysis of variance (ANOVA) followed by Dunn's post hoc test. ANOVA test from t0 to t180 was statistically significant for all anticoagulants. *p<0.05 vs t0; #p<0.05 vs previous time; °p<0.05 vs K2EDTA a t0.

FIGURE 1

Figure 2 Variation over time of MPV in blood samples in different anticoagulants from 100 **thrombocytopenic patients.**

	Anticoagulant			
Time	K2EDTA	CTAD	CPT	Mg-Ac
t0	9.5 (8.5-10.5)	9.4 (8.5-10.5)	9.2 (8.2-10.4)°	9.0 (8.1-9.9)°
t20	10.5 (9.3-11.5)*#	9.6 (8.7-11.0)	9.6 (8.6-10.5)*#	9.1 (8.3-10.2)*#
t40	10.7 (9.5-11.8)*#	9.6 (8.6-10.5)	9.8 (8.6-10.8)*	9.2 (8.4-10.2)*#
t60	10.8 (9.6-11.5)*	9.8 (8.6-10.8)	9.9 (8.7-10.7)*	9.3 (8.3-10.5)*
T120	10.9 (9.8-11.5)*#	9.5 (8.6-10.6)	9.6 (8.6-10.9)*	9.4 (8.6-10.6)*
t 180	11.1 (19.4-11.9)*	9.5 (8.7-10.8)	9.6 (8.6-10.6)*	9.5 (8.7-10.5)*

Median values in fL (IQR). Friedman's analysis of variance (ANOVA) followed by Dunn's post hoc test. ANOVA test from t0 to t=180 was statistically significant for all anticoagulants. *p<0.05 vs t0; #p<0.05 vs previous time; °p<0.05 vs EDTA a t=0.

FIGURE 2

PB 1597 | Cytosolic Calcium Concentrations in Platelets from Patients with Pulmonary Embolism and Implications for Therapy

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Background: Prior work has shown platelet hyperactivity with evidence of apoptosis in acute pulmonary embolism (PE). Cytosolic [Ca++] is a key mediator in platelet activation and apoptosis.

Aims: Link cytosolic [Ca++] to morphological and functional changes associated with hypercoagulability in PE.

Methods: Patients had image-proven submassive PE within 24 h of diagnosis with blood analyzed within 60 min. Patient informed consent was obtained in this IRB-approved study. Thromboelastography (TEG, Haemoscope 5000) was performed on whole blood, and cytosolic [Ca++] was measured in Fluo-8 loaded platelets using spectrofluorometry and fluorescence microscopy with agonist stimulation or antagonist inhibition (PAF and PAR receptor antagonists WEB 2086 and SCH79797) or soluble guanylate cyclase activation with cinaciguat. Comparisons (2-way ANOVA, Dunnett's) were made to platelets from healthy controls.

Results: On TEG, PE platelets had shorter R time, higher angle and MA, both without or with platelet agonists (arachidonic acid and ADP), indicating global hypercoagulability. On fluorescence microscopy, unstimulated PE platelets were hypoactive with lower basal [Ca++] (90±45 nM vs controls 177±95 nM, P< 0.001). With agonists (arachidonic acid, ADP and thrombin), PE platelets had more fragmentation,

pseudopod formation, and disintegration, with a similar relative increase in [Ca++]: 324±250, 148±88*, and 118±78*, respectively, vs 499±332, 309±155, and 224±75 in controls (*P< 0.001). WEB 2086, SCH79797, and cinaciguat reduced thrombin-stimulated [Ca++] to 95±46, 124±77, and 122±35, respectively, vs 381±419, 166±45, and 190±95 in controls.

Conclusions: Patients with PE have a globally hypercoagulable TEG profile with platelets that show an apoptotic morphology, lower basal [Ca++], and preserved [Ca++] increase with thrombin that was effectively blocked by multiple antagonists. These data suggest a large proportion of apoptotic but not necrotic platelets in PE and emphasize the need and options for early platelet protection.

PB 1598 | Probable Risks and Benefits of Second Line Therapy Splenctomy versus TPO Mimetics in Chronic Idiopathic Thrombocytopenic Purpura an Experience of a Third World Country, Pakistan

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Background: Immune Thrombocytopenia (ITP) is an autoimmune disease of low platelet count and a tendency to bleed. Difference between splenectomy and TPO receptor agonists is that the first prolongs platelets' lifespan by eliminating platelets destruction, second stimulates platelet production. Our study compares changing standards of care from surgical to non surgical approach of treatment.

Aims: To evaluate the usefulness of splenectomy in patients of chronic ITP in the era of new treatment modalities in a developing country.

Methods: It was a descriptive Cross Sectional Study. In this study we analyzed data of 32 cases of chronic ITP for complete response (CR), partial response (PR) and no response (NR) to therapy. Three approaches were used i.e splenctomy, combination therapy (non-approved medical BCSH) and TPO agonist as group one, two and three respectively. The data was analyzed using SPSS 20.

Results: There were 71% females and 28% males. In group one (21 patients) 66% had CR, 24% showed PR and NR was noted in 9.5 % patients. While in group two (7 patients) 28% had CR, PR was 57% and NR was 14%. In group three (4 patients) 75% had CR and 25% had PR.

Conclusions: Our study predicts splenectomy to be cost effective and safe second-line treatment for chronic ITP. TPO agonists have become the standard in developed world but further research is required in developing countries for their cost effectiveness and long term safety.

PB 1599 | Sonoclot Signature Analysis In Thrombocytopenic Patients

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Background: Patients of thrombocytopenia behave differently when it comes to risk of bleeding. The biggest puzzle remains how to predict the risk of bleeding. Though there is a correlation of platelet count with the bleeding risk, a study which can determine platelet function can help stratify these patients. Platelet function studies usually require normal platelet counts. A global test of coagulation like sonoclot can be handy for the clinician if it can show differences between bleeders and non bleeders amongst thrombocytopenic patients.

Aims: To look for utility of sonoclot signature in predicting risk of bleeding in thrombocytopenic patients.

Methods: In this prospective observational study, a total of 50 patients with thrombocytopenia with or without a history of bleeding were included. All patients included had platelet counts lower than 20000/μL. Thirty of them had no history of any recent (last 7days) bleeding (Non-Bleeders) while 20 of them had history of (WHO grade ≥2) bleeding (Bleeders). They were evaluated as per unit protocol for the etiology of thrombocytopenia. The diagnoses included both non-malignant and malignant conditions. All these patient samples were evaluated by Sonoclot. Sonoclot measures activated clotting time (ACT), clot rate (R1) and platelet function (PF).

Results: Both the groups were matched in terms of patient age, diagnoses, platelet count, prothrombin time and activated partial thromboplastin time. The ACT was significantly longer in the bleeders compared to non-bleeders. The platelet function was significantly lower in the bleeders than the non-bleeders. The clot rate was non-significantly lower in the bleeders. (Table 1 and Figure 1)

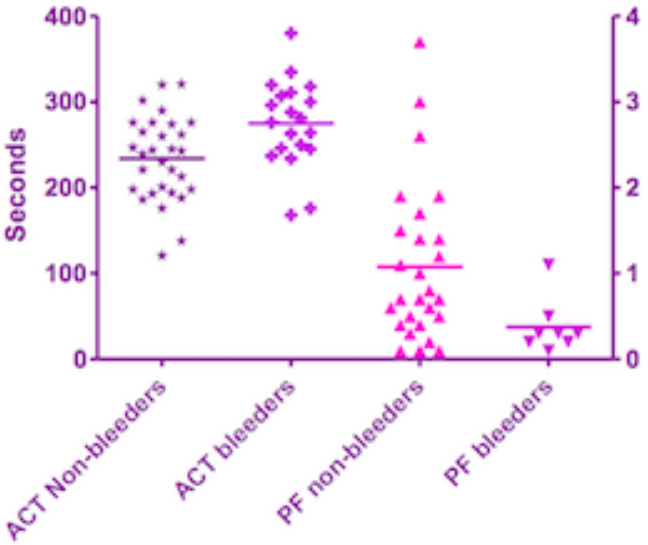

FIGURE 1 Sonoclot Analysis

TABLE 1 Comparison of non-bleeders with bleeders

		Non-bleeders (n=30)	Bleeders (n=20)		
S No	Parameters	Median±SD	Median±SD	P value	Normal value
1	Age (years)	39.5±18.91	40.5±14.76	NS	
2	Platelet(/µL)	11500±4735	7000±4593	NS	150-450 x103
3	Prothombin time (seonds)	14±1.921	14±2.294	NS	11-14
4	Activated partial thromboplastin time (seconds)	29±2.141	28±2.447	NS	25-35
5	ACT (seconds)	241±49.15	278±50.83	0.009	100-155
6	CR (R1) (unit)	18.5±6.042	16.5±4.041	0.06	9-35
7	Platelet function (unit)	0.7±0.916	0.3±0.351	0.01	>1.5

Conclusions: This is the first study to the best of our knowledge demonstrating the use of sonoclot in severe thrombocytopenic patients. Sonoclot offers ease of use as compared to other global tests of coagulation (TEG/ ROTEM). Sonoclot is a handy outpatient or bed side tool to predict risk of bleeding in thrombocytopenic patients.

PB 1600 | High Incidence of Platelet Dysfunction is unmasked in Newly-diagnosed Multiple Myeloma

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Background: Multiple myeloma (MM) is a plasma cell disorder that causes monoclonal protein (M-protein). M-protein can affect coagulation factors and platelets leading to coagulopathy and platelet dysfunction.

Aims: We aimed to study the incidence of bleeding symptoms in newly-diagnosed MM patients as an initial presentation and the effect of M-protein on platelet function.

Methods: The newly-diagnosed MM patients who did not receive antiplatelets had been prospectively enrolled. Baseline characteristics and laboratory results were collected. Platelet aggregation was determined by changes of optical density. Platelet activation assessed by P-selectin expression at resting and activating state was measured by flow cytometry. Impaired platelet aggregation to ^{3} 2 agonists was classified as abnormal platelet function group (group 1) whereas patients with normal platelet aggregation test were classified as group 2.

Results: During September to December 2016, eighteen patients were enrolled. The median age was 65 years (range 42-90). The M-protein consisted of IgG kappa (4), IgG lambda (4), IgM kappa (1), IgA lambda (1), IgA kappa (2) and free light chain (kappa 5, lambda 1). Only one patient (5.5%) presented with abnormal uterine bleeding. Six patients (33.3%) had abnormal platelet aggregation test (group 1). Compared between two groups, there was no significant difference of baseline characteristics including age, sex, and comorbidities. Likewise, there was slightly lower the mean value of P-selectin expression in group 1 than in group 2 (21.7 vs. 28.3%; p=0.6). In contrast, the mean value of M-protein in group 1 was significantly higher than in group 2 (6.35 vs. 2.69 g/dL; p=0.01).

Conclusions: Although bleeding manifestations in MM patients are uncommon, high incidence of platelet dysfunction is seen in this study. The high level of M-protein significantly predicts acquired platelet dysfunction.

PB 1601 | Effects of Time and Different Anticoagulants on Platelet Count in Blood Donors and in Thrombocytopenic Patients

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Background: The accuracy of platelet count (PC) measurement is pivotal for the diagnosis and management of patients with thrombocytopenia. We showed that some degree of time-dependent formation of platelet agglutinates in EDTA-blood occurs in some patients with thrombocytopenia, leading to underestimation of their platelet count.

Aims: Aim of the study was to assess the variation of PC over time in different anticoagulants, in blood donors (BD) and thrombocytopenic patients (TP).

Methods: PC was measured in blood samples from of 100 TP and 100 BD, collected in different anticoagulants: EDTA, Citrate-Theophylline-Adenosine-Dipyridamole (CTAD), Citrate-Tris-Pyridossalphosphate (CPT) and magnesium-anticoagulant (Mg-AC). Blood cell count was performed (COULTER® LH 750 Hematology Analyzer) in each sample within 10 min after sampling (t0), and at 20 (t20), 40 (t40), 60 (t60), 120 (t120) and 180 (t180) min. PC was also measured by flow cytometry (FC), the gold-standard technique for PC measurement, in non-anticoagulated blood immediately after sampling.

Results: At t0, there were no differences between PC measured by FC and by cell counter in antico agulated samples, except for CTAD-anticoagulated samples from TP (figure 1 and Figure 2). In TP, PC decreased significantly at t20 in EDTA-anticoagulated samples, and only at t120 in samples with the other anticoagulants (Figure 2). A similar

figure 1. Variation over time of PC in blood samples in different anticoagulants from 100 **thrombocytopenic patients.**

Platelet count (x 10^9/L) expressed as mean values (SD). *p<0.05 vs t0. ° p<0.05 vs flow cytometry a t0.

		Anticoagulant			
Time	Flow cytometry	EDTA	CTAD	CPT	Mg-AC
t 0	77 (37)	75 (35)	73 (36)*	75 (37)	74 (35)
t 20	-	73 (35)	72 (36)	75 (36)	74 (36)
t 40	-	72 (35)*	72 (35)	74 (36)	73 (36)
t 60	-	69 (34)*	72 (35)	73 (36)	73 (35)
t 120	-	70 (34)*	70 (34)*	72 (36)*	71 (35)*
t 180	-	68 (34)*	68 (33)*	72 (37)*	70 (34)*

FIGURE 1

figure 2. Variation over time of PC in blood samples in different anticoagulants from 100 **blood donors.**

		Anticoagulant			
Time	Flow cytometry	EDTA	CTAD	CPT	Mg-AC
t 0	251 (62)	252 (64)	254 (66)	253(66)	253 (66)
t 20	-	249 (64)	253 (66)	253 (66)	252 (65)
t 40	-	249 (65)	253 (67)	251 (65)	250 (65)
t 60	-	248 (63)*	253 (66)	250 (66)	251 (65)
t 120	-	249 (64)	251 (66)*	252 (65)	250 (63)
t 180	-	249 (65)*	249 (67)*	252 (65)	251 (65)

Platelet count (x 10^9/L) expressed as mean values (SD). *p<0.05 vs t0. ° p<0.05 vs flow cytometry a t0.

FIGURE 2

pattern was observed with BD, although the exetnt of PC decrease was lower than in TP (figure 1).

Conclusions: PC decreased over time in blood samples from TP, independently of the anticoagulant used, although the effect was more remarkable with EDTA. PC was more stable in BD. When EDTA is used to collected blood samples for cell counts, measurement of PC should be performed with 20 min from sampling to obtain accurate results.

PB 1602 | In vitro Ibrutinib Effect (Inhibitor of Bruton´S Tyrosine Kinase) on Platelets Function. A Single Centre Study

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Background: Ibrutinib is a Bruton´s tyrosine kinase (BTK) inhibitor , in use for the treatment of B- cell malignancies and shown to increase the risk of hemorraghic complications in this patient setting. BTK is expressed in platelets and plays an important role in platelet signaling pathways.

Aims: To sttudy the effect of ibrutinib on platelets function in vitro, in patients (pts) with B cell malignancies receiving treatment with the drug.

Methods: 14 patients under Ibrutinib were included. Platelets function was examined before and at least one month after treatment. Impedance Aggregometry (Multiplate analyzer-IA) in whole blood was performed using ADP, collagen, ristocetin arachidonic acid (AA) and thrombin (TRAP-6) as agonists. Light Transmission Aggregometry (LTA) was also performed in platelet rich plasma (PRP) and washed patient platelets with ADP and colllagen (two different concentrations) epinephrine, ristocetin and AA. TxB2 (thromboxane B2) was measured by elisa (ENZO) in washed patient platelets after aggregation using AA for 6 min and the addition of EDTA as stop solution.

Results: Collagen aggregation was reduced i) In pts whole blood IA (mean before treatment (A) 26U, mean after treatment (B) 25U and controls mean (C) 63U.

ii) LTA in washed pts platelets with concentration 20 µg/ml B:32% vs C:81% (p< 0.0001) and with 10 µg/ml B:18% vs C:73% (p< 0.0001).

Ristocetin in whole blood (IA) in pts with platelets >10^9/L (n=8) showed A: 34U, B:21U vs C: 109U.

LTA with washed platelets using AA as agonist showed reduced aggregation B:49% vs C:59% p=0.014. TXB2 was found reduced in pts vs controls (B:750ng/ml vs C:1112ng/ml).

Conclusions: Ibrutinib has a more pronounced effect on platelets aggregation with collagen and ristocetin and a mild effect on AA aggregation. Patients in our cohort didn´t show a bleeding phenotype. IA is simple and fast and could be used to advice pts programmed to undergone hemostasis challenges.

PB 1603 | The Platelet Function Analyzed by Flow Cytometry in Psoriatic Patients on Reference Therapy

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Background: Platelets have an important role in hemostasis and thrombosis as well as in increasing inflammation. The pathogenesis of psoriasis may involve platelet activation.

Aims: Aim of the study was to identify platelet function activity in patients with a psoriasis on reference therapy by flow cytometry accurate measurement method.

Methods: The study included 13 psoriatic patients at the age of 44±9 years and 25 healthy volunteers at comparable age. All patients received the reference therapy and the selection phototherapy. The amount of receptor GPIIb-IIIa and P-selectin on the platelet surface were assessed in whole blood and platelet-rich plasma before and after activation of 10 uM ADP by flow cytometer Cytomics FC500 (Beckman Coulter). The fluorescence-labeled monoclonal antibodies to CD61 (GPIIb-IIIa) and CD62 (P-selectin) were used.

Results: The pool of platelets expressed P-selectin after 20 uM ADP activation was significantly lower in psoriatic patients prior to treatment in comparison to healthy volunteers (4,4±1,9% vs 26,7±2,0%; p<0.05). The expression of P-selectin on the activated platelets membrane increase up to 7,9±4,4% (p<0.05) to Day 10 of reference therapy but has not reached the level of healthy individuals. The same results were obtained for GPIIb-IIIa: before ADP-activation 5,9±0,7 MFI, after ADP-activation 18,2±1,9 MFI; in healthy volunteers - 9,6±0,4 MFI and 25,1±1,4 MFI, respectively; p<0.05.

Conclusions: The decrease in the number of ADP-activated platelets may reflect their exhaustion as a result of long circulation and involving in inflammatory processes in skin. Against the background of the carried-out therapy the P-selectin expression on platelets surface increase significantly, caused by emergence of functionally full-fledged platelets replenished from bone marrow.

Thus, system reference therapy of psoriasis in combination with phototherapy, leads to platelets function normalization trend according to decrease in inflammatory presentation of skin.

PB 1604 | PANDA, a Biomarker for Diagnostic of Leaky Gut Syndrome

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Background: Platelets are anucleate cells that play a crucial role in primary hemostasis. In addition, they also play a role in inflammation and immunity by expressing Toll-like receptors (TLRs) to sense and bind microbial products, such as LPS. In patients with leaky gut syndrome (LGS), a condition also referred to as „increased intestinal permeability", LPS leaks into the intestinal microcirculation and binds to platelet´s surfaces. Heparins also interact with platelets via the GP IIIa, that is a part of the fibrinogen receptor (GPIIb/IIIa). After binding of heparin molecules on GPIIIa, platelets are partially activated and started platelet-platelet interactions with adhesive proteins. The activation threshold of LPS-bound platelets by heparin is lower than that of LPS-free platelets. For this reason, we propose that the **P**latelet **A**nalyzed **N**umber in **D**ifferent **A**nticoagulants (or PANDA) could be an indicator (biomarker) for LGS.

Aims: The aim of this work was to investigate whether differential platelet aggregation in heparin and EDTA blood could be a biomarker for diagnostic of LGS.

Methods: Platelet count was determined in EDTA- and heparin-blood from 40 patients with LGS-associated gastrointestinal diseases using an automatized hematological counter. Platelet count was also determined in healthy adult volunteers (control group).

Results: A highly significant difference ($p < 0.0001$) in platelet count in heparin and EDTA blood was seen in patients with gastrointestinal diseases. In contrast, in healthy volunteers the platelet number in both blood samples was very similar.

Conclusions: Our results indicate that the PANDA test can be used as Biomarker for LGS-associated gastrointestinal diseases.

PB 2357 | Knock down the CD70 Expression in DCs Could Inhibit the CD4+ T Cells Differentiating to Tregs in ITP

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Background: CD70 expressed on T cells causes a decrease in T-cell response, an impairment of autoreactive T-cell immune tolerance.

Aims: This study aims to assess the biological properties and functions of CD70 costimulatory molecule on dendritic cells(DCs) from patients with chronic immune thrombocytopenia (ITP).

Methods: DCs were generated from monocytes isolated from peripheral blood. Chemically synthesized siRNA was then transferred into the cells to block CD70 expression. The CD4+ T cells were co-cultured with DCs to assess the suppression capacity of DCs in the proliferation of CD4+ T cells and the production of IL-10 and IFN-γ by CD4+ T cells. The CD4+ T cells were co-cultured with DCs to assess the ability of DCs in the induction of Regulatory T cells (Tregs).

Results: Dendritic cells were transfected with FITC-labelled scrambled siRNA for 4-6h. The transfection efficiency was average 60%. After transfection (48 h), the results of real-time RT-PCR analysis showed strong and specific downregulation of CD70 mRNA. DCs from transfected group could inhibit the proliferation of PHA-activated CD4+ T cells than the negative control in ITP patients($p=0.008$), but showed no difference in group of normal control ($p=0.08$). In chronic ITP patients and normal controls, the DCs from transfected group could inhibit the expression of IFN-γ compared with the group of negative control ($p=0.018$, $p=0.043$) and increase the expression of IL-10 ($p=0.012$, $p=0.043$). In chronic ITP patients and normal controls, the DCs from transfected group were defective in inducing the CD4+ T cell differentiating to CD4+CD25+CD127low Tregs compared to the negative control ($p=0.012$, $p=0.043$).

Conclusions: In our study, the DCs from siRNA group could inhibit the CD4+ T cells and induce the CD4+ T cells differentiating to Tregs. This approach is a useful tool by which costimulatory molecules of DC can be studied as well as a potential therapeutic option for autoimmune disease.

PB 2358 | IL-10 Producing B Cells Induced by CD72 Ligation Suppress Th1 and Th17 Polarization in Primary Immune Thrombocytopenia

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Background: Primary immune thrombocytopenia (ITP) is characterized by reduced platelet count secondary to immune-mediated destruction. Impaired regulatory T cell (Treg) and B cell (Breg) compartment and skewed T helper cells (Th)1 and possibly Th17 responses have been described in ITP patients. CD72, a co-receptor of B cell, is involved in the pathogenesis of ITP. The role of CD72 on defect of Breg and aberrant T-cell polarization in ITP remains unknown.

Aims: We aimed to investigate the contribution of CD72 in control of interleukin (IL)-10 producing B cells and Th development in chronic ITP.

Methods: Distribution of IL-10 producing B cells was tested in ITP patients and healthy controls. Peripheral blood mononuclear cells (PBMCs) or PBMCs depleted of $CD19^+$ B cells were activated by anti-CD40 and IL-21 in the presence or absence of anti-CD72 to elucidate the effects of CD72 on IL-10 producing B cells and $CD4^+$T cells. The potential regulatory mechanism of CD72 was also investigated in co-culture of isolated $CD4^+$T cells with $CD19^+$B cells.

Results: The frequency of IL-10 producing B cells was decreased in ITP patients and was positively correlated with platelet counts.In culture of PBMCs with anti-CD72, the frequency of IL-10 producing B cells was elevated and $CD40^+$T cell proliferation was inhibited in patients and controls. Furthermore, under this stimulatory condition, $CD4^+$ T cells from ITP patients produced less interferon-γ (IFN-γ) and IL-17 but more IL-4, whereas their conversions into Tregs and IL-10^+T cells remained unchanged. Depletion of B cells completely reversed the effect of B cells mediated by CD72 on $CD4^+$ Th cells and cytokine production. Moreover, the observed regulatory effect of B cells mediated by CD72 was partly dependent on IL-10 release and cell-to-cell contact.

Conclusions: This study showed that the ligation of CD72 might suppress effector T cells by enhancing IL-10 producing B cells in ITP. This underscores the critical role of targeting CD72 in the amelioration of ITP.

PB 2359 | Impact of Platelet Transfusion on Thromboembolic Events in Patients with Acute Heparin-induced Thrombocytopenia

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Background: Platelet concentrate (PC) transfusion is considered to be relatively contraindicated for heparin-induced thrombocytopenia (HIT), since transfused platelets (plts) could be activated by HIT antibodies (Abs), triggering thromboembolic events (TEEs). However, it remains unclear if PC transfusion is a risk for TEEs in HIT.

Aims: To clarify the impact of PC transfusion on TEEs in HIT patients (pts) whose diagnosis was confirmed with the washed plt activation assay, considering the temporal relationship between PC transfusion and TEEs.

Methods: Sera of pts that activated plts at a therapeutic (but not high) heparin concentration were defined as positive. Of these, sera that activated plts within 30 min or in the absence of heparin were defined as strongly positive (SG+). The remaining sera were considered weakly positive (WK+).

Results: We analyzed 609 pts clinically suspected of having HIT (Table 1). Pts with stronger plt-activating Abs had a higher incidence of TEEs after heparin exposure than those who tested negative (NEG) [60% (70/116) in SG+, 48% (33/69) in WK+, and 31% (130/424) in NEG, p< 0.001). In each group, 20-29% of pts received PC transfusion for thrombocytopenia that led to HIT suspicion. Among transfused pts, the incidence of TEEs after heparin exposure was higher in pts with stronger plt-activating Abs [52% (14/27) in SG+, 43% (6/14) in WK+, and 27% (33/121) in NEG, p=0.031], but the incidence of TEEs after PC transfusion was almost similar [22% (6/27) in SG+, 21% (3/14) in WK+, and 15% (18/121) in NEG, p=0.51]. In HIT pts, 89% (8/9) of TEEs after PC transfusion occurred before starting non-heparin anticoagulants.

Conclusions: Among pts who received PC transfusion for thrombocytopenia that led to HIT suspicion, the overall incidence of TEEs was

TABLE 1 Association of platelet transfusion with thromboembolic events in patients clinically suspected of having HIT

Patients clinically suspected of having HIT (n = 609)	enrolled in a nationwide HIT registry	approved by the Ethics Review Committees		χ^2 or Fisher´s exact test
Washed platelet activation assay	Strongly positive	Weakly positive	Negative	
Diagnosis	HIT	HIT	non-HIT	
Number of patients	116 (19.0%)	69 (11.3%)	424 (69.6%)	609 (100%)
TEEs after heparin exposure	70/116 (60.3%)	33/69 (47.8%)	130/424 (30.7%)	p<0.001
PC transfusion for thrombocytopenia leading to HIT suspicion	27/116 (23.3%)	14/69 (20.3%)	121/424 (28.5%)	p=0.24
Among patients with PC transfusion				
TEEs after heparin exposure	14/27 (51.9%)	6/14 (42.9%)	33/121 (27.3%)	p=0.031
TEEs after PC transfusion	6/27 (22.2%)	3/14 (21.4%)	18/121 (14.9%)	p=0.51

higher in pts with HIT Abs with more robust plt-activating properties. However, PC transfusion appeared not to increase TEEs significantly in HIT pts even with stronger plt-activating Abs, especially in those undergoing non-heparin anticoagulant therapy.

PB 2360 | Isolation of a Monoclonal IgG Kappa with Functional Autoantibody Activity against Platelet Factor 4/Heparin from a Patient with a Monoclonal Gammopathy of Undetermined Significance and Clinically Overt Heparin Thrombocytopenia

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Background: A 62-year-old man followed for several years for chronic thrombocytopenia (platelets 80 G/L) and monoclonal gammopathy of undetermined significance (MGUS, IgG1 kappa peak 3.8 g/l) was hospitalized for cerebral thrombophlebitis. At day 6 of treatment with unfractionated heparin, he developed extensive thrombosis of the right radial and ulnar vein, which made suspect heparin-induced thrombocytopenia (HIT). IgG antibodies against platelet factor 4 / heparin (PF4/H) were strongly positive (>3000AU, Zymutest®HIA IgG; Hyphen BioMed). Heparin-induced platelet agglutination assay (HIPA) was positive at therapeutic concentrations of heparin (0.2 and 0.5 IU/ml) and negative at 50 IU/ml, confirming the diagnosis of HIT. Platelet activation was observed in the absence of heparin. This result, combined with the presence of thrombocytopenia and thrombosis prior to treatment with heparin, suggested spontaneous HIT. We tested patient plasma collected before initiation of heparin treatment and found a high titer of anti-PF4/H that persisted beyond 100 days after the thrombotic episode.

Aims: To show that patient monoclonal IgG and anti-PF4/H antibodies are one same entity.

Methods: Anti-PF4/H antibodies were purified by affinity chromatography.

Results: Immunofixation carried out on the fraction obtained at pH 2.5 showed that it contained a monoclonal IgG kappa. This fraction tested in ELISA showed a strong affinity for PF4/H but not for PF4 alone and induced platelet activation in the presence and absence of heparin.

Conclusions: This is the first reported case of MGUS associated with a human monoclonal IgG with anti-PF4/H activity responsible for a clinically overt HIT. This human monoclonal IgG has the specificity of activating platelets even in the absence of heparin, which distinguishes it from other monoclonal anti PF4/H such as KKO. We therefore aim to further characterize the reactivity of this monoclonal IgG in order to identify its precise endogenous antigenic target.

PB 2361 | Anticoagulation Stewardship Program for Heparin Induced Thrombocytopenia: A Cooperative Effort between a Community Hospital System Pharmacy Anticoagulation Service and an Associated Reference Laboratory

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Background: Heparin induced thrombocytopenia(HIT) is a rare and potentially life- or limb-threatening complication. HIT misdiagnosis may cause exposure to high-cost, unindicated anticoagulants with high bleeding risk. Anticoagulation treatment is typically initiated when a heparin induced platelet antibody(HIPAB) is ordered. Prior studies including a pharmacy/lab intervention have demonstrated a reduction in inappropriate ordering of HIT labs by utilizing the 4T score and contacting the physician for low risk patients.

Aims: We hypothesized that a pharmacy-led, multidisciplinary team-based intervention with 4T pre-test probability would reduce inappropriate HIPAB tests, reduce costs, and decrease exposure to high-risk anticoagulants. A previous retrospective study of our system revealed that 81% (47/58) of patients tested in a 3 month period were low risk and unindicated.

Methods: In March 2016, the laboratory began notifying the pharmacy when HIPAB tests were ordered. A pharmacist would calculate a 4T score, contact the ordering provider and suggest test cancellation if low (≤3) risk. Special coagulation pathologists provided clinical support to the pharmacist if needed.

Results: After eight months, only 14.8% (16/108) of HIPAB tests were unindicated. The intervention resulted in a decrease of inappropriate HIT testing by 66.2% and estimated cost savings of $45,000. Average monthly tests decreased from 23 ordered and 23 processed to 14.5 and 7.1 per month, respectively in the same time frame, with an 86% acceptance of cancelling HIPAB for recommended patients. Overall ordered HIT labs decreased from 219 in 2015 down to 50 in 2016 increasing HIPAB yield from 10% to 44%.

Conclusions: A joint multidisciplinary team based intervention with Pharmacists, Pathologists, and special coagulation technicians can result in improved patient care and cost savings. This study provides validation that a pharmacist intervention and multidisciplinary approach in multiple hospitals systems can improve patient care as related to HIT.

PB 2362 | IP-10 and MCP-1 Gene Polymorphisms in Chinese Patients with Chronic Immune Thrombocytopenia

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Background: Aberrant Th1/Th2 polarization is considered to play a crucial role in the abnormal immune state of primary immune thrombocytopenia (ITP). IFN-γ-inducible protein of 10 kilodaltons (IP-10) and Monocyte chemoattractant protein-1(MCP-1) gene are involved in enhancing the Th1 and Th2 immune response, respectively. The serum levels and biological activity of the IP-10 and MCP-1 proteins may be regulated by a single nucleotide polymorphism occurring at position -201 and position -2518 of the IP-10 and MCP-1 gene promoters.

Aims: We designed our study on investigating the relationship between the distribution of IP-10 (-201G/A) and MCP-1(−2518A/G) polymorphisms and the susceptibility of ITP.

Methods: A total of 235 ITP patients and 162 healthy donors were enrolled in this study, and the distributions of polymorphisms were investigated mainly by polymerase chain reaction-restriction fragment length polymorphism. Stratified analyses were conducted based on gender and onset age.

Results: The percentage of AA + AG genotype and the frequency of A allele of IP-10 in the ITP group was significantly elevated compared to the control group, while no differences were revealed in the genotype and allele distributions of MCP-1 between the two groups. Additionally, a comparable association between the AA + AG genotype and the ITP disease was observed in the adult cohort in IP-10. In the female cohort, a more evident discrepancy with regard to allelic and genotypic frequency distributions was observed in the IP-10 gene.However, there was no significant difference in either genotypes or allelic distribution of IP-10 in the male cohort.

Conclusions: In this study we demonstrated that the A allele and AA+AG genotype of IP-10 were associated with an increased risk of chronic ITP in Chinese people, indicating that individuals with A allele on IP-10 might have a higher risk of chronic ITP. This genetic change may be partly associated with the Th1 dominant immune response and female prone of the development of adult chronic ITP.

PB 2363 | Decreased TLR4 Expression on Monocytes May Cause Regulatory T Cells Abnormality in Patients with Primary Immune Thrombocytopenia

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Background: TLR4, a key member of the TLRs, has been reported to be implicated in autoimmune diseases. An increasing number of studies have indicated that the TLR4 signaling pathway might interact with Tregs. However, the effect of TLR4 on Treg differentiation in chronic ITP remains unclarified.

Aims: The aim of the study was to explore the effect of TLR4 on Treg differentiation in chronic ITP.

Methods: Expression of TLR4 on monocytes in ITP patients and healthy controls was determined by flow cytometry. PBMCs were cultured in complete RPMI 1640 medium with or without anti-TLR4 and meanwhile stimulated with lipopolysaccharide (LPS). Tregs and Th17 cells were detected by flow cytometry. Levels of IL-10, IL-17A and TGFB1 in cell culture supernatants were measured by ELISA.

Results: Expression of TLR4 on monocytes was significantly decreased in patients with ITP than that in healthy controls and it had positive correlation with platelet count. The result of further experiments *in vitro* showed that activation of TLR4 with LPS could promote differentiation of Treg cells and anti-TLR4 attenuated this effect. There was no significant difference about Th17 cells among three subgroups. However, the Th17/Treg cell ratio was decreased after stimulation with LPS and increased with anti-TLR4. Moreover, activation of TLR4 with LPS could significantly promote the secretion of IL-10 and TGFB1, while anti-TLR4 significantly suppressed the secretion of them. Nevertheless, the secretion of IL-17A did not reach the statistical difference among three subgroups.

Conclusions: Decreased TLR4 appears to cause Tregs abnormality in ITP by modulating Tregs differentiation and immunoregulatory cytokines.

PB 2364 | The Abnormal Expression of Rap1 in Various Cells of Patients with Immune Thrombocytopenia

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Background: Primary immune thrombocytopenia (ITP) is an autoimmune disease involved in the abnormal immunity and megakaryopoiesis. Rap1 plays a dominant role in the regulation of cell-cell interactions and MAP kinase (MAPK) activity in lymphocytes and megakaryocytes, which are important pathogenesis of ITP.

Aims: This study aimed to investigate the role of Rap1 played in ITP.

Methods: Megakaryocytes were induced from bone marrow $CD34^+$ cells of ITP patients and healthy controls (HC) in vitro. The expression of Rap1 in lymphocytes and megakaryocytes were measured by flow cytometry. The expression and subcellular localization of the Rap1 in immature and mature megakaryocytes were analyzed by confocal microscopy.

Results: The results showed that the expression of Rap1 in $CD4^+$ T, $CD8^+$ T, and $CD19^+$ B lymphocytes of ITP patients were significantly lower than that of healthy controls ($P = 0.004$, $P = 0.005$, $P = 0.009$, respectively). And the expression levels of Rap1 were increased obviously of ITP patients in remission

($P = 0.022$, $P = 0.019$, $P = 0.019$, respectively). Rap1 was predominantly detected on internal α-granule and the plasma membrane. High levels of Rap1 were detected in the majority of immature

megakaryocytes (ITP versus HC, $P > 0.05$), while the expression levels were decreased with the maturation of megakaryocytes (ITP versus HC, $P < 0.05$).

Conclusions: This is the first study indicates that abnormal expression of Rap1 in lymphocytes and megakaryocytes might participate in the pathogenesis of ITP. These findings suggest Rap1 as a therapeutic agent for ITP to be assessed in the future.

PB 2365 | Resequencing of GWAS Loci Identifies New Candidate Genes on Chromosome 5 for Heparin-induced Thrombocytopenia

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Background: Treatment with unfractionated (UFH) or low molecular weight (LMWH) heparins is an important strategy in the prevention and treatment of thrombotic diseases. Heparin-induced thrombocytopenia (HIT) is the most important specific adverse drug reaction (ADR) in response to heparin therapy resulting in a paradoxical prothrombotic state.

Aims: We conducted a pharmacogenetic genome-wide association study (GWAS) to explore whether a genetic predisposition contributes to the individual risk for HIT.

Methods: Within a surveillance case-control study program at a single pharmacovigilance center in Berlin, Germany, we selected 182 suspected HIT cases and 182 controls (matched for age, sex and exposure to the same class of heparin) for the pharmacogenetic study consisting of a GWAS (96 cases and 96 controls) and a replication in additional 86 cases, followed by imputing and overall fine mapping analysis. In order to find variants not detected in our GWAS but contributing to the detected association signals, we applied next generation sequencing (NGS)-based targeted resequencing approach in a subgroup of 73 HIT patients and 23 controls.

Results: One single nucleotide polymorphism (SNP, rs1433265) from initially 16 identified SNPs in our GWAS was successfully replicated ($P=1.5\times10^{-4}$) and remained the most strongly associated SNP ($P=3.5\times10^{-5}$) after imputing genotypes on chromosome 5. Fine mapping revealed two significantly associated haplotypes with an odds ratio of 0.63 (95% CI, 0.46-0.88; $P=5.6\times10^{-3}$) and 2.41 (95% CI, 1.64-3.55; $P=4.9\times10^{-6}$). We applied targeted resequencing for the regions with the 16 most strongly HIT-associated SNPs. A C-alpha test was applied to perform a gene-based test for the impact of rare variants in our targeted region and we were able to detect two candidate genes, *ICE1* (P = 0.010) and *ADAMTS16* (P= 0.005) containing 17 and 23 rare variants, respectively.

Conclusions: These results provide a basis for further studies that aim to characterize the genetic predisposition to HIT.

PB 2366 | Elevated Plasma sCXCL16 Correlated with Th1 Polarization in Patients with Primary Immune Thrombocytopenia

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Background: CXCL16, a CXC subtype member of the chemokine superfamily, exists in two forms, the membrane-bound CXCL16 and the soluble CXCL16 (sCXCL16). CXCL16 is the sole ligand for the receptor CXCR6. sCXCL16 functions as a chemotactic factor for functional-CXCR6-expressing Th1 and Tc1 cells. Moreover, sCXCL16 has been showed to be modulated by Th1 type cytokines, such as IFN-γ and TNF-α and implicated in immune response of autoimmune diseases. However, the role of sCXCL16 in ITP has not been clarified yet.

Aims: The aim of the study was to explore the role of plasma sCXCL16 in ITP.

Methods: Plasma sCXCL16, IFN-γ and IL-4 were measured by ELISA. Expression of CXCR6 on lymphocyte subsets were determined by flow cytometry. The mRNA expression of CXCL16 and CXCR6 were detected based on real-time PCR. In addition, plasma sCXCL16, CXCL16 and CXCR6 mRNA levels of 8 patients were monitored before and after treatment.

Results: Plasma sCXCL16 levels were significantly increased in active ITP patients compared to remission patients and healthy controls. And elevated plasma sCXCL16 levels positively correlated with IFN-γ, IFN-γ/IL-4 ratio and negatively correlated with IL-4, platelet counts in ITP patients. Besides, expression of CXCR6 on lymphocyte subsets and mRNA levels of CXCL16 and CXCR6 in PBMCs were higher in active ITP patients compared to remission patients and healthy controls. Additionally, plasma sCXCL16 and IFN-γ levels were reduced while CXCR6 mRNA expression were down-regulation after effective treatment compared with those before treatment.

Conclusions: Elevated plasma sCXCL16 might be implicated in the pathogenesis of ITP by correlated with Th1 polarization and disease activity.

PB 2367 | Time to Disappearance of Platelet-activating Antibodies Depends on the Strength of Their Platelet Activating Properties in Patients with Heparin-induced Thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is caused by platelet-activating antibodies (HIT Abs), which decline to an

Fig

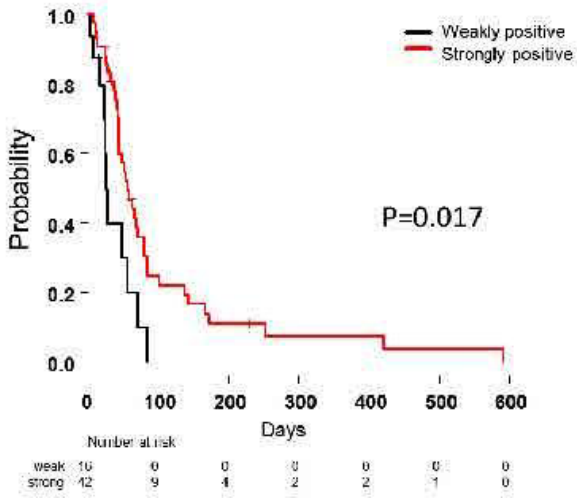

FIGURE 1

undetectable level within a couple of months. Since evidence for an anamnestic response on heparin re-exposure is lacking in patients (pts) with a history of HIT, heparin can be re-administered during cardiovascular surgery (CVS) when HIT Abs are no longer detectable. Therefore, predicting the time to the disappearance of HIT Abs is crucial in acute HIT patients who require CVS.

Aims: To clarify the time to the disappearance of HIT Abs with the washed platelet activation assay.

Methods: In a nationwide registry of pts clinically suspected of having HIT, 196 pts whose sera activated platelets at a therapeutic (but not high) heparin concentration were diagnosed with HIT. The current analysis included 58 pts in whom subsequent sera were available to study changes in levels of HIT Abs at least twice over time. Sera that activated platelets within 30 min or in the absence of added heparin were defined as strongly positive (pos). The remaining positive sera were considered weakly pos.

Results: Among the 58 pts, 42 and 16 tested as strongly and weakly pos, respectively, when HIT was diagnosed. Figure 1 shows the proportion of pts over time in whom HIT Abs remained detectable. The time to disappearance of HIT Abs was significantly longer in pts who initially tested strongly pos than those who tested weakly pos (p=0.017, log-rank test). The median time to a negative test according to Kaplan-Meier analysis was 55 and 26 days in pts who tested strongly vs weakly pos, respectively.

Conclusions: Higher strength platelet-activating properties of HIT Abs were significantly associated with longer time to Ab disappearance. Based on these results, we recommend that if acute HIT patients require CVS, those who are weakly pos should wait for a couple weeks while those who are strongly pos should undergo plasma exchange or use an alternative anticoagulant.

PB 2368 | Predictive Factors for Successful Splenectomy in Immune Thrombocytopenic Purpura

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Background: In the era of new drugs a proper patient's selection for splenectomy is particularly necessary. However, it is not clear whether there are pre or post-operative parameters able to predict splenectomy response.

Aims: To determine the pre and postoperative factors with potential to predict successful splenectomy outcome.

Methods: We retrospectively analyzed the data on 123 ITP patients (median age 43 years, range 19-74; 84/39 female/male; median follow-up from splenectomy 112 months (range: 2-364)) who underwent splenectomy between 1986 and 2015.

Results: The median pre-operative platelet count (PC) was 99×10^9/L (range 20-320×10^9/L). Complete remission (CR) was achievedin in 95/120 (79%), partial remission (PR) in 10/120 (7.5%) and 15/120 (112.5%) patients were refractory. Thirteen of the 110 (11.8%) responsive patients relapsed.

By univariate analysis younger age (41 vs 48, p= 0.025), lower number of pre-splenectomy therapies

(1 vs 2, p=0.028), response to steroids (69.5% vs 22.7%, p=0.021), higher PC at the time of splenectomy (90×10^9/L vs. 37×10^9/L, p=0.021), higher PC on the first and seventh days after splenectomy (387×25^9/L vs. 25×10^9/L, p=0.0035) and platelet destruction in spleen (86% vs 0%, p=0.0003) were predictive for good response. By multivariate analysis younger age (p=0.003), and the PC on the first (p=0.043) and seventh days (p=0.0013) after splenectomy were predictive of a favorable response.

Relapsed patients had significantly lower CR rate (53% vs. 93%, p=0.002), PC three weeks (150×10^9/L vs. 340×10^9/L, p=0.048) and three months (130×10^9/L vs. 278×10^9/L, p=0.007) after splenectomy. Only CR rate retained statistical significance (p=0.0023) by multivariate analysis.

Conclusions: Splenectomy is effective in approximately two thirds of patients with ITP. Our study have been identified some potential predictive factor for successful splenectomy. However, large population based studies for testing a predictive cut-off of age, pre and post-splenectomy PC values are needed.

PB 2369 | The Th17/Treg Imbalance and Fatigue in Primary Chronic Immune Thrombocytopenia

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Background: Fatigue is an important symptom for patients with primary chronic immune thrombocytopenia (ITP). However, the biological mechanisms underlying ITP related-fatigue are unknown.

Aims: This study aimed to determine the relationship between ITP related-fatigue and Th17/Treg imbalance.

Methods: We assessed fatigue in patients with chronic ITP using the Functional Assessment of Chronic Illness Therapy-Fatigue scale (FACIT-Fatigue). Peripheral blood was collected to assess Th1, Th2, Th17 and Treg cell populations by Flow cytometry and to measure the levels of interleukin-10 (IL-10), IL-6, IFN-γ, IL-4, IL-17A, and IL-1β by ELISA.

Results: Thirty-seven patients were recruited including fifteen men and twenty-two women aged between 18 and 65 years (mean age 45.6 ± 12.6 years). Participants were dichotomized into severe fatigue (n = 12) and low-moderate fatigue (n = 25) groups. There were no differences between the two groups stratified by fatigue severity with regard to age, gender, disease duration, platelet counts, comorbidities, and previous treatment regimens. The ratio of Th17 to Treg was significantly higher in the severe fatigue group compared to the low to moderate fatigue group (0.30±0.25 versus 0.42±0.21, p=0.038). Plasma IL10 level was significantly higher in low to moderate fatigue patients than in severe fatigue patients (1.48±0.51 versus 0.91±0.47, p=0.002). Furthermore, plasma IL-6 level in severe fatigue group was significantly higher in low to moderate fatigue group (2.08±1.28 versus 1.49±0.51, p=0.019). In contrast, there was no significant difference between IFN-γ, IL-4, IL-17A, and IL-1β cytokine concentration between the two groups. Th17/Treg ratio correlates with plasma IL-6 levels (r=0.407, p= 0.013). Higher IL-10 levels were associated with a lower Th17/Treg ratio (r = −0.408, p = 0.025).

Conclusions: Our preliminary findings suggest that an imbalance in the Th17/Treg ratio is associated with the fatigue severity of chronic ITP.

PB 2370 | Predictors of Fatigue among Individuals with Primary Immune Thrombocytopenia in China

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Background: Fatigue is an aspect of primary immune thrombocytopenia (ITP), which is important to patients.

Aims: This cross-sectional study was conducted to examine a range of variables potentially associated with fatigue among individuals with ITP and to determine which variables best predict subjective fatigue.

Methods: Two hundred and seven patients with ITP and 213 matched healthy controls (MHCs) were assessed for patient-reported fatigue with the Chinese version of the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue scale. In addition, to further investigate other factors potentially predicting fatigue; sleep quality (the Pittsburgh sleep quality index (PSQI)), physiological conditions (the Hospital Anxiety and Depression scale (HADS)) and bleeding score(the ITP-specific Bleeding Assessment Tool (ITP-BAT)) were also evaluated. Correlations between fatigue and other variables were examined, and the predictors of fatigue were determined with multiple regression analyses.

Results: The mean age was 42.1±10.9 years and 40.5±11.1 years in ITP patients and healthy controls, respectively (p=0.864). The FACIT-F score was significantly lower in ITP patients (37.5±9.05) compared to 217 MHCs (45.8±6.02) (p=0.001).

The FACIT-F score in ITP patients was negatively correlated with the PSQI (r=-0.654, p 0.001), HADS-depression (r=-0.598, p 0.001), HADS-anxiety (r=-0.616, p 0.001) and bleeding severity (r=-0.276, p 0.001). Platelet count was positively correlated with the FACIT-F score (r=0.307, p 0.001). A multiple linear regression analysis revealed that ITP-related fatigue could be explained by sleep quality, platelet count, depression, and bleeding severity.

Conclusions: Fatigue severity was mainly associated with disease-related factors but also patient-related variables, indicating that the etiology of fatigue in ITP is multifactorial. Interventions addressing depressive symptoms, sleep quality, bleeding symptoms and platelet count could be potential avenues for treatment of fatigue in patients with ITP.

PB 2371 | Elevated Plasmacytoid DCs Proportion and Plasma IFNα Concentration in Active ITP Patients

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Background: Dendritic cells (DCs) are key factors in linking innate and adaptive immunity. Among them, Myeloid DCs (mDCs) are highly potent in antigen capture, presentation and therefore can effectively stimulate T-cells. plasmacytoid DCs (pDCs) release copious amounts of type I IFNs following activation and induce differentiation of plasma cells. DCs abnormalities are involved in many autoimmunities. However, whether the distribution of DCs is abnormal in ITP was still unknown.

Aims: To detect the distribution of DCs and associated cytokines expressions in ITP patients.

Methods: A total of 23 untreated active ITP patients, 25 ITP patients in remission and 28 healthy donors (HCs) were enrolled in this study. Our research was approved by the hospital-based ethics committee and comply with the declaration of Helsinki. In addition, the proportion of mDCs (lin-HLA-DR+CD11c+CD123-) and pDCs (lin-HLA-DR+CD11c-CD123+) were detected by FACS. The plasma IFNα, IL-6 and TNFα levels were analyzed by ELISA. And the mRNA expressions of Interferon regulatory factor (IRF) 5, IRF7, Toll like receptor (TLR)7 and TLR9 in peripheral blood mononuclear cells were detected by real-time PCR.

Results:

(1) The percent of pDCs in peripheral blood from active ITP patients (16.64±3.04) was higher than those of patients in remission (10.24±1.39) and HCs (9.56±1.43), and no difference were found between patients in remission and HCs. However, although mDCs proportion seems decreased in active ITP compared with HCs, there was no difference was found.

(2) the plasma IFNα concentration in active ITP patients (40.15±8.42 pg/mL) was elevated compared with this in patients in remission (15.15±3.75 pg/mL) and HCs (8.78±2.67 pg/mL), and no difference was found between patients in remission and HCs .Nevertheless, the concentrations of IL-6 and TNFα were not different among these three subsets.

(3) No difference of IRF5, IRF7, TLR7 and TLR9 mRNA expressions were found.

Conclusions: elevated proportion of pDCs may be involved in ITP by promoting IFNα secretion.

Figure 1

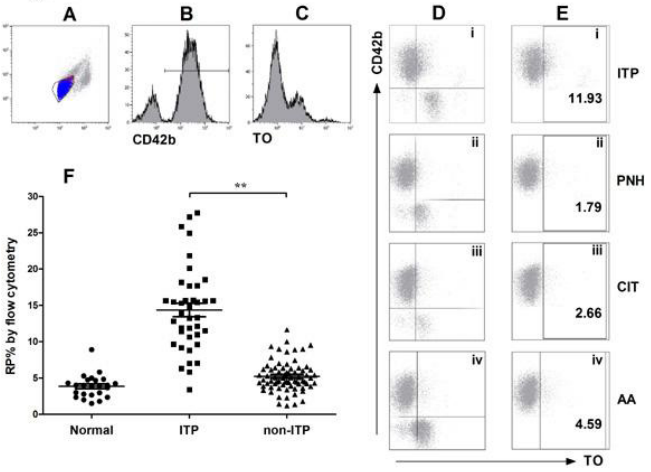

FIGURE 1 Representative flow images of RP% in patients with ITP and other thrombocytopenic disorders

PB 2372 | A Prospective, Double-blind, Multicenter Study on FCM-based Measurement of Circulating Reticulated Platelets as a Diagnostic Assay for Immune Thrombocytopenia

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Background: Reticulated platelets (RP) are immature platelets that contain residual RNA and represent the youngest platelets in the circulation. Based on differential diagnosis, immune thrombocytopenia (ITP) still lacks a definitive test to support the identification of active patients.

Aims: To distinguish ITP from isolated thrombocytopenia due to aplastic thrombocytopenic disorders or chemotherapy-induced thrombocytopenia.

Methods: We performed a 6-month, prospective, multicenter validation study (NCT02967328) involving 6 clinical sites and 524 patients each with a platelet count less than 60×10^9/L. Informed consent was obtained from all participants and the study was approved by the medical ethics committee of Qilu Hospital. The percentages of reticulated platelets (RP%) were analyzed by flow cytometry using thiazole orange that binds cytosolic RNA. Results of a central, blinded clinical review of the entire cohort served as the reference standard. The differences between mean values were evaluated using Student *t* test.

Results: Of the 524 tested patients, 39 were classified as ITP by the review board. The FCM-based measurement of RP% identified 34 out of 39, and RP% above 8.6% (mean + 3SD of controls) had a negative predictive value of 98.8% (95% confidence interval [CI], 97.2 to 99.6) with a sensitivity of 87.2% (95% CI, 71.8 to 95.2) and a specificity of 88.0% (95% CI, 84.7 to 90.7). ITP patients with RP% above 8.6% demonstrated significantly higher levels of immature platelets (Fig. 1), lower concentrations of serum thrombopoietin (Fig. 2) and big platelet morphology showing brilliant cresyl blue-stained granules, compared to other thrombocytopenic patients. Bone marrow cytologic analysis of the 5 patients with false negative results revealed 4 with insufficient numbers of megakaryocytes.

Conclusions: These data suggested that FCM-based measurement of RP% might be valuable in identifying thrombocytopenic disorders due to early platelet destruction such as ITP.

Figure 2

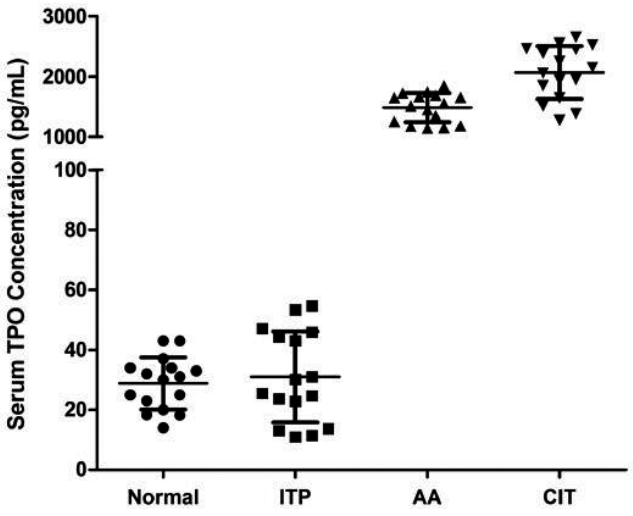

FIGURE 2 Serum TPO levels of enrolled patients and controls

PB 2373 | Comparison Head-to Head of Two Functional Assays in the Assessment of Heparin-induced Thrombocytopenia

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Background: Diagnosis of Heparin-induced thrombocytopenia (HIT) is difficult to achieve especially in cardiac surgery and requires laboratory confirmation using functional tests.

Aims: To compare the performance of 2 functional assays: serotonin release assay (SRA) and heparin-induced platelet activation test (HIPA) in patients suspected of HIT in cardiac surgery and medical setting.

Methods: 115 patients suspected of HIT (78 after cardiac surgery and 37 in medical setting) were included between 2010 and 2015. Criteria of inclusion were: anti-PF4/heparin IgG (OD>0.5 Zymutest®HIA IgG; Hyphen BioMed), HIPA and SRA results. Two senior hematologists established final clinical diagnosis according to kinetics of platelet count, existence of any other cause of thrombocytopenia, +/- thrombotic event, anti-PF4/H levels, HIPA and SRA results (HIPA was positive if patient's plasma activated at least 2 donor washed platelets in the presence of 0.2 or 0.5 IU/ml of heparin but not at 0 and 50 IU/ml of heparin, SRA was positive if the release was ≥20% at 0.1 or 0.5 IU/ml of heparin with no release at 0 and 10 IU/ml heparin from washed platelets of at least one donor).

Results: Among the 115 patients, 51 (44%) had a diagnosis of HIT based on HIPA and/or SRA positive results. Sensibility and specificity for SRA were 0,92, and for HIPA 0.84 and 1, respectively. Positive and negative predictive value of HIPA and SRA were very similar (table1). In 11 patients with HIT diagnosis, discordant results were observed: SRA was the only positive test in 7 out of the 11 and HIPA in 4 others. In 3 patients without HIT confirmed, SRA was the only positive functional test. No patient had only HIPA positive test in this group.

TABLE 1 Tests performances for diagnosis of Heparin-induced thrombocytopenia (HIT) of HIPA and SRA in all patients

	HIPA	SRA
Sensitivity, % (95% CI)	84,4 (70,5-93,5)	92,2 (81,1-97,8)
Specificity, % (95% CI)	100 (93,0-100)	92,3 (81,5-97,9)
Positive predictive value, % (95% CI)	100 (90,8-100)	92,2 (81,1-97,8)
Negative predictive value, % (95% CI)	87,9 (76,7-95,0)	92,3 (81,5-97,9)

Conclusions: We observed good and similar performance of HIPA and SRA. Interestingly, sensibility and specificity seem better in cardiac surgery than medical setting (table2). Comparison head-to-head of HIPA and SRA demonstrates that HIPA could be used to confirm the HIT diagnostic in all clinical settings.

TABLE 2 Tests performances for diagnosis of HIT of HIPA and SRA in patients in cardiac surgery and medical setting

	Cardiac surgery		Medical setting	
	HIPA	SRA	HIPA	SRA
Sensitivity, % (95% CI)	90,6 (75,0-98,0)	94,3 (80,8-99,3)	69,2 (38,6-90,9)	87,5 (61,7-98,5)
Specificity, % (95% CI)	100 (89,4-100)	97,0 (84,2-99,9)	100 (81,5-100)	84,2 (60,4-96,6)
Positive predictive value, % (95% CI)	100 (88,1-100)	97,1 (84,7-99,9)	100 (66,4-100)	82,4 (56,6-96,2)
Negative predictive value, % (95% CI)	91,7 (77,5-98,3)	94,1 (80,3-99,3)	81,8 (59,7-94,8)	88,9 (65,3-98,6)

PB 2374 | Regulation of FcγRIIA-mediated Platelet Activation by a Cytochalasin-sensitive Cytoskeletal Rearrangement: its Clinical Application to the Detection of Functional HIT Antibody

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Background: The signaling pathway resulting from FcγRIIA is essential in platelet activation induced by heparin-induced thrombocytopenia (HIT) antibody (Ab), i.e. anti-platelet factor 4/heparin.

Aims: We examined the effect of cytochalasin on the activation of FcγRIIA on the assumption that a unique cytoskeletal rearrangement may be employed in this signaling pathway.

Methods: A mouse monoclonal HIT Ab (mouse HIT MoAb) used in this study was derived from clone 5A1, and the aggregation of washed platelets, which were collected from healthy donors, was observed by using PA-200 (Kowa). This study was approved by the Institutional Research Ethics Committee of the Faculty of Medicine, the University of Tokyo.

Results: A long lag time before the onset of platelet aggregation was observed for that induced by a mouse HIT MoAb. Platelet aggregation induced by the mouse HIT MoAb was dependent on the concentrations of this antibody and heparin and the platelet count, and was completely inhibited by high concentrations of heparin and anti-human CD32 antibody (clone IV.3). When high concentrations of the mouse HIT MoAb were added, platelet aggregation occurred in the absence of heparin, which may be analogous to spontaneous HIT syndrome. When platelets were preincubated with suitable concentrations

(around 0.5 μg/mL) of cytochalasin B (CB), the lag time was markedly shortened before the onset of platelet aggregation induced by the mouse HIT MoAb; even heparin-independency was induced by the CB treatment. Finally, the effects of the mouse HIT MoAb was mimicked by HIT Ab from the patients with HIT. Our present results indicate that platelet activation via FcγRIIA, including that by the mouse HIT Ab, involves a cytochalasin-sensitive cytoskeletal rearrangement and that CB enhances platelet activation induced by HIT Ab.

Conclusions: In a practical viewpoint, the use of CB may be useful for the improvement of sensitivity in the detection of functional HIT Ab.

PB 2375 | Vitamin D Receptor Gene Polymorphisms in Adult Primary Immune Thrombocytopenia

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Background: Recently, several studies have demonstrated the role of vitamin D receptor (VDR) polymorphisms in the development of autoimmune diseases. Vitamin D affect both innate and adaptive immune responses that have been blamed in immune thrombocytopenia (ITP) pathogenesis.

Aims: The aim of this study is to assess the association of vitamin D receptor gene polymorphism BsmI in cases of adult primary immune thrombocytopenia.

Methods: Vitamin D receptor polymorphism *BsmI* (rs1544410) was detected by Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP). Deoxyribonucleic acid (DNA) samples were extracted from peripheral blood of 40 ITP patients and 60 geographically and ethnically matched healthy controls.

Results: Statistically significant difference was found in the BsmI polymorphism between ITP patients and controls ($\chi 2 = 8.77$, P value=0.01). The BsmI polymorphism B allele was higher in ITP group than that in controls but in statistically insignificant difference ($\chi 2 = 2.125$, P = 0.145). bb genotype played a protective role in ITP incidence.

Conclusions: This is the first published report on VDR gene polymorphisms in adult primary ITP patients. The BsmI genotype was associated with increased risk for ITP incidence with no obvious effect on bleeding severity, platelet count nor site of bleeding.

PB 2376 | Anti-PF4/Heparin Antibodies and Platelet Derived PF4 Bearing Microparticles in Acute Sepsis

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Background: Heparin induced thrombocytopenia (HIT) is due to antibodies (ab) against platelet factor 4 (PF4) bound to heparin. A possible pre-immunization by bacterial antigens mimicking PF4/heparin complexes (PF4/H) has been suggested with anti-PF4/H ab generation during infection. Platelet derived microparticles (MPs) were shown both in HIT and in sepsis, as markers of platelets activation.

Aims: To evaluate the development of anti-PF4/H ab and the levels of total MPs and platelet derived PF4 bearing MPs (PF4-MPs) in acute septic patients.

Methods: Thirty-four patients admitted for sepsis (cases) confirmed by clinical and laboratory exams, 28 inpatients treated with heparin without infection (C1) and 65 healthy subjects (C2) were studied. Anti-PF4/H ab, either IgG-IgM-IgA or specific IgG, expressed by optical density (OD), were assayed by ELISA methods; MPs were measured using flow cytometry. In septic patients anti-H/PF4 ab and MPs were measured at admission (T0) and 7 days later (T1).

Results: Anti-PF4/H IgG-IgA-IgM levels were significantly higher in cases (T0 0.341 OD, T1 0.367 OD) compared to both C1 (0.224 OD, $p < 0.05$) and C2 (0.303 OD, $p < 0.05$), whereas anti-PF4/H IgG levels in cases showed a non significant increase at T1, and were slightly higher respect to both control groups. Total MPs median levels in cases were significantly increased as compared to C2 (cases T0 3251 MP/μL and T1 2044 MP/μL vs C2 1728 MP/μL, $p < 0.001$), but not to C1 (2388 MP/μL). PF4-MPs levels were significantly higher only in cases at T0 (391 MPs/uL) than in both C1 (94 MPs/uL, $p < 0.001$) and C2 (93 MPs/uL, $p < 0.001$). No correlation was seen between anti-PF4/H ab and MPs, clinical and biochemical parameters.

Conclusions: In sepsis we showed a modest but significant development of anti-PF4/H ab, likely IgM type suggesting an immunization. In addition, we found for the first time the generation of PF4-MPs as a result of platelets activation, and the role of these MPs in sepsis needs to be clarified.

PB 2377 | Monocyte Subsets and Platelet Apoptosis in Patients with Primary Immune Thrombocytopaenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder. In its pathophysiology, autoantibodies against platelet glycoproteins and abnormalities in T lymphocytes-mediated immunity are involved. Platelet-monocyte cross-talk participates in many immune-inflammatory diseases and their interaction might regulate life span of they both (Pawelski et al, Front Biosci, 2014,6:75-91).

Aims: We aimed to study monocyte subsets distribution in ITP patients and their correlation with platelet apoptosis.

Methods: Blood samples of 29 patients with primary chronic ITP and 32 healthy controls sex and gender matched were included.

A multi-parametric flow cytometry panel (BD Biosciences) were used for the characterization of monocyte subsets. Platelet-surface exposure of phosphatidylserine (PS) and active caspase-3, -8 or -9 were assessed by flow cytometry (FITC-labelled Annexin V, BD Pharmingen and Millipore respectively).

E-selectin and plasminogen activator inhibitor-1 (PAI-1) were determined by ELISA.

Results: Intermediate (IM, CD14++CD16+), but not non-classical (NCM, CD14+CD16++) and classical (CM, CD14++CD16-) monocytes were increased in ITP patients (Table 1). The lower the platelet count the higher increase in IM count (Spearman r = -0.6, p < 0.001).

Platelets from ITP patients significantly exposed more PS than controls and activity of their caspases -3, -8 and -9 were increased (Table 1).

TABLE 1 Results are expressed as Mean±SD or Median (range). *P<0.05; **P<0.001

	CONTROL	ITP
CLASSICAL MONOCYTES/µL	67 (57-111)	75 (42-110)
INTERMEDIATE MONOCYTES/µL	98 (64-141)	596 (394-824)**
NON CLASSICAL MONOCYTES/µL	501 (410-631)	513 (352-757)
ANNEXIN V BINDING (%)	59.3±8.4	69.0±14.6*
CASPASE-3 (%)	47.4±8.9	60.8±10.0*
CASPASE-8 (%)	47.8±9.7	63.4±13.0*
CASPASE-9 (%)	47.5±8.3	58.0±13.1*
PAI-1 (ng/ml)	10.4 (6.6-23.4)	27.01 (16.2-43.8)**
E-Selectin (ng/ml)	10.69 (6.57-14.47)	30.49 (17.53-45.33)**

These apoptosis markers significantly correlated to IM and NCM counts (Table 2).

TABLE 2 Correlation calculated with Spearman test. "R" values are shown. All of them are significant with P<0.001

	ANNEXIN V (%)	CASPASE 3 (%)	CASPASE 8 (%)	CASPASE 9 (%)
INTERMEDIATE MONOCYTES/µL	0.425	0.608	0.721	0.663
NON CLASSICAL MONOCYTES/µL	0.422	0.633	0.759	0.705

Since monocyte-platelet interaction might be involved in endothelial damage, E-selectin and PAI-1, markers of endothelial injury, were measured and increased plasma levels of they both were found (Table 1).

Conclusions: Our results showed that IM subset was expanded and correlated with thrombocytopaenia and platelet apoptosis signs in ITP patients. Moreover, their interaction might react against vascular endothelium in ITP patients as reported for other autoimmune diseases.

PB 2378 | BAFF-TACI Signaling Promotes the Differentiation of Terminal Effector T Cells in Primary Immune Thrombocytopenia (ITP)

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Background: Primary immune thrombocytopenia (ITP) is an autoimmune disease. Its pathogenesis involves complex immune dysfunctions. Besides autoantibody-secreting B cells, $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells and aberrant cytokine profiles also play important roles. Our previous study has shown that B cell activating factor (BAFF), via binding to TACI (transmembrane activator and CAML interactor), is involved in ITP by influencing the homeostasis and polarization of $CD4^+$ T cells.

Aims: To explore how BAFF-TACI signaling influences T cell memory and migration.

Methods: Peripheral blood mononuclear cells form ITP patients and healthy controls (HCs) were co-cultured with allogeneic dendritic cells under stimulation of IL-2 and BAFF with/without BAFF-receptor blocking antibodies.

Results:

1. BAFF -TACI interaction did not change the differentiation profiles of central memory T cells (T_{CM}) and effector memory T cells (T_{EM}) among $CD4^+$ cells.
2. The proportion of $CCR7^-$ cells, whose migrating ability toward draining lymphoid nodes is tampered owing to lost of CCR7, was significantly increased in $CD8^+$ T cells in both patients and HCs under BAFF-TACI signaling.
3. The increase of $CD8^+CCR7^-$ cells was not accompanied by changes of the activation status of $CD8^+$ T cells, but rather by transformational biases towards different $CD8^+$ T-cell subsets. Specifically, BAFF-TACI interaction caused a preferential differentiation of terminal effector cells ($T_{TermEff}$, $CD45RA^+CCR7^-CD27^-$) in ITP; whereas a $CD8^+$ T_{EM} ($CD45RA^-CCR7^-CD27^-$) transformation dominated in $CD8^+$ cells of HCs.

Conclusions:

1. BAFF-TACI signaling has no influence on $CD4^+$ memory cell profiles.
2. BAFF-TACI interaction weakens the homing ability of $CD8^+$ T cells in both HCs and ITP by promoting the transformation of $CCR7^-$ cells.
3. BAFF-TACI signaling increases the cytotoxicity of $CD8^+$ T cells in ITP by interfering with their differentiating direction, which leads to an aberrant over-transformation of terminal effector cells.

PB 2379 | Delayed Clearance of Argatroban in Patients with Heparin Induced Thrombocytopenia

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Background: Argatroban is a direct thrombin inhibitor used in the treatment of Heparin Induced Thrombocytopenia (HIT). Argatroban has advantages including a short half-life (45 minutes), ease of monitoring with the activated partial thromboplastin time (APTT) and limited renal clearance. However, patients with HIT typically have significant co-morbidities including hepatic dysfunction or multi-organ failure which have been associated with a lower dose requirement for Argatroban.

Aims: To quantify the risk of drug accumulation in patients treated with Argatroban.

Methods: Patients were identified from laboratory records of Heparin Induced Platelet Aggregation (HIPA) results. Data collected included: age, gender, clinical diagnoses, dose and duration of Argatroban, presence of significant organ dysfunction, evidence of drug accumulation during treatment and thrombotic or bleeding complications.

Results: Eight patients were identified with a positive HIPA who were treated with Argatroban between 2013-2016. Starting doses of Argatroban were: 2mcg/kg/min (n=1, 14.5%), 1mcg/kg/min (n=5, 71%), 0.5mcg/kg (n=1, 14.5%), missing data n=1. During treatment with Argatroban, four patients required dose adjustments; increased in two and decreased in two (by 10% and 90%). Two patients showed evidence of clinically important delayed clearance of Argatroban with therapeutic levels of Argatroban at more than 16 hours after cessation of the infusion. In one patient, delayed clearance occurred without the presence of hepatic dysfunction or multi-organ failure. Delayed clearance resulted in postponement of a planned procedure but no bleeding.

Conclusions: Two of eight patients treated with Argatroban showed evidence of drug accumulation, in one case without typical risk factors. Clinical management of patients with HIT in a critical care setting may be complicated by delayed clearance.

PB 2380 | Sleep Quality in Chinese Patients with Primary Immune Thrombocytopenia: Contributing Factors and Effects on Health-related Quality of Life

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Background: Normal sleep is paramount for a healthy lifestyle and high quality of life. Sleep modulates the immune system and thus affects the course of several chronic inflammatory conditions.

Aims: The objective of this study was to examine the prevalence of sleep disturbance, contributors of sleep disturbance and the association between sleep quality and health related quality of life (HRQoL) in patients with ITP independent of known predictors of HRQoL.

Methods: We enrolled 249 patients with ITP in the cross-sectional study. Self-report sleep quality (Pittsburgh Sleep Quality Index [PSQI]), and HRQoL (36-item Short Form [SF-36]) were evaluated for all patients. Independent samples t-tests, Chi square analysis, logistic regression modeling and linear regression were used to analyze these data.

Results: Our study found that one hundred and thirty-eight (71%) patients with ITP were ‚poor sleepers' (global PSQI≥7). There was a significant positive correlation of global PSQI score with fear of bleeding among ITP patients (r=0.670, p 0.001). Meanwhile, logistic regression models identified fear of bleeding (odds ratio 4.383 [95% confidence interval 2.941; 6.533]) as predictor of poor sleep quality. Poor sleepers in ITP had a significantly lower Physical Component Scale (PCS) scores, Mental Component Scale (MCS) scores and total SF-36 scores than good sleepers. The global PSQI score was a significant independent predictor of the MCS (β= -0.549, p 0.001) and PCS (β= -0.634, p 0.001) after controlling for age, gender, fear of bleeding, platelet count at diagnosis and current treatment measures in multivariate analysis.

Conclusions: Sleep disturbance is common for patients with ITP in China, which significantly impaired their HRQoL. The findings suggest that fear of bleeding contributed significantly to sleep disturbances in ITP and the importance of objective interventions to improve their sleep quality and finally to improve their HRQoL.

PB 2381 | Immune Thrombocytopenia in Patients with Chronic Hepatitis C Virus Infection and Non-alcoholic Fatty Liver Disease

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Background: Thrombocytopenia(TP) is a challenging clinical problem in patients with chronic hepatitis C virus infection(HCV) and nonalcoholic fatty liver disease(NAFLD). Development of TP in these patients is multifactorial but one of the main cause is immune genesis. Previous studies showed that amount of macrophage-lymphocyte rosettes (MLR) formation in vein blood leukocyte culture correlates with immune reactivity of organism(in norm it equals 37,4±2,2%, while in patients with immune hemolytic anemia or immune thrombocytopenia(ITP) it increases up to 60-85%).

Aims: Our aim was to reveal immune genesis of TP in patients with chronic HCV and NAFLD.

Methods: Fibroscan and Fibrotest were used for assessment METAVIR stages of fibrosis. We studied 15 patients with NAFLD(9 with cirrhosis(F4), 6 with F2-F3) and 25 with chronic HCV(15 with cirrhosis(F4), 10 with F2-F3). All patients had moderate(50-75×10^3/μL) or severe(< 50×10^3/μL) TP. In all was studied MLR formation in vitro.

Results: Among patients with NAFLD in 5(3 cirrhosis, 2-F3) MLR was in the range of 20-25%, pointing to nonimmune genesis of TP,

whereas in the rest(6 cirrhosis, 4 with F2-F3) MLR reached 69-78% revealing ITP. In chronic HCV patients low MLR was revealed in 2 with cirrhosis and 3 with F2, while in all the rest (13 with cirrhosis, 7 with F2-F3) was revealed high indices of MLR(62-83%) pointing to ITP. It is notable that in all cases of nonimmune TP, TP was moderate, while in ITP cases it was severe or moderate. In chronic HCV patients treated with Sofosbuvir or Ledipasvir/Sofosbuvir amount of platelets increased in all cases. Platelet count reached low level of norm in non-immune TP, while in patients with ITP mild thrombocytopenia was remained.

Conclusions: In conclusion, MLR formation in vitro can be used successfully for the revealing ITP in patients with chronic liver disease to tailor more precise treatment for each patient, especially in cases of severe TP, when platelet transfusions can cause platelet refractoriness due to HLA alloimmunization.

PB 2382 | Epidemiology and Management of Immune Thrombocytopenia: A Nationwide Population-based Study in Korea

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Background: The epidemiology of immune thrombocytopenia (ITP) is not well characterized in an Asian population.

Aims: We aim to investigate the nationwide incidence and practice patterns of ITP.

Methods: From 2010 to 2014, ITP patients were identified using the Korean Health Insurance Review and Assessment Service database.

Results: The overall incidence rate of ITP was 5.3 per 100,000 person-years (95% CI: 5.1-5.5). The overall incidence rate ratios of children under 15 years old to adults and females to males were 3.9 (95% CI: 3.7-3.9) and 1.3 (95% CI: 1.2-1.4), respectively. Of the total 10,814 patients, 3,388 patients (31%) needed treatment for ITP. Among treated patients, 1,821 patients (54%) continued treatment for more than three months. First-line therapy consisted of corticosteroids (CS) in 42%, immunoglobulin (IVIg) in 35%, CS with IVIg in 19%, and other immunosuppressive agents (ISA) in 4%. Among treated patients, 75% of adults and 33% of children continued treatment for more than three months. After three months, the most frequently used drug was CS in 63% of patients, followed by CS with IVIg in 17%, ISA in 15%, and IVIg in 5%. Only 104 patients underwent splenectomy; of these, 51% received salvage treatment after a median of one month after surgery (range: 0-27). Platelet transfusions were performed in 1,082 patients. The proportion of patients who received platelet transfusions of 12 units or more per month for at least two consecutive months was significantly higher among patients treated for more than three months compared with patients who completed treatment within three months.

Conclusions: This population-based study is the first to describe the incidence of ITP and its treatment reality for patients in Korea.

PB 2383 | Laboratory Identification of Heparin Induced Thrombocytopenia: A Re-evaluation

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Background: Heparin induced thrombocytopenia (HIT) is a feared complication of heparin therapy, assessed clinically and facilitated by laboratory testing, which comprises both immunological and functional assays. Clinical evaluation is aided by pre-test probability assessment, for example by 4Ts score. Neither 4Ts nor lab testing has 100% sensitivity or specificity for HIT, and combining both in a sequential process offers the highest likelihood of appropriate diagnosis or exclusion of HIT.

Aims: To (re)evaluate HIT diagnosis/exclusion using contemporary laboratory methods combined with 4Ts.

Methods: Prospective study combining 4Ts with a battery of contemporary methods, including ELISA, chemiluminescence (AcuSTar), later flow (STiC), serotonin release assay (SRA), light transmission aggregometry (LTA) and whole-blood aggregometry (Multiplate).

Results: 80 patients referred for HIT testing were evaluated. Immunological methods (ELISA, AcuSTar, and STiC) showed limited agreement with one another (r^2 values < 0.5), despite yielding similar proportions of negativity/positivity (Table 1). Overall, STiC yielded many equivocal findings, and ELISA higher levels of positives. Three functional methods also showed limited agreement, although Multiplate showed better correlation with SRA than LTA. Using SRA as gold standard, all immunological methods showed reasonable sensitivity but poor specificity to HIT. Multiplate showed higher sensitivity than LTA to HIT. Stronger reactivity in all immunological methods showed better sensitivity to HIT than lower reactivity. Overall, a combination of immunological methods, taking into consideration reactivity strength, plus 4Ts showed highest likelihood of appropriate diagnosis or exclusion of HIT.

TABLE 1 Differential test patterns in HIT investigation

Grade, rank	Means	STIC (%)	AcuStar (%)	ELISA (%)	SRA (%)	Multiplate (%)
-, 0	negative	44.7	73.0	59.4	64.4	69.7
+/-, 1	equivocal	28.9	NA	NA	NA	NA
+, 2	positive	15.4	9.0	12.9	5.9	11.8
++, 3	strong positive	11.1	18.0	27.7	29.7	18.4
0 or 1	neg or equiv	73.5	73.0	59.4	64.4	69.7
1 or 2	all positive	26.5	27.0	40.6	35.6	30.3

Conclusions: Laboratories should be aware of the strengths and limitations of individual methods for detecting HIT. A composite and sequential approach provides the best opportunity for effective diagnosis/exclusion of HIT.

PB 2384 | Experience in Diagnostic Assays for Heparin-induced Thrombocytopenia

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Background: The diagnosis of heparin-induced thrombocytopenia (HIT) requires clinical data and laboratory detection of platelet activating factor 4/heparin (PF4/H) antibodies by immunological or functional assays. Although antigen screening assays are widely used, the functional assays are performed only by several expert labs.

Aims: To analyze the experience of a tertiary medical center in clinical and laboratory diagnosis of suspected HIT.

Methods: A retrospective review of the Hematology Laboratory database on patients evaluated between 2008-2016 at Rambam, identified 412 individuals with clinical suspicion of HIT. Till 2011, 135 cases were screened using particle gel PaGIA (Biorad) and between 2012-2016, 277 cases were screened by lateral flow Milenia (Biotec GmbH). All patients diagnosed with HIT were treated with Fondaparinux (Arixtra). Functional assay with heparin/LMWH induced platelet aggregation was performed using light transmission aggregometry (Helena AggRAM) to validate borderline or positive results in indistinct cases.

Results: From the tested samples, 63% vs. 75% were negative in PaGIA and Milenia, respectively (P=0.03), and were considered negative for HIT. During 2008-2011, only 38% of cases with non-negative immunoassay results underwent functional aggregation, whereas, in 2012-2016, 83% of such cases were further evaluated. None of the borderline PaGIA samples was positive in the functional assay compared to 13.3% borderline Milenia results. 10.5% of positive PaGIA and 51.7% of positive Milenia were confirmed by a positive functional HIT assay (P=N.S.). The survival rate among 14 patients with a positive functional assay was 42.7 % (6 patients).

Conclusions: The Milenia assay introduced at our lab in 2012, has improved the screening process. The functional assay provides a more accurate HIT diagnosis. Combined approach of an optimal laboratory and clinical investigation is crucial to obtain a precise HIT diagnosis.

PB 2385 | Prevalence of Heparin Induced Thrombocytopenia among Iranian Patients with Cardiac Surgery

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Background: Heparin-induced thrombocytopenia (HIT) is a life-threatening, pro-thrombotic syndrome caused by anti-PF4/ heparin IgG antibodies(PF4 Ab). It's incidence is different among patients population with higher incidence of PF4 Ab in post-cardiac surgery. HIT diagnosis in Iran is almost always based on clinical criteria and rarely with considering the results of immunological assays (very limited centers performing first-line HIT diagnostic tests but no functional assays). This approach may lead to over-diagnosis of HIT and inappropriate use of alternative anticoagulants.

Aims: To determine the prevalence of HIT among patients undergo CPB, along with planning for setting up heparin induced platelet aggregation (HIPA) functional assay to establish a national referral HIT diagnostic lab for the first time in Iran.

Methods: We followed 92 patients that had been undergone CPB from 9/2015 to 5/2016.The signs and symptoms of HIT were evaluated by 4Tscoring system as a probability testing. From all cases with 4Tscore ≥ 4, an anticoagulant free blood sample was obtained and sent to the referral coagulation lab of IBTO for detection of Anti/Heparin PF4 IgG Ab (ELISA) and antibodies with ability of platelet activation (HIPA). Subjected patients were considered as HIT if they showed all three criteria: 4TScore≥4, Positive PF4 Ab (OD≥0.2) and Positive HIPA. All laboratory tests were rechecked in Hospital of Greifswald University, Germany (kindly supported by Professor A. Greinacher).

Results: 14 patients had 4TScore≥4 (15 %). 8 of 14 patients (8.6%) = PF4 Ab positive. 5 of all cases fulfil all criteria
(5.4 % incidence of HIT in our study).

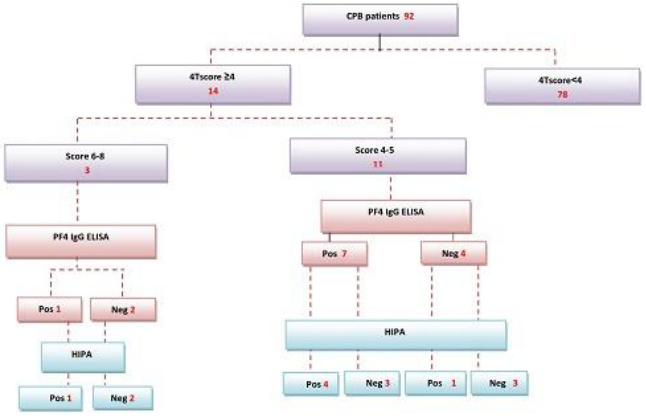

FIGURE 1 Flowchart of clinical and laboratory results of patients

TABLE 1 clinical and laboratory data of 5 patients with definite HIT

Patient no.	Age (years)	4T,s-score	Anti-PF4 / heparin (OD units)	HIPA test	Thrombosis event
1	64	5	0.617	Positive	No
2	63	5	0.470	positive	No
3	66	5	0.286	Positive	No
4	61	5	1.028	Positive	Aortic valve thrombosis
5	72	6	1.088	Positive	Recurrent stroke

Conclusions: Our study indicated that for accurate diagnosis of HIT, considering clinical criteria along with complete laboratory diagnostic panel (including immunological as well as functional assays) are necessary to prevent over-diagnosis of HIT and for this purpose establishment of at least one national referral lab is needed in our country with about 80 million population.

PB 2386 | HIT or Miss: Improving the Quality of Diagnosing Heparin-induced Thrombocytopenia at an Academic Medical Center

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Background: Heparin-induced thrombocytopenia (HIT) is a rare but serious complication seen in hospitalized patients. The 4T score is a validated tool to assess the pretest probability of HIT. Although a low 4T score has been shown to have a high negative predictive value, previous studies suggest that the 4T scoring system is often underutilized prior to ordering heparin-platelet factor 4 (PF4) antibodies or the platelet serotonin release assay.

Aims: To assess the efficiency of calculating the 4T score prior to heparin-PF4 antibody testing when HIT is suspected.

Methods: We performed a retrospective chart review of patients over the age of 18 who had heparin-PF4 antibody testing ordered between January and August 2016. 4T scores were calculated retrospectively from data in the electronic medical record (EMR).

Results: 181 heparin-PF4 antibody tests were performed, of which 146 (81%) were negative (low dose optical density < 0.40), 31 (17%) positive and 4 (2%) equivocal. The 4T score was significantly higher in patients with positive or equivocal heparin-PF4 antibody values compared to those with negative values (p< 0.0001) when analyzed either as dichotomous or continuous variables [Figure 1]. ROC curves demonstrated that the 4T score was highly predictive of a positive or equivocal heparin-PF4 antibody value [Figure 2: AUC 0.804, 95% CI, 0.736 to 0.884]. A 4T score of ≤2 and ≤3 had negative predictive values of 96% and 92%, respectively. 123/181 (68%) of heparin-PF4 antibody tests were ordered in patients with 4T scores ≤3, amounting to a cost of $45,387 (estimated annual cost: $68,081), excluding the additional costs of managing HIT. Subsequently, a data-driven algorithm utilizing a 4T calculator prior to heparin-PF4 antibody testing was implemented within the EMR. We plan to reevaluate utilization in 6 months.

Conclusions: Incorporating routine 4T scoring prior to heparin-PF4 antibody testing for HIT has the potential to be an efficient and cost-effective management tool at large medical centers.

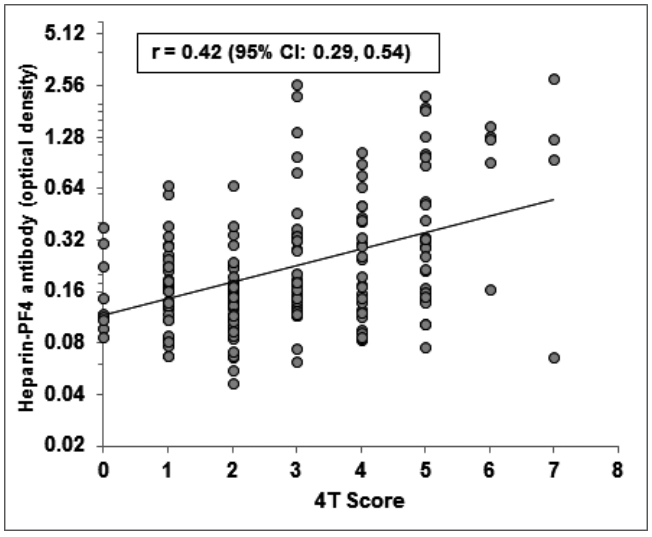

FIGURE 1 Correlation of 4T score with heparin-PF4 antibody (measured by optical density).

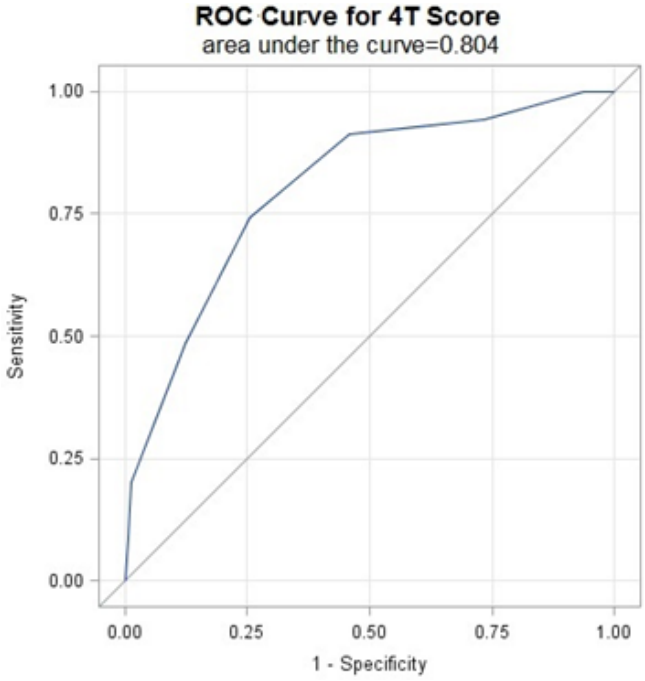

FIGURE 2 ROC curve of 4T score as a predictor of equivocal/positive heparin-PF4 antibody test.

PB 2387 | Practical Limitations of Diagnostic Algorithms in the Management of Heparin Induced Thrombocytopenia: Clinical Case Report

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Background: Heparin-induced thrombocytopenia (HIT) can be associated with devastating complications such as life-threatening

thrombosis making it one of the most serious adverse drug reactions.

Aims: The diagnosis of HIT is challenging due to a number of practical issues and methodological limitations.

Methods: We report on a clinical case of HIT which demonstrates the limited sensitivity of a rapid assay and the 4Ts score, respectively, emphasising the need for a clear diagnostic algorithm in the clinical practice.

Results: A 61 year old woman with a spinal cord tumour was admitted to our hospital with pain and tenderness in the right leg. Clinical examination and laboratory markers were suggestive for thrombosis. Despite treatment using low molecular weight heparin (LMWH) the patient developed a progressive thrombosis involving calf, popliteal, femoral, and vena cava. The diagnosis of HIT was suspected. First evaluation of the patient by the treating physician revealed a 4Ts score of 3. The rapid immunoassay (Lateral Flow Immunoassay) was negative. Although these results were suggestive against HIT, we initiated further laboratory investigation and decided to switch heparin to argatroban due to the progressive thrombosis. Strong anti-PF4/heparin IgG antibodies were detected using ELISA (OD 2.458) and the Heparin induced platelet activation (HIPA, platelet activation 4/4 cells). Critical review of the medical records and direct patient´s interview revealed that the 4Ts score was miscalculated on day 7 because of

(1) previous treatment with LMWH heparin (initially reported as s.c. insulin) was not recognized and rapid-onset HIT was not considered, and

(2) chemotherapy was considered to be a sufficient reason for thrombocytopenia. The 4Ts score was re-calculated revealing 6/8 points (high risk).

Conclusions: In clinical practice, we recommend an integrated diagnostic approach combining clinical assessment (the 4Ts score), immunoassays and functional assays as well.

PB 2388 | Low Serum Vitamin D Levels in Egyptian Adults with Chronic Primary Immune Thrombocytopenia: A Single Center Study

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Background: Immune thrombocytopenia (ITP) is a disorder characterized by immune-mediated accelerated platelet destruction and suppressed platelet production. Low vitamin D levels have been found in several autoimmune diseases, such as rheumatoid arthritis, SLE. The mechanisms underlying the link between vitamin D with autoimmunity are not completely understood. No currently available studies about vitamin D status in primary ITP patients.

Aims: To evaluate vitamin D levels in patients with primary chronic ITP and compare these levels with normal control subjects and thrombocytopenia due to other non immune causes.

Methods: The study included 80 adult subjects, 40 ITP patients (They were segregated into 20 responders and 20 non-responders), 20 cases of thrombocytopenia due to non ITP causes and 20 healthy control subjects. Measurement of serum 25vitamin D was done with ELISA.

Results: Vitamin D levels were significantly lower in patients with ITP (range=2-40ng/ml; mean±SD=17.29±10.96 ng/ml) and thrombocytopenia due to non-ITP causes (range=10-40ng/ml; mean±SD=21.05±8.31 ng/ml) in comparison to normal healthy controls (range = 10-65 ng/ml; mean±SD=36.70±16.30 ng/ml) (P=0.000), but there was no statistically significant difference between levels in ITP versus non- ITP thrombocytopenia (P=0.225). There was no statistical significant difference between responders & non-responders.

Conclusions: Vitamin D levels are lower among ITP patients in relation to healthy control subjects which might point out to a role in aetiopathogenesis of ITP attributable to the sunshine vitamin deficiency.

PB 2389 | Efficacy and Safety of a New 10% Intravenous Immunoglobulin Product in Patients with Primary Immune Thrombocytopenic Purpura

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Background: Intravenous immunoglobulin (IVIg) is a key drug in the treatment of primary immune thrombocytopenia (ITP). We recently developed IVIG-SN 10%, a 10% intravenous immunoglobulin formulation.

Aims: This study aimed to investigate the efficacy and safety of IVIG-SN 10% in adult patients with primary ITP.

Methods: It was a non-randomized, open-label, single arm, multicenter prospective study (ClinicalTrials.gov identifier: NCT02063789). Patients received 10% IV-Globulin SN formulation with a dose of 1g/kg/day for two consecutive days; infusion rate was initially 0.01mg/kg/minute and then doubled every 30 minutes to a maximum of 0.08 ml/kg/minute. The primary endpoint was the response after treatment with 10% IV-Globulin SN formulation.

Results: One hundred five patients with severe ITP were enrolled. Among 81 eligible patients, 31 patients were newly diagnosed, 7 patients had a persistent ITP, and 43 patients had a chronic ITP. Their median time to response was 2 days and the mean duration of platelet count ≥ 50 x 10^9/L was 8.80 ± 7.90 days. Response rates were not significantly different when compared according to the phase of ITP or previous treatment for ITP. The drug was well tolerated and the frequency of mucocutaneous bleeding was decreased during the study period.

Conclusions: 10% IV-Globulin SN formulation was efficacious in adult prmary ITP patients regardless of their disease status as well as safe given that the resolution of bleeding and manageable adverse events.

PB 2390 | Quantification of Specific T and B Cells Immunological Markers in Children with Chronic and Transient ITP

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Background: : Immune Thrombocytopenia (ITP) is characterized by a transient (non-chronic) or permanent (chronic) decline in the number of platelets. Predicting the course of ITP, at the time of diagnosis, is of importance. Here we studied at diagnosis, clinical and immunological parameters in order to distinguish between different courses. The latter included the measure of new B and T cells using quantification of kappa-deleting recombination excision circles (KRECs) and T-cell receptor excision circles (TRECs), respectively.

Aims: The purpose of this study was to look at clinical and immunological parameters which at the time of diagnosis will enable one to distinguish between patients with chronic and non-chronic courses of ITP. Another goal was to evaluate the immune function in patients by measuring neogenesis of B and T cells using quantification of KREC and TREC, respectively.

Methods: Blood samples were collected from 44 children with a clinical diagnosis of ITP. Real Time PCR was performed in order to quantify the number of copies of TREC and KREC followed by collection of clinical data from medical files. The children were retrospectively divided into two groups: chronic and non-chronic.

Results: 24 patients (54%) were classified as non-chronic ITP and 20 patients (46%) were classified as chronic ITP. We confirmed some clinical parameters (e.g. gender, age) but not others (e.g. preceding infection, level of thrombocytopenia) that distinguish patients with chronic and non-chronic course. While KREC quantification was similar in patients regardless the outcome of their disease, it was significantly higher than the level of controls ($p<0.05$). TREC quantification was not different between patients and controls.

Conclusions: KREC but not TREC levels are different in patients comparing to controls, pointing to an overreaction of B cell development as a role in the pathogenesis of ITP. These results may shed more lights on the immune mechanism of ITP.

PB 2391 | Risk of Cancer Occurrence and Survival in Patients with Primary Immune Thrombocytopenia

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Background: It has been shown that platelet-derived serotonin supports hepatocellular carcinoma development in mice chronically exposed to carbon tetrachloride. Mouse models of acute viral hepatitis in which hepatocellular damage is resolved in a few days to show that platelets are present at sites of organ damage; their depletion reduces the number of virus-specific CD8 T cells ameliorating disease severity; and this positive outcome is reversed by reconstituting thrombocytopenic animals with normal but not dysfunctional platelets.

Aims: The aim of our study is to investigate whether the number of platelet is associated with cancer development and progression.

Methods: We analyzed data of 968 patients with primary immune thrombocytopenia (ITP) retrieved from Taiwan's National Health Insurance Research Database between 1997 and 2013, by comparing variables to 9680 age- and gender-matched healthy individuals from the general population.

Results: There were 103 ITP patients and 866 individuals of general population with newly diagnosed cancer (Hazard ratio 0.871, 95 % CI 0.659-1.151). The cumulative incidences of cancer in ITP and the general population were 10.6 % and 9.0 %, respectively. The survival time after acquiring cancer is shorter in ITP cohort (HR 1.955, 95 % CI 1.377 - 2.777). After excluding patients who died of bleeding and infection, no difference of survival time after acquiring cancer was found between the two cohorts (HR 1.507, 95 % CI 0.700 - 3.245).

Conclusions: The study suggests that platelet count may not be associated with cancer occurrence and prognosis. However, a prospective, large scale, and controlled study is needed to elucidate this issue.

PB 2392 | Bleeding Outcome during Pregnancy in Patients Diagnosed with Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is a common cause of thrombocytopenia in women of reproductive age. Prior studies investigated the natural history of ITP and birth outcome. However, the data regarding maternal bleeding complications is limited.

Aims: To investigate the prevalence and predictive factors of bleeding complications during pregnancy in ITP patients.

Methods: A retrospective study of pregnant women diagnosed with ITP was performed.

Results: A total of 99 women with 106 pregnancies was included. The mean age was 29±6.1 years. Forty-three pregnancies (40.6%) were primigravida and 27 (25.5%) were newly diagnosed ITP. Primary ITP was diagnosed in 93 pregnancies (87.7%). The median time of ITP diagnosis before pregnancy was 36 months (range 8-96 months). The median platelet (plt) count during the 1st, 2nd and 3rd trimesters was 73.3 x10^9/L (range 45.0-158.0 x10^9/L), 86.7 x10^9/L (range 55.0-142.0 x10^9/L) and 74.5 x10^9/L (range 42.0-122.0 x10^9/L), respectively. ITP treatment was given in 52 pregnancies (49%). The median plt count prior to the delivery was 72.0 x10^9/L (range 34.3-141.5 x10^9/L). Severe thrombocytopenia (plt count < 30,000 x10^9/L) was found in 20 out of 100 pregnancies (20%). Bleeding occurred in 25 pregnancies (23.6%), of which 72% happened in prenatal period and 4.7% was major bleeding. The prevalence of obstetric (OB) and non-OB bleeding was 13.2% and 11.3%, respectively. Mucocutaneous bleeding was the most common type of non-OB bleeding. Pregnancies with prenatal bleeding had significantly lower levels of platelet count in 3rd trimesters compared to those without bleeding (p=.021). However, there was no association between plt count at delivery and PPH. In addition, prenatal bleeding was significantly correlated with bleeding manifestations at the diagnosis of ITP (OR 4.8, 95% CI 1.02 - 22.29, p=.047).

Conclusions: Severe bleeding was uncommon during pregnancy in ITP patients. Bleeding manifestations at the diagnosis of ITP was the predictive factor for prenatal bleeding.

PB 2393 | Development of PF4/Heparin Dependent Antibodies in Patients Undergoing Extracorporeal Membrane Oxygenation and Clinical Impact

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Background: Extracorporeal membrane oxygenation (ECMO) provides circulatory support in patients with severe cardiac failure and anticoagulation with unfractionated heparin (UFH) is necessary in these patients with a high risk for heparin-induced thrombocytopenia (HIT).

Aims: The aim of this study was to prospectively evaluate the incidence of "pathogenic" anti-platelet factor 4/heparin (PF4/H) antibodies in patients with ECMO and their impact on platelet count (PC) and clinical evolution.

Methods: From July 2014 to August 2016, 27 patients treated longer than 5 days by UFH for ECMO were studied. Plasma samples collected after initiation of ECMO were systematically analysed for the presence of antibodies (Abs) to PF4/H by ELISA (HAT 45®, GTI, cut off value: 0.4). Serotonin release assay (SRA) was performed with samples positive in ELISA.

Results: Demographic data and clinical characteristics of patients are presented in table 1.

TABLE 1 Demographic and clinical results of patients with ECMO according to the presence of anti- PF4/H Abs.

	ECMO n = 27	Patients without anti-PF4/H Abs n = 9	Patients with anti-PF4/H Abs n = 18
Age median years [range]	56 [17-76]	53 [17-76]	57 [36-69]
Sex ratio (M/F)	20/7	6/3	14/4
Setting of ECMO medical/surgical	17/10	7/2	10/8
ECMO duration (median days) [range]	14 [6-28]	17 [8-28]	12 [6-26]
SOFA score (median)	11 [7-14]	11 [7-14]	11 [8-13]
Issue : Favourable/ death/Bridge by heart turbine	8/11/8	2/4/3	6/7/5
Thrombotic complications	5	3	2
Sepsis	18	8	10

Significant levels of Abs to PF4/H were present in 2/27 patients before ECMO. Sixteen of 25 additional patients (64%) then developed Abs to PF4/H three to ten days after initiation of ECMO, with optical densities in ELISA higher than 1.0 in 7 cases. Serotonin release assay was positive in only one patient, therefore considered as having HIT. PC decreased (< 100 G/L) early, from day 1 to day 4 in 25 patients despite Abs to PF4/H were absent in most cases. PC recovered in 20 cases although Abs to PF4/H were present in 12 of them including the patient with HIT in whom UFH was replaced by argatroban. Thrombosis and death were not more frequent in patients with Abs to PF4/H.

Conclusions: Development of anti-PF4/H Abs is frequent in patients receiving heparin for ECMO, but these antibodies are rarely pathogenic and not associated with less favourable outcome.

PB 2394 | Platelet Survival in Treatment of ITP

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Background: Splenectomy is used as a 2nd line therapy of immune thrombocytopenia. Even though it is the most effective therapeutical option, approximately one third will eventually relapse. There are some important undesired side effects such as increased risk of thrombosis and risk of overwhelming postsplenectomy infection.

Aims: Predictive factors are needed to maximize probability of ITP remission after the splenectomy.

Methods: We searched in ITP registry for platelets survival and turnover before the splenectomy and compare the data with remisions after the procedure.

Results: We found 27 splenctomized patients during years 2003-2015 with complete pre-splnenectomy platelet examination. Mean age of patients was 41 years. Median of survival time was 5 hours, spleen/liver destruction ratio was 4,8. Complete remission was observed in 92% of patients. Long-term remission without need of any other therapy was 76%. Patients with relaps were older (mean age 38 vs. 51, p=0,18). There was no statistical difference in platelet survival times or place of platelet destruction between the groups.

Conclusions: Splenectomy is still used in treatment of ITP. Predictive factors are missing and platelet survival may be helpful for patient's and physician's decision. More data are needed before statistical significance can be reached.

PB 2395 | When Cancer Patients are HIT: A Literature Review of Heparin-induced Thrombocytopenia (HIT) and Clinical Outcomes in Cancer Patients

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Background: Thrombosis is the second leading cause of death in cancer patients. Cancer patients are at 4-7 times higher risk of thrombosis compared to the general population. HIT poses a thrombosis risk of 30-50% in the first month after diagnosis in the general population. The thrombosis risk in cancer patients affected by HIT is not known.

Aims: We conducted a literature review to determine the thrombosis outcomes in cancer patients affected by HIT.

Methods: We performed a literature search through January 2, 2017 with search terms "heparin-induced thrombocytopenia AND cancer" in PUBMED and EMBASE databases. Manuscripts with details unavailable, remote history of cancer, remote history of HIT, other thrombophilia, and in other languages were excluded. The primary endpoint was the thrombosis rate in the first month of HIT diagnosis. Secondary endpoints were recurrent thrombosis rate after 1 month, anticoagulant-associated bleeding rate and overall survival (OS).

Results: Of 509 citations screened, 44 manuscripts/abstracts (55 patients) were eligible for analysis. M:F, 1.0:2.6; solid cancers vs hematologic cancers, 2:1; median 4T scores of 6. HIT diagnosis by antibody-based tests vs functional assays vs both, 7.5:1.0: 5.0. Unfractionated heparin caused HIT in 67% of cases.Thrombosis rate in the first month was 80% with a recurrent thrombosis rate of 6.8% at months 2 to 6. The overall clinically relevant, major and nonmajor, bleeding rate was 5.7%. Median OS for available cases was 4 months with 95% CI [2.6-5.4].

Conclusions: The thrombosis risk appears very high in cancer patients affected by HIT. The recurrent thrombosis rate beyond 1 month is comparable to that of cancer patients without HIT. Likely due to high thrombogenicity from both cancer *per se* and HIT, the anticoagulant-associated bleeding risk in this population appears to be acceptable. Larger studies are required to confirm our findings.

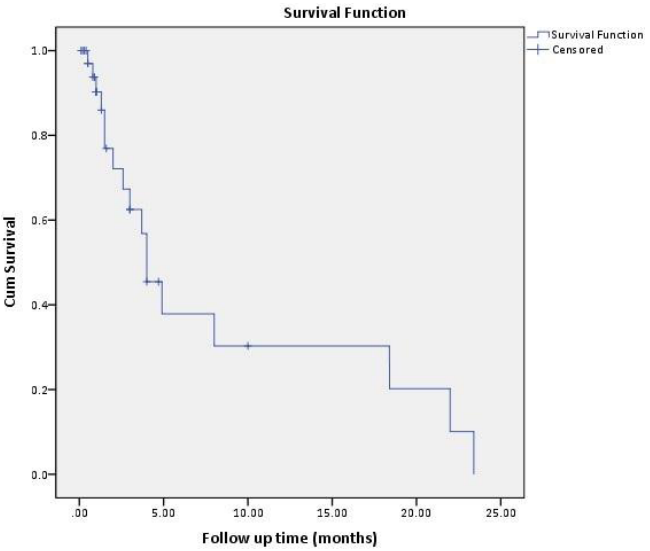

FIGURE 1 Overall Survival

TABLE 1 Patient Characteristics and Clinical Outcomes

Total number of cases	55
Age (years), Median [IQR]; Female, N (%)	63[53-67]; 40 (72.7%)
Solid malignancy, N (%); Hematologic, N (%); Solid + hematologic, N (%)	36 (65.5%); 18 (32.7%); 1 (1.8%)
4T score: Median [IQR]	6 [5-7]
Diagnostic testing: Antibody-based screening only, N (%); Functional assay only, N (%); Both screening and functional assays, N (%)	30 (54.5%); 4 (7.3%); 21 (38.2%)
Culprit medications: Heparin, N (%); Low molecular weight heparin, N (%); Heparin/LWMH, N (%)	37 (67.3%); 14 (25.4%); 4 (7.3%)
All thrombotic events within 4 weeks from HIT, N(%)	44 (80.0%)
Recurrent thrombotic events after 4 weeks from HIT,N(%)	3 (6.8%)
Alternative anticoagulation treatment: Anticoagulation during the first 4 weeks since HIT, N(%); Long term anticoagulation (>4 weeks since HIT), N(%)	53 (96.4%); 34 (61.8%)
Bleeding complications during alternative Anticoagulation, N(%); Clinical relevant non-major bleeding, N; Major bleeding, N	3 (5.7%); 1; 2

PB 2396 | Evaluation of Increased Incidence of Heparin Induced Thrombocytopenia at a Large Academic Teaching Hospital

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TABLE 1 PF4 and SRA Results in Patients with Heparin Induced Thrombocytopenia

Year	PF4 Positive	PF4 Optical Density 0.40 - 0.99	PF4 Optical Density 1.0 - 1.99	PF4 Optical Density ≥ 2.0	SRA Positive	SRA Negative	SRA Not Sent
2015 - n(%)	19	9 (47.4%)	7 (36.8%)	3 (15.8%)	6 (31.5%)**	12 (63.2%)	1 (5.2%)
2016 - n(%)	22	9 (41.0%)	6 (22.7%)	7(31.8%)	13(59.1%)*	6 (27.2%)	4(18.2%)***

*Two patients from 2016 had an initial negative PF4 and then SRA was positive within 10 days ** Two patients from 2015 had an initial negative PF4 then SRA was positive within 7 days ***Of the four in 2016 who did not have an SRA sent, had OD 2.42, 1.97, 0.5, and 0.733.

Background: An increase from 2% to 3.7% in PF4 positive heparin induced thrombocytopenia (HIT), defined as OD >0.4, from calendar year 2015 to 2016, was observed by our hemostatic and antithrombotic stewardship (HAT) service.

Aims: To identify patient factors associated with the observed increase in the number of HIT cases.

Methods: A retrospective chart review of patients with suspected HIT treated with a direct thrombin inhibitor (DTI) was performed comparing 2015 to 2016. Patients' baseline characteristics, history and type of heparin product exposure, calculated 4-T score, PF4 and SRA results, and additional risk factors were compared between groups. A bivariate logistic regression analysis was used to identify risk factors in PF4 and SRA positive HIT cases.

Results: A total of 118 patients were treated with a DTI for suspected HIT prior to PF4 result: 54 in 2015 and 64 in 2016. The number of PF4s sent per year was significantly decreased by 37.4%. In 2015, there were 19/54 (35.2%) PF4 positive patients' versus 22/64 (34.4%) in 2016. There was a 1.7% increase in the number of positive PF4s in 2016 compared to 2015. Of those who were PF4 positive, 6 (31.6%) were SRA positive versus 13 (59.1%). A multivariate analysis showed sepsis and previous heparin exposure within the past 100 days had the highest incidence of HIT, whereas those receiving renal replacement therapy or cardiopulmonary bypass were lower.

Conclusions: Screening completed by the HAT service decreased the number of PF4 tests sent without affecting the yield of a positive PF4 test. Though the sample size was small, we were not able to detect statistical differences in patient characteristics related to incidence, though higher rates noted in those with sepsis and previous heparin exposure within the past 100 days. Additional analysis is anticipated.

PB 2398 | rhTPO is Conductive to Remission from Acute Hemorrhage Phase of Children Severe ITP

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Background: Severe bleeding is always a concern in treatment of children SITP. rhTPO, which can stimulate thrombopoiesis, is applied to children ITP in their acute hemorrhage phase.

Aims: To discuss the effect of rhTPO in acute hemorrhagic phase of children SITP.

Methods: We collected 22 cases of children SITP, who received rhTPO or combination of rhTPO and immunosuppressor with the IBLS of score 2. After observation for 2 weeks, we examined the PLT and IBLS regularly to accessed the effect of rhTPO in hemorrhagic phase of children SITP.

Results: ①Evaluation of therapeutic effect: OR was 68.2% and the median time for PLT rising to more than 30×10^9/L was 6 days. Rate of patients whose IBLS declined to score 1 was 18.2%, those down to 0 was 77.3%. ②Adverse events: One C-SITP evaluated as no response died eventually with IBLS remained to be score 2, others had no serious hemorrhagic event and drug-related adverse events. ③Correlation between combination with immunosupressor therapy and effect of rhTPO: OR of those received rhTPO alone was 25.0%, that of those received combination therapy was 72.2%, effect of the latter was better than the former (P=0.014). Rate of those received rhTPO alone with IBLS declined to score 0 and 1 was 25.0% and 75.0% respectively, those received combination therapy with IBLS declined to score 0 and 1 was 88.9% and 5.6% respectively, the latter was better than the former in controlling hemorrhage (P=0.013).

Conclusions: As for children SITP in acute hemorragic phase, rhTPO could rise PLT and control hemorrhage in short period effectively, getting children SITP through hemorragic phase safely, without any serious hemorrhagic event and drug-related adverse events. Combination with immunosupressor could be more effective in rising PLT and controlling hemorrhage, though it need further verification from large prospective data in future.

PB 2399 | Neuropilin-1 Expression on Regulatory T Cell Can Affect its Immunosuppressive Function in ITP

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Background: Primary immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by lots of immune dysfunctions . Abnormalities of regulatory T cells (Treg) have been proved in the

pathologic process of ITP. Neuropilin-1 (NRP-1) plays an vital role in the immunoregulation and expresses on Treg in ITP patients.

Aims: However it's unclear whether NRP-1 is related to the dysfunctions of Treg in ITP.

Methods: We found that anti-NRP-1 only inhibit the proliferation of $CD4^+$T cell instead of $CD8^+$T cell, and it also increased the apoptosis of Treg rather than $CD4^+$T cell, $CD8^+$T cell and platelet.

Results: Our results indicated that NRP-1 have no functions on Th1/Th2 polarization with no unchanged interleukin-10(IL-10) level, and mRNA level of T-bet and GATA-3,except decrease of interferon-γlevel. Also, we confirmed that dexamethasone (DXM) could promoted the expression of NRP-1 on Treg, but higher expression of NRP-1 have no effect on Foxp3 expression.

Conclusions: NRP-1 might play an important role in ITP by influence immunosuppressive function of Treg which mediate the interaction between dendritic cell(DC) and T cell.

PB 2400 | Thrombotic Events in Patients Treated with Eltrombag

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease (AID). The diagnosis is by exclusion and a major concern is determining whether the ITP is primary or secondary to an underlying condition that might benefit from treatment.

Eltrombopag is an agonist of thrombopoietin and is an option to patients who have failed to corticoids, splenectomy or Rituximab. Five percent of thrombotic events during treatment are described.

Aims: We submit 2 cases of thrombosis.

Methods:

CASE 1: A 15-year-old girl was diagnosed of ITP. She had Antinuclear Antibodies (1/640) but AID was ruled out. She had several relapses treated with corticoids and immune globulin (IG). Presented Cushing syndrome so she asked for a therapeutic alternative. She responded to 50mg/24h dose of Eltrombopag. After 4 weeks, she developed an acute pulmonary thromboembolism (fig.1). Eltrombopag was stopped and platelet levels were monitored. During the hospitalization presented nefrotic syndrome and anti-phospholipid antibodies were found. She was treated with corticoids and LMWH. The final diagnosis of AID is still pending.

CASE 2: A 51-year-old woman was diagnosed of primary ITP. She developed steroid dependence, so started Eltrombopag as bridge to splenectomy, which was chosen as second line of treatment. Prophylactic LMWH were suspended 4 weeks later. She relapsed 2 months after surgery and was treated with Eltrombopag and prednisone. A month later, she developed portal and superior mesenteric vein thrombosis (fig.2). She received corticoids and IG in order to maintain therapeutic doses of LMWH. Nowadays, she has normal platelet levels without specific treatment.

Results: Thrombosis is an unfrequent complication described with Eltrombopag, but we should not forget ITP by itself has increased thrombotic risk. Both cases had thrombotic risk factors: splenectomy and anti-phospholipid antibodies.

Conclusions: We should not underestimate the prothrombotic effect of ITP.

We must take into account other risk factors, not only the side effects of the drugs.

PB 2401 | Familial Thrombocytopenia - Course of 3 Subsequent Pregnancies in an Idiopathic Thrombocytopenic Purpura (ITP) Patient; Case Report

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Background: Idiopathic thrombocytopenic purpura (ITP), a relatively common platelet disorder when sensitization with autoantibodies leads to premature removal of platelets from circulation. Patients present easy bruising, petechiae, manorrhagia or hemorrhage. In pregnacy and particularily during peripartum period it is essential to prevent and control acute bleeding.

Aims: To highlight the need for sources of complications during pregnancy, delivery and puerperium.

Methods: A case report.

Results: A 34 year old patient with familial ITP. Her three pregnancies were all complicated with extremely low platelet counts - (below $10x10^3$μL). The patient responded poorly to high-dose corticosteroids. As result of repeat high-dose intravenous immunoglobulin administration the platelet count increased to over $20x10^3$μL. This may have been caused either by blockage of Fc receptors on macrophages or inhibition of antiplatelet antibody biosynthesis. Before each of her C-sections performed due to hazards of fetal thrombocytopenia, platelet transfusion was ordered. Despite sharp decline in platelet count (< $10x10^3$μL) 4 hours after the procedure - no major bleeding episode occurred in the puerperium. In the otherwise healthy offspring thrombocytopenia was the only complication of an early neonatal period. Each of the infants had platelets nadir in the first day of life: - 12 ,27 ,$110x10^3$μL respectively.

Conclusions: It is recommended to refer to a multidisciplinary approach in severe cases of familial ITP during pregnancy and puerperium. Delivery should be planned in special perinatal care units and careful surveillance in the neonatal period is highly advisable.

PB 2402 | Treatment Options and their Outcomes in Pediatric Immune Thrombocytopenic Purpura Patients

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Background: There are several therapeutic options available in immune thrombocytopenic purpura (ITP). Its efficacy varies significantly.
Aims: The aim of this study was to determine the treatment options and their outcome in pediatrics ITP patients.
Methods: A case series conducted at NIBD from 2008-2016**.** Total 67 ITP patients of either gender having isolated thrombocytopenia were included. ITP was classified into acute (remission occur within 3 months), persistent (spontaneous remission or patients not maintaining complete response from 3-12 months) and chronic (platelets < $150X10^9$/L persisting >12 months after diagnosis). Prednisone or IVIG were first-line and Azathioprine, Rituximab were second-line treatment. Splenectomy was done in chronic ITP patients with significant bleeding or if the first-line treatment failed. Outcome was evaluated as Complete Response (CR), Partial Response (PR) and No Response (NR).
Results: Out of total 67 patients, 29.85% patients achieved complete remission in acute ITP phase, 36.17% achieved complete remission in persistent ITP phase while chronic ITP was noticed in 30 children. Overall 47.45% children showed CR with Prednisone, 44.44% with Azathioprine and 43.75% with Anti-D. Splenectomy was done in 7 patients, among them response was observed in 71.42% children.
Conclusions: ITP was found safe disease with lower rate of morbidity and no mortality. Most of the pediatric ITP (55.22%) resolved before chronic stage. Future work on pathophysiology and in mechanism of ITP is required to identify patient tailored therapy.

PB 2404 | Childhood Immune Thrombocytopenic Purpura (ITP) in Gorgan Taleghani Hospital

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Background: ITP is the main reason of Thrombocytopenia in children which is most prevalent in 1 to 4 years old children And it´s incident is not gender dependent. Initial treatments are applied to patients with platlet count lower than 20000/mm3 or symptomatic cases. The initial treatments include Prednisolone and IVIG. In 20 % of cases disease manifestation becomes chronic (longer than 12 months).
Aims: We decided to investigate finding in our ITP patients in Gorgan Taleghani Hospital and compared them with references.
Methods: we investigated in-patients files from December2014 to March2016 in Hematology department of Taleghani Hospital and recorded demographic data and patients contact information. Then these patients files were followed up in out-patients files.
Results: 49 patients with ITP diagnosis were recorded between 2014-2016 .patients were from 22days to 15years with mean age of 8.3 years. male to female ratio was 1.33/1. platlet counts had minimum and maximum of 1000 /^{mm3}and 55000/^{mm3} respectively with mean value of 18820/^{mm3}. Initial treatment of patients found out to be only Prednisolone in 0 patients (0%), IVIG in 7patients (14.3%), and both Prednisolone & IVIG in 42patients (85.7%). in 1 patients (2.04%), disease took a chronic form (lasted longer than 12 month). in 1 patients (2.04%) with ITP initial presentation, definite diagnosis turned out to be AML M_7.
Conclusions: our derived data had good correspondence with global findings except chronicity.Taking into account the fact that in 1 patient with ITP initial presentation, definite diagnosis turned out to be AML M_7 , we recommend to pay enough attention to differential diagnosis in ITP cases.

PB 2405 | Pharmacokinetic Variations, Fc Receptor Polymorphisms and their Impact on Response to IVIG in Pediatric Immune Thrombocytopenia

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Background: 30-40% of pediatric patients with immune thrombocytopenia (ITP) are non-responders to intravenous immunoglobulin (IVIG), but it remains unclear which factors determine a patient´s responsiveness. IVIG clearance varies significantly between individuals and has been shown to affect the clinical outcome in Guillain-Barre syndrome. This variation may depend on variable levels of saturation of the neonatal Fc receptor (FcRn) for immunoglobulin G (IgG) and polymorphism affecting its expression. In addition, it is possible that polymorphisms of myeloid Fc-receptors (FcγR) may be involved.
Aims: To investigate whether variations in IVIG pharmacokinetics and Fc receptor polymorphisms could explain clinical outcome in newly diagnosed childhood ITP.
Methods: Blood samples were analyzed from 134 children with newly diagnosed ITP allocated to careful observation or administration of 0.8g/kg IVIG in a multicenter randomized controlled trial. Increases in serum IgG levels (ΔIgG) were measured by nephelometry 1 week and 12 weeks after trial inclusion. Polymorphisms in the neonatal Fc

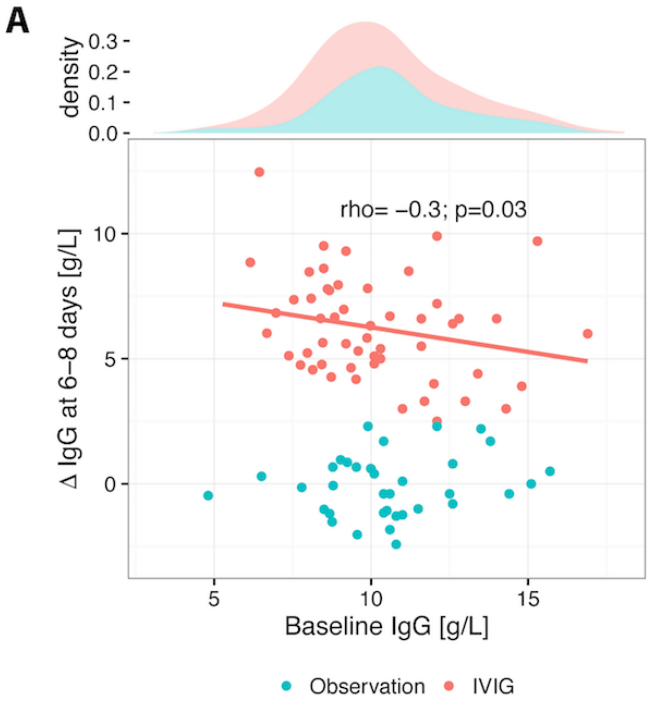

FIGURE 1 Changes in IgG after 1 week vs baseline IgG levels in children with newly diagnosed ITP.

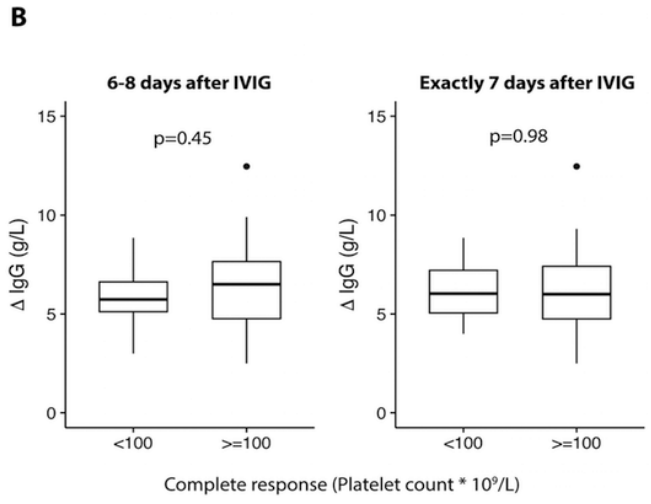

FIGURE 2 Increases in serum IgG 1 week after IVIG and resolution of thrombocytopenia (platelet count > 100 * 10^9/L).

receptor (FcRn) promoter and a panel of FcγR polymorphisms were determined.

Results: Pediatric patients with ITP showed a high interindividual variation in ΔIgG.

Lower ΔIgG levels may partly be explained by high pre-treatment serum IgG and saturation of FcRn-dependent IgG recycling. No association between affinity- and expression-determining polymorphisms in FcRn or FcγR and ΔIgG was detected. Our data indicate that ΔIgG between responders and non-responders were similar (Bayesian t-test; BF_{10} = 0.3, BF_{01} = 2.8).

There was no association between ΔIgG and increased Buchanan bleeding scores.

Conclusions: Post-IVIG increases in IgG did not explain heterogeneity in the clinical response in childhood ITP. We were unable to confirm that polymorphisms in FcRn or FcγR affected IVIG responsiveness. Therefore, from our data, neither dose adjustment nor genotyping of Fc receptors are tangible strategies to decrease the number of non-responders.

PB 2406 | Fetal/Neonatal Alloimmune Thrombocytopenia - An Underestimated Life-threatening Clinical Condition

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Background: In fetal/neonatal alloimmune thrombocytopenia (FNAIT), platelets (PLT) are destroyed by maternal antibodies directed against fetal antigens. Thrombocytopenia may be severe and lead to intracranial hemorrhage in about 10% of cases. FNAIT is estimated to be markedly underdiagnosed, partly due to the fact that complete blood count (CBC) is not routinely done in all neonates.

Aims: To evaluate the degree of awareness of FNAIT in an attempt to decrease the risk of this devastating condition.

Methods: A retrospective analysis.

Results: A retrospective analysis of 322 suspected FNAIT cases sent over the past 4 years to our reference laboratory from the majority of medical centers in the country revealed a low referral rate of 39%. In addition, 50% of the families with a thrombocytopenic newborn were referred for evaluation weeks after discharge, when the antibody titer could already be below the detection level and 27% of the mothers with a previously suspected FNAIT pregnancy were referred for such evaluation only during a subsequent pregnancy. A supplementary retrospective analysis performed at our center between 2010-2015 showed that a CBC test was done for various clinical reasons only in 7370 (23.1%) of 31952 newborns. Thrombocytopenia (< $150x10^9$ PLT/L) was found in 2505 (34%) of these babies, being severe (< $50x10^9$ PLT/L) in 220 (2.98%) of them. Extended evaluation of the latter subgroup demonstrated that 45 newborns were term and had no other possible causes for the low PLT count. Yet, only 7 babies were referred for FNAIT assessment: 4 of them were found positive for PLT antibodies. Thus, in the current analysis, 84% of the newborns with severe thrombocytopenia were not assessed for FNAIT.

Conclusions: Awareness of physicians and cooperation of the multidisciplinary team (neonatologists, pediatric hematologists and gynecologists) involved are crucial for FNAIT diagnosis. Development of uniform guidelines for the evaluation of this life-threatening clinical condition is warranted.

PB 2407 | Variable Impairment of Platelet Function in Patients with Severe, Genetically Linked Immune Deficiencies

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Background: Based on studies with mice, in young patients with severe immune deficiency linked to mutations in *STIM1* (calcium-regulating protein), *ORAI1* (Ca^{2+} channel) or *FERMT3* (integrin-regulating protein kindlin-3), platelet dysfunction is expected.

Aims: Assessment of the consequences of homozygous or heterozygous mutations in these 3 genes on overall platelet hemostatic functionality.

Methods: Platelet function analysis of patients (3 children, 1 adult, 5 parents) with mutations in *ORAI1*, *STIM1* or *FERMT3*. Measurement of platelet calcium signaling. Multi-parameter assessment of whole blood thrombus formation (arterial or venous shear rate) on microspots, activated via GPVI (collagen), CLEC2 (rhodocytin) or GPIb/$\alpha_{IIb}\beta_3$ (von Willebrand factor/fibrinogen). Comparison with data from healthy donors.

Results: In platelets from 4 out of 6 immune-compromised patients (including parents) with a mutation in *ORAI1* or *STIM1*, store-operated Ca^{2+} entry (SOCE) was substantially or completely reduced in comparison to control platelets. Platelets from patient with heterozygous mutation in *STIM1* showed normal thrombus formation, in spite of reduced SOCE. Platelets from 5 patients with mutations in *ORAI1* were reduced in thrombus formation, most discriminative for surfaces acting via CLEC2 and GPIb/$\alpha_{IIb}\beta_3$. Phosphatidylserine exposure was consistently low, irrespective of surface and shear rate. Platelets from family members with mutation in *FERMT3* scored lowest in thrombus formation at all surfaces. Bone marrow transplantation of patients (n=2) resulted in an overall improvement of platelets function and thrombus parameters.

Conclusions: Cumulative parameters of thrombus formation were more severely reduced in blood from patients plus relatives in the order of $STIM1^M$<$ORAI1^M$<$FERMT3^M$. The reduced hemostatic potential is primarily (n=7) linked to signaling dysfunctions and secondarily (n=1) to a low platelet count.

PB 2408 | Whole Blood Aggregometry to Assess Platelet Function in Children on Cardiopulmonary Bypass

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Background: Cardiopulmonary bypass (CPB) causes platelet destruction and dysfunction; therefore, platelets are often transfused empirically to pediatric patients to reduce bleeding risk. Whole blood aggregometry (WBA) is a rapid test of platelet function and has safely reduced transfusions without increased bleeding in adults, but is not routinely used in children.

Aims: Use WBA to measure change in platelet function in children on CPB.

Methods: This IRB-approved study consented children 1-60 months old undergoing surgery with CPB at Children's Hospital of Wisconsin 2015-2016. WBA uses electrical impedance to measure platelet aggregation after agonist activation. Agonists used were thrombin receptor activating peptide (TRAP) and ADP. WBA was performed pre-CPB, on CPB after surgical repair completed, and post-CPB (after platelet transfusion). ANOVA used for >2 groups (Dunn's test for multiple comparisons), Wilcoxon rank sum test was used for 2-group comparisons.

Results: 63% of patients (27/43) received a platelet transfusion. Platelet count decreased in most subjects (median: -176x10^9/L in transfused, -96x10^9/L in non-transfused patients). Patients had a post-transfusion increase (median, 82x10^9 platelets/L), whereas

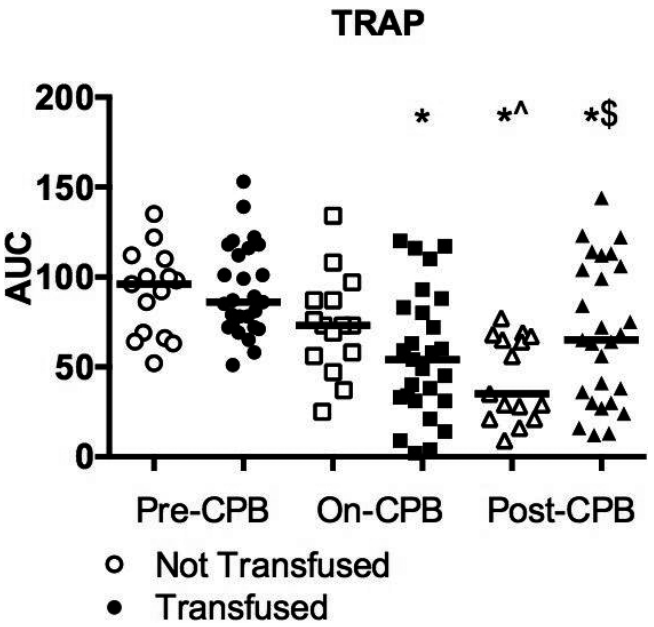

FIGURE 1 Change in TRAP response due to CPB. Significant difference compared to *pre-CPB, ^on-CPB, and $ non-transfused patients at the same time point.

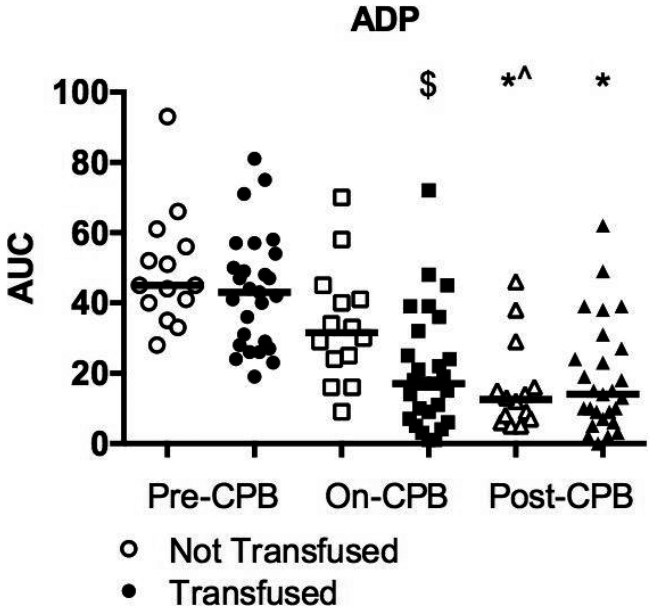

FIGURE 2 Change in ADP response due to CPB. Significant difference compared to *pre-CPB, ^on-CPB, and $ non-transfused patients at the same time point.

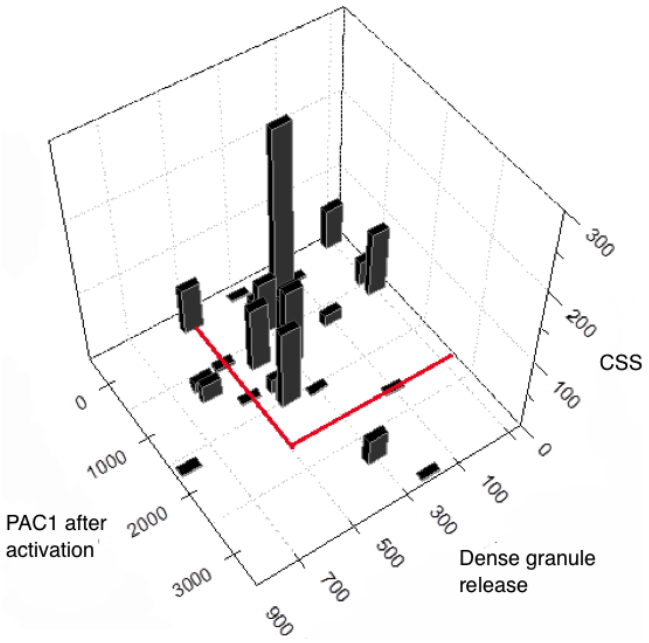

FIGURE 1 Correlation between CSS, PAC1 after activation and dense granule release

non-transfused patients had a further decrease in platelets post-CPB (median: -27x10^9/L). WBA in response to TRAP mirrored changes in platelet count (Fig 1), whereas response to ADP was not improved despite platelet transfusion (Fig 2). Two of three subjects that had a very poor response to TRAP (AUC < 20) pre- and post-transfusion had excessive postoperative bleeding.

Conclusions: CPB decreased platelet count and response to TRAP and ADP. Platelet count and TRAP response improved after platelet transfusion. ADP response remained poor even in transfused patients, suggesting that stored platelets have decreased reactivity to ADP. Poor response to TRAP despite transfusion was associated with bleeding. Future studies are needed to evaluate using WBA to guide platelet transfusions and hemostatic management.

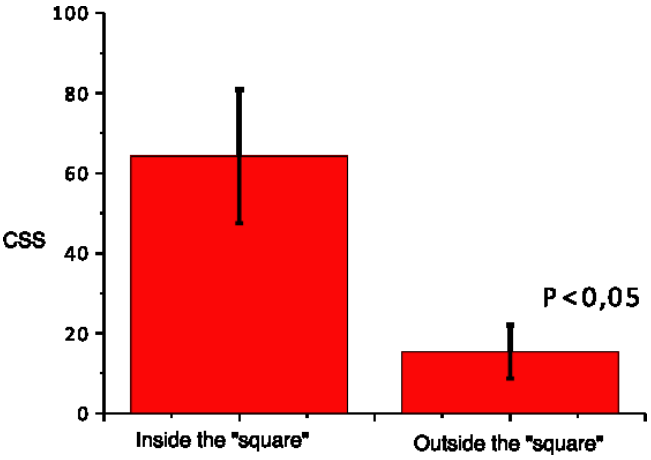

FIGURE 2 Comparison of patients inside and outside the

PB 2409 | Platelet PAC1 expression and dense granules function reflects bleeding severity in children

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Background: There is no clear data on the correlation between the degree of in vitro platelet function failure (PFF) and bleeding severity (BS) in children with suspected IPD7.

Aims: To analyze the correlation between PFF degree determined using flow cytometry (FC) and BS in children.

Methods: 32 patients (1-17 yo) with bleeding without Willebrand disease, factor XIII deficiency, or other coagulopathy were included. PF was evaluated with FC before and after stimulation with CRP plus TRAP. Fluorescencently labeled antibodies against CD42b, CD61, CD62P and PAC-1 were used; dense granule release (DGR) was studied using loading with mepacrine; procoagulant activity was evaluated using annexin V. To standardize BS a clinical score (CS) system was developed and calculated with formula: i*[∑(SLa*SSa)+ …+ (SLx*SSx)], where i is a systemic index which equals 5 when three or more bleeding localizations persist, and equals 1 when two or less bleeding localizations persist. The term (SL*SS) reflects the score for each localization (SL, 1 to 10 points) and severity (SS, 1 to 10 points). All comparisons were performed with OriginPro 8.0 (OriginLab, MA, USA) software using Mann-Whitney U-test.

Results: Out of the 32, three patients were excluded because of normal results or preanalytical issues. Among the remaining 29 patients, four children were diagnosed with CD61 deficiency, and 25 had different combined defects. We used a two-parameter statistical model to analyze the relationship between PFF and CSS (fig. 1).

The majority of platelets with CSS≥ 10 were situated in the square 0< PAC1< 2000, 0< DGR< 500 (all units are arbitrary).

A comparison between CSS among those patients in this ´´square´´ with those who were outside the the region, revealed a statistically significant difference (fig 2).

We did not find any statistically significant correlation between CSS and other studied parameters.

Conclusions: PFF degree determined as a combination of integrin activation and DGR in vitro correlates with BS in children.

PB 2411 | Utility of Small Volume Whole Blood Platelet Function Assays in Adolescent Women with Heavy Menstrual Bleeding

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Background: Qualitative platelet disorders (QPDs) have been reported in up to 40% of young women with heavy menstrual bleeding (HMB). Diagnosing QPDs remains challenging due to the limitations of currently available clinical platelet function assays. Herein we applied a panel of small-volume whole blood assays to a population of adolescent women with HMB.

Aims: To determine the utility of the platelet function assays in evaluating HMB in adolescent women.

Methods: The study was approved by the Institutional Review Board. Written informed consent and/or assent was obtained. Citrated venous whole blood from each participant was used in a panel of assays: activation of GPIIbIIIa integrin as measured by PAC-1 mAb; platelet granule secretion as measured by P-selectin expression; platelet aggregation; platelet static adhesion on collagen or von Willebrand Factor (vWF); and platelet adhesion to collagen or vWF under low or high shear flow. The activation, aggregation, and static adhesion assays were assessed in response to the following agonists: ristocetin, TRAP-6, U46619, ADP, calcium ionophore, collagen-related peptide, and epinephrine. In addition, participants underwent evaluation for HMB per the clinical standard at the treating institution.

Results: Twelve participants with clinical bleeding symptoms were included in the study of which 7 were diagnosed with a clinically-identifiable bleeding disorder (BD). Participants diagnosed with a BD exhibited lower GPIIbIIIa activation and P-selectin expression in response to calcium ionophore compared to subjects without a BD diagnosis. A dramatic reduction in platelet adhesion was observed in flow assays for the participant diagnosed with thrombocytopenia.

Conclusions: Our assays detected differences among participants with and without clinically-identifiable BDs. These results demonstrate the potential clinical utility of our small volume whole blood platelet function panel.

PB 2412 | Novel Mutations in Thai Patients with Glanzmann Thrombasthenia

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Background: Glanzmann thrombasthenia (GT) is an autosomal recessive platelet disorder, caused by defects of the platelet integrin αIIbβ3 (GPIIb/IIIa) resulting from pathogenic mutations in either *ITGA2B* or *ITGB3*. It is characterized by spontaneous mucocutaneous bleeding. Molecular features of GT in Thailand have not been identified.

Aims: This study aimed to determine the clinical and molecular features of unrelated Thai patients with GT.

Methods: Four patients with clinically suspected GT were recruited at the Division of Pediatric Hematology/Oncology, King Chulalongkorn Memorial Hospital. The diagnosis was based on clinical and hematological parameters as well as molecular genetic analysis. Whole exome sequencing (WES) was performed in all cases.

Results: Of the four patients studied, the median age at first suspicion of GT was 2.5 years. All presented with severe bleeding symptoms (WHO bleeding scale 3). Flow cytometry to assess the surface GPIIb/IIIa complex showed reduced expression with the value being less than 5% in all cases, thus categorized into GT type 1. By WES, we successfully identified seven mutant alleles in *ITGA2B*. One alteration, the c.2911_2912insC was detected in two unrelated families. One patient was found to be homozygous for the c.617T>A. Of five different causative variants identified, four have never been previously described. These include missense and frameshift (c.617T>A, c.1522_1531del, c.2911_2912insC, and c.2377C>T). The novel missense variants were absent in an in-house database of 200 Thai exomes.

Conclusions: This study identified seven causative variants in *ITGA2B* in four unrelated patients with GT (87.5%). Four novel pathogenic variants were identified, expanding the genotypic spectrum of *ITGA2B* causing GT.

PB 2413 | Chemotherapy Induced Thrombocytopenia and Bleeding in Children and Young Adults with Cancer

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Background: Chemotherapy induced thrombocytopenia (CIT) has long been recognized as a significant complication of cancer therapy,but there is still lack of consensus about the optimal approach to prophylaxis and/or treatment of this important morbidity.

Aims: The aim of the study is to estimate the frequency and features of CITand associated clinically significant bleeding in children and young adults.

Methods: For this retrospective, hospital-based study, children (0-18y) and young adults (19-40y) with differenttypes of solid tumors and hematologic malignancies who received chemotherapy at the Clinic of Chemotherapy of Muratsan Hospital Complex of Yerevan State Medical Universitywere identified from the patients' database and included in the study (overall 122 patients). Thrombocytopenia was defined as a decrease of platelet count below < $100x10^9$/L. For assessing bleeding WHO scale (0 = no bleeding, 1 = no petechiae, 2 = mild blood loss, 3 = gross blood loss, 4 = debilitating blood loss) was used.

Results: Overall the whole group of patients received 430 chemotherapy cycles.During 131 (31.6%) chemotherapy cycles patients developed CIT. The study revealed statistically significant negative correlation between the age of the patient and the severity of CIT. Another important finding of the study was that the patients, who previously were exposed to radiation therapy, were more likely to develop CIT,than those, who haven't received radiation therapy. From 430 cycles of chemotherapy only 31 (7.2%) cycles reported to have a bleeding incidence.

Conclusions: As a result of the study we concluded, that although CIT is a common complication of cancer therapy, clinically significant thrombocytopenia and bleeding are quite rare.

PB 2414 | Pregnancy Management of Fetal Alloimmune Thrombocytopenia (NAIT): A Case Report

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Background: NAIT is caused by maternal immunization against platelet antigens (HPAs) and represents the most frequent cause of isolated fetal/neonatal thrombocytopenia. Clinical neonatal manifestations ranges from mild asymptomatic to severe thrombocytopenia that can lead to spontaneous intracranial hemorrage (20-25% of cases).

Aims: We describe a case of this rare underdiagnosed condition.

Methods: Clinical, laboratory and radiological informations were collected on medical record

Results: *A 35*-year-old pregnant woman with a previous miscarriage and 1 pregnancy complicated by NAIT with severe thrombocytopenia and minor bleeding symptoms referred to our clinic Serological tests confirmed maternal anti-HPA-1b, anti-HPA-5a, anti-HLA-class1 antibodies. Amniotic liquid showed HPA-1a/1a and HPA-5a/5b antigenic profile. Maternal antibodies were monitored every two weeks, an increase of anti-HPA-1b antibodies was observed from week 16. At week 18 she started prednisone 0.5 mg/Kg/die and IVIG 1gr/Kg/iv per week.

Maternal anti-HPA-1b was performed from the beginning of IVIG therapy to delivery every 2 weeks, showing a progressive reduction in titre. From week 32, IVIG dose was increased to 2gr/Kg/iv per week until delivery. A cesarean section was performed at week 38. The newborn, actively clinically monitored during the first week, didn't developed major or minor bleeding manifestations and always showed normal platelet count and negative Ab anti HPA-1b.

Conclusions: This case showed a successfully management of NAIT during a second pregnancy and the necessity of increase general knowledge on this condition. At present, no screening test on platelet antigen profile on both parents is indicated before a first pregnancy but they are required after a first NAIT to plan the following pregnancies. Since this syndrome shows high risk of recurrence and greater severity in subsequent pregnancies there is a general consensus on antenatal management with maternal first-line therapy using intravenous immunoglobulins (IVIG) and steroids.

TRANSFUSION & BIOTHERAPEUTICS

PB 307 | Plasma Derived FVIII Concentrates Induce Neutrophil Extracellular Trap (NET) Formation

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Background: Previously we showed pdFVIII induced platelet and neutrophil activation.

Aims: Here we studied whether pdFVIII may boost inflammation by the formation of NETs.

Methods: Three pdFVIII products and two rFVIII products were studied. Neutrophils were gently isolated from fresh human blood. Whether FVIII concentrates induced generation of reactive oxygen species from non-activated neutrophils was determined as the linear rate of superoxide dismutase-inhabitable reduction of cytochrome C. Activation of CD11b was analysed by flow cytometry using an activation specific antibody against CD11b labelled with PE. Associate formation in whole blood between platelets and neutrophils was measured by flow cytometry. Effect of factor VIII products on DNA release from isolated human neutrophils was measured by three methods, fluorescent dye Syto13 binding to nucleic acids, a sandwich ELISA against histone-DNA complexes and microscopy.

Results: Significant activation of CD11b was only observed on neutrophils treated with (0.1-1 U/ml) pdFVIII, but not on neutrophils treated with rFVIII. pdFVIII induced neutrophil ROS production as well as the

release of extracellular DNA, which was not observed with rFVIII. Fluorescence microscopy showed the pdFVIII induced NETs present alpha defensins (HNP1-3) and myeloperoxidase. In addition to experiments with isolated neutrophils, we studied the effect of FVIII on neutrophils in melagatran anticoagulated whole blood. Two of three tested pdFVIII clearly and significantly induced the formation of associates between neutrophils and platelets. In contrast, rFVIII had no effect. The activation effects of pFVIII on neutrophils were more pronounced in the presence of platelets.

Conclusions: Based on these in vitro results pdFVIII products in contrast to rFVIII products seem to be proinflammatory by activating neutrophils. Further research aims to investigate the effect of factor VIII products ex vivo.

This work was supported by a grant of Bayer Vital GmbH, 51368 Leverkusen, Germany

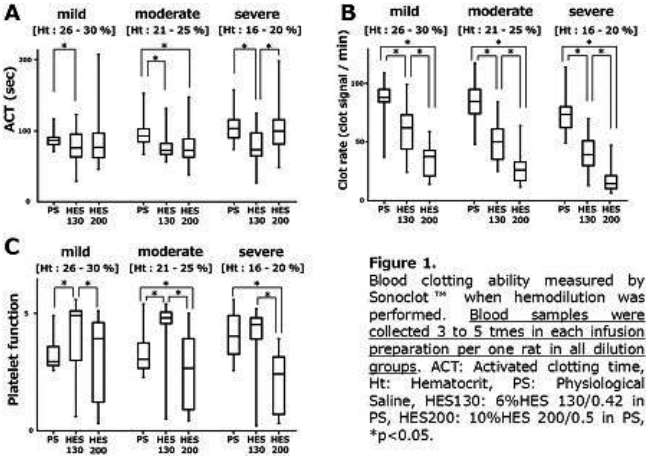

Figure 1.
Blood clotting ability measured by Sonoclot™ when hemodilution was performed. Blood samples were collected 3 to 5 tmes in each infusion preparation per one rat in all dilution groups. ACT: Activated clotting time, Ht: Hematocrit, PS: Physiological Saline, HES130: 6%HES 130/0.42 in PS, HES200: 10%HES 200/0.5 in PS, *p<0.05.

FIGURE 1

PB 308 | Vlidation of Hemostatic Impairment Induced by Hydroxyethyl Starch *in vivo*

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Background: Precedent studies suggest that hemodilution using hydroxyethyl starch (HES) may inhibit blood coagulation. However, **resolution by α-amylase or the possibility of ´effective´ dilution was not considered** because most of the studies were *in vitro* or retrospective clinical studies in which the dilution level was not standardized.

Aims: We compared the blood clotting ability using Sonoclot™ when hemodilution was performed with physiological saline, 6% HES 130/0.42 and 10% HES 200/0.5 in order to clarify the influence of HES on coagulation ***in vivo* when diluted equally**.

Methods: Thirty male Wistar rats were divided into 3 groups of 10 rats. After tracheotomization, continuous monitoring of blood pressure were performed under artificial respiration. Each infusion preparation was given at 0.5 ml/h, and additional intravenous administration was permitted without limit in order to maintain blood pressure. Blood samples of 1.5 ml were taken for blood gas analysis and Sonoclot™ every 30 minutes. Hemodilution was continued until the hematocrit level was reduced to 16-20% without 20% decrease of mean blood pressure. Activated clotting time, clot rate (CR) and value of platelet function (PF), were recorded in each hematocrit and were considered to indicate the degree of hemodilution, divided into 3 groups (mild:26-30%, moderate:21-25%, severe:16-20%). Factor Ⅹ and soluble GPⅡb/Ⅲa were assayed by ELISA. Statistical analysis was performed using the Kruskal-Wallis H-test followed by the Newman-Keuls-type test for multiple comparisons. P values < 0.05 were considered statistically significant.

Results: CR was significantly reduced by the larger molecular weight HES despite equal dilution (Fig. 1B). PF was rather high in HES 130/0.42(Fig. 1C). Factor X tended to be reduced in HES 200/0.5 (Fig. 2A).There was no significant difference in GPⅡb/Ⅲa (Fig. 2B) .

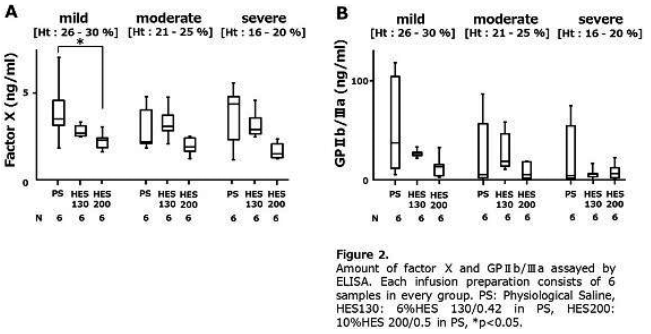

Figure 2.
Amount of factor X and GPⅡb/Ⅲa assayed by ELISA. Each infusion preparation consists of 6 samples in every group. PS: Physiological Saline, HES130: 6%HES 130/0.42 in PS, HES200: 10%HES 200/0.5 in PS, *p<0.05.

FIGURE 2

Conclusions: Hydroxyethyl starch impairs coagulation but all of HES do not inhibit platelet function necessarily. This cannot be explained by amount of factor X or GPⅡb/Ⅲa.

PB 309 | Ex Vivo Haemostatic Capacity of Thawed Plasma Beyond 24 Hours: Comparison between FFP and RTFP24

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Background: Extending shelf-life of thawed plasma (TP) stored at 1-6°C to >24 hours (h) allows rapid plasma availability in massive transfusion protocol with less wastage. Most studies involved FFP (plasma frozen within 8h) or frozen plasma (FP) prepared from whole blood (WB) stored at 1-6°C up to 24h, which supported TP's storage up to 5 days in USA. Data on RTFP24 (FP prepared after ambient storage of WB up to 24h) is limited. We recently switched from FFP to RTFP24 for its operational benefits after validating its comparability to FFP at time of thaw.

TABLE 1 Comparison of FII, FV, FVII-FXI and FXIII levels at D0 and D4 (*p<0.05 compared to D0, ** p<0.05 compared to corresponding FFP value).

	FFP (N=20 for all except N=19 for FV: one FV data excluded for erroneously low level at D0)			RTFP24 (N=20 for all except N=19 for FV: one FV data excluded for erroneously low level at D0)		
Parameter	D0: Median (range)	D4: Median (range)	Percentage decrease from baseline: Median (range)%	D0: Median (range)	D4: Median (range)	Percentage decrease from baseline: Median (range)%
FII (IU/dL)	101 (65-121)	103 (64-123)	0 (-7.8 to 6.6)%	100 (85-120)	101 (81-120)	0.9 (-5.4 to 10.4)%
FV (IU/dL)	109 (92-135)	100 (63-121)*	11.6 (-30.1 to 42.2)%	98 (68-164)	90 (48-141)*	9.9 (-5.8 to 49)%
FVII (IU/dL)	112 (81-143)	81 (56-107)*	27.1 (20.7 to 35.6)%	114 (82-174)	87 (61-152)*	25.6 (9.0 to 37.4)%
FVIII (IU/dL)	97 (60-135)	51 (27-79)*	46.8 (40.2 to 55)%	82 (50-120)	52 (30-80)*	40.5 (27.3 to 45.2)% **
FIX (IU/dL)	87 (68-112)	89 (57-117)	-1.1 (-21.5 to 18.6)%	87 (61-120)	85 (49-106)*	6.1 (-10.4 to 20.0)%
FX (IU/dL)	97 (74-129)	92 (75-117)*	5.9 (-1.4 to 9.5)%	98 (70-135)	97 (73-149)	-1.0 (-16.2 to 11.4)% **
FXI (IU/dL)	82 (65-124)	74 (58-115)*	8.1 (-1.4 to 15.2)%	98 (67-151)	90 (70-129)*	6.3 (-9.0 to 16.8)%
FXIII (IU/dL)	90 (67-129)	85 (64-134)*	5.4 (-3.9 to 19.8)%	110 (85-151)	106 (85-152)	0 (-6.3 to 6.6)% **

TABLE 2 Comparison of remaining parameters & thrombin generation at D0 and D4 (*p<0.05 compared to D0, ** p<0.05 compared to corresponding FFP value).

	FFP (N=20)			RTFP24 (N=20)		
Parameter	D0: Median (range)	D4: Median (range)	D4: Percentage decrease from baseline: Median (range)%	D0: Median (range)	D4: Median (range)	Percentage decrease from baseline: Median (range)%
vWF:Ag (IU/dL)	109 (48-165)	105 (47-155)*	3.0 (-7.0 to 23.7)%	96 (52-160)	95 (53-164)	-1.8 (-7.1 to 15.3)%
Fibrinogen (g/L)	2.4 (1.8-3.2)	2.4 (1.9-3.3)*	-2.2 (-7.2 to 1.2)%	2.5 (1.8-3.0)	2.3 (1.7-3.2)	1.1 (-6.4 to 9.5)% **
Protein C (IU/dL)	109 (86-139)	106 (87-136)	0.9 (-3.6 to 21.3)%	102 (83-154)	98 (82-154)*	3.1 (0 to 6.8)% **
Protein S (IU/dL)	99 (70-116)	74 (51-104)*	24.4 (-5.1 to 43.7)%	76 (57-102)	79 (48-93)	4.6 (-18.7 to 30.4)% **
ATIII (IU/dL)	88 (78-100)	87 (75-129)*	1.2 (-29 to 5.1)	94 (73-112)	92 (77-108)	0 (-5.5 to 9.6)%
Lag time for thrombin generation (Min)	2.8 (2.5-4.0)	3.5 (3.0-5.0)*	-20.0 (-50 to -4.9)	2.5 (2.0-4.0)	3.0 (2.5-5.0)*	-24.2 (-40 to 0)%
Endogenous Thrombin Potential (ETP)(Min nM)	1363.9 (1174.4-1605.7)	1189.4 (1015.3-1495.2)*	10.8 (5.1 to 22.1)%	1238.8 (870.0-1823.2)	1090.5 (750.4-1489.0)*	14.6 (-22.8 to 31.3)%
Peak of thrombin generation (nM)	182.3 (107.1-251.0)	124.7 (68.8-189.5)*	34.3 (23.6 to 45.6)%	154.7 (102.6-331.4)	103.3 (62.5-303.6)*	35.5 (-0.6 to 53.5)%

Aims: Study RTFP24's ex vivo haemostatic capacity >24h post-thaw and compare to FFP.

Methods: 20 units (U) each of RTFP24 and FFP (blood group O:8U, A:5U, B:5U, AB:2U) were thawed ≥3 weeks after preparation, stored at 1-6°C till 4 days post-thaw (D4) and measured for FII, FV, FVII-XI; FXIII (chromogenic); fibrinogen; vWF:Ag; protein C (PC), ATIII; protein S (coagulometric); thrombin generation (calibrated automated thrombogram without PC activator) at time of thaw (D0) and D4. Labile factors (FV, FVII, FVIII) were also measured on D1-D3. Data was analysed for significant differences from corresponding baselines within each TP type and between both TP types at D4 using non-parametric tests.

Results: FV, FVII and FVIII declined from D1 (p< 0.001) except that FV declined from D2 in FFP (p< 0.01). ≥95% of FFP and RTFP24 U maintained clotting factors within reference ranges at D4 (except FV and FVIII) with no significant decline in proportion of such U from D0 (except FVIII). However, proportion with abnormal thrombin generation (using our reference ranges) increased at D4 for both TP types (p< 0.05). At D4, RTFP24 was comparable to FFP except for lower ETP (p=0.03) and higher FXIII (p=0.01). Of these, only FXIII was different between RTFP24 and FFP at baseline (p=0.03). See tables for other results.

Conclusions: While comparable to FFP, RTFP24 may have less efficacy at D4 from lower ETP. We do not recommend universally extending RTFP24's shelf-life to >24h post-thaw.

PB 310 | Effect of Handling on the Functional Utility of Stored Platelet Units

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Background: 7000 units of stored platelets are infused daily into patients with significant bleeding all over the United States. The platelet units commonly need to be urgently delivered to the critical care team with the requirement that platelets remain functional in order to immediately improve hemostasis. A pneumatic tubing transport system is frequently used to facilitate a rapid and controlled delivery of reagents including platelet units within a hospital. However, the potential detrimental effect of shear forces due to gravitational effects platelets may experience during this delivery method is not well understood, leasing many hospitals to not permit platelet transportation using pneumatic systems.

Aims: Determine whether pneumatic tubing transport has a functional effect on stored platelet units.

Methods: Stored platelet units were divided into 3 groups: pre-transport, pneumatic tubing and ambulatory transport. Platelet activation (P-selectin expression) and microaggregation (CD41+/CD31+ events) and glycoprotein (GP) Ib receptor expression levels were quantified using FACS in the presence of select agonists. The hemostatic function of platelets was assessed by quantifying platelet adhesion and aggregation following perfusion of whole blood over collagen under shear. VWF multimer levels were assessed via western blot.

Results: While the mode of transport did not affect platelet GPIb receptor or VWF multimer levels, our results show that transport of stored platelet units through pneumatic tubing led to a decrease in platelet adhesion on collagen and vWF as compared to platelet units transported by ambulatory transport.

Conclusions: Our results suggest that subjecting platelets to high velocities within pneumatic tubing may diminish the ability of stored platelets to form a hemostatic plug on exposed extracellular matrix proteins and thus reduce their efficiency at staunching blood loss at an injury site.

PB 311 | Coagulations Parameters and Need for Transfusion in Patients with Veno-arterial Extracorporeal Membrane Oxygenation in Clinical Hospital Center Rijeka

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Background: Adult patients supported by veno-arterial extracorporeal membrane oxygenation (V-A ECMO) have high blood products requirements. The main indication are severe cardiac failure and cardiopulmonary resuscitation.

Aims: Analysis of coagulation parameters and need for transfusion in ECMO patients during three years period.

Methods: Retrospective analysis and statistics elaboration of medical and laboratory data regarding adult patients during V-A ECMO treatment.

Results: In our hospital 38 adult patients were treated by V-A ECMO from 2014 to 2016; 9 women and 29 men. Mean ECMO time was 6 days (1 to 19). Surviving patients were 22 and 16 died. The mean values of coagulation parameters in first day were: prothrombin time (PT) 68%, International Normalized Ratio (INR) 1.36, activated partial thromboplastin time (aPTT) 76s, fibrinogen 3.4 g/L, antithrombin III (ATIII) 63%.The highest transfusion requirements patients had during the first day. Red blood cells (RBCs) were transfused to 36 patients (mean units in died 11 vs. 6 in survivors). Eighteen patients received 185 units of fresh frozen plasma (FFP), mean units in died were 8 vs. 6 in survivors. The same number of patients received transfusion of 122 units of platelets (mean units in died were 4 vs. 3 in survivors). Antithrombin III was the most frequently used blood derivate during the first ECMO day in 9 patients (mean units in died 469 vs. 227 in survivors). During monitoring of coagulation parameters normalization of all values is obvious, except of ATIII which is under lower limits all the time.

Conclusions: The values of PT/INR and antithrombin III are the most subjected to the changes during the initial period of ECMO treatment. Transfusion requirements of blood components and derivates were the mostly intense in the first day. The difference between patients who died and survivors was clinically significant although statistically can not be determined due to small sample.

PB 312 | Blood Loss and Transfusion of Blood Products in Children Undergoing Craniofacial Surgery

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Background: Craniosynostosis (CS) is a condition where the cranial sutures ossify prematurely. To avoid brain damage, CS needs to be corrected surgically within the first years of life. This intervention is associated with substantial blood loss, which can exceed the child´s total blood volume. Reviewing international literature, the reported amount of blood loss during these operations differs significantly.

Aims: The overall aim was to investigate whether the amount of blood loss and transfusion of blood products have changed during a 15-year period. This was done by comparing two cohorts of children (2000-2006 vs 2013-2014) operated for CS at our institution.

Methods: All medical records of children who underwent craniofacial reconstruction during the periods 2000-2006 (n=123) and 2013-2014(n=52) were reviewed. Demographic parameters and clinical information was obtained. The intraoperative blood loss was registered as evaluated by the attending anesthesiologist, while the postoperative blood loss was reported as the total output in surgical drains

during ICU-stay. Information on the amount of transfused blood products was obtained from the anesthesia and ICU records.

Results: Per February 1st, 2017, the data of the first 111 patients (2000-2006: n=85; 2013-2014: n=24) were analyzed. These preliminary results show no difference has yet been detected in terms of intraoperative (25 ml/kg vs 26 ml/kg p=0.94) or postoperative blood loss (26 ml/kg vs 27 ml/kg p=0.27) while a numerically, though not significantly, reduced amount of transfused blood products during surgery (median 19 ml/kg vs 15 ml/kg p=0.25 for red blood cells; median 18 ml/kg vs 13 ml/kg p=0.27) for fresh frozen plasma.

Conclusions: The present preliminary results suggest that the amount of transfused blood products has dropped, though not significantly, between the analyzed time periods.

PB 313 | Inappropriate Fresh Frozen Plasma Transfusion in Children

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Background: Fresh frozen plasma (FFP) is a product extracted from whole blood. Blood products should be used according to transfusion guidelines to reduce the transfusion related complications.

Aims: In this study our aim is to evaluate inappropriate FFP transfusion in children.

Methods: Data were obtained from FFP transfusion records between April -December 2016. We based on National Blood Products Guideline for FFP transfusion indications. Thus inappropriate FFP transfusion ratio according to clinical department was determined.

Results: Ninety male (61.6%) and 56 (38.4%) female patients with a mean age of 3.5±5.1 years were enrolled in this the study. Totaly 286 units of FFP in 146 transfusion episods were used. Forty-one (28.1%) and 105 (71.9%) of products were used in surgical and pediatric services respectively. Twenty nine (19.8%) of 146 transfusion episods were inappropriate . The ratio of inappropriate transfusions according to services were seen in table 1. The reason for inappropriate FFP transfusion were as follows: 16 (%39,3) patients for operation, seven (24.1%) patients for burn, five (17.3%) patients for alongation of coagulation parameters and one (3.5%) patients for gastrointestinal bleeding with normal coagulation parameter. There were no transfusion related complication during FFP transfusion periods.

Conclusions: Collection of blood and blood products based on voluntary donation and the cost is high. Transfusion of blood products has troublesome side-effects. In this study inappropriate FFP transfusion rate was found 19.8% . This ratio could increase up to 60% in adult groups. Different educational programs based on needs may raise awareness among clinicians for appropriate FFP transfusion.

TABLE 1 The ratio of inappropriate fresh frozen plasma transfusions according to services

	Pediatric services	Surgical services	Total
Appropriate	98 (67.1%)	19 (13.1%)	117 (80.2%)
İnappropriate	7 (4.8%)	22 (15.0%)	29 (19.8%)
Total	105 (71.9%)	41 (28.1%)	146 (100%)

PB 314 | Knowledge, Attitude and Practice Regarding the Voluntary Blood Donation among the Young Student Population of Karachi

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Background: Safe blood is a crucial and irreplaceable component in the medical management of many diseases. The Voluntary non-remunerated blood donation is the ideal sources of quality blood, which forms less than 15 % of the demand of the blood in Pakistan.

Aims: To assess the knowledge, attitude and practice regarding the voluntary blood donation among the young student population of Karachi.

Methods: A cross sectional prospective study was conducted among 600 students from different universities and colleges of Karachi. A well-structured and pre-tested questionnaire, in English, was used to access the knowledge, attitudes and practices about voluntary blood donation. A scoring mechanism was used to understand overall knowledge level. Obtained data was analyzed.

Results: The sample population consisted of 54% male and 46% female students in the age group of 18-28 years. Only 65 % of the students have heard about voluntary blood donation and 28 % of the students have given blood once in their lifetime and among them 19 % are blood donors at the moment. 42 % of the participants believed that there is a specific reason why they don´t donate blood and 59 % believed that there is a risk involved for the donors, when donating blood. 80 % students wanted to promote voluntary blood donation. Fear and lack of awareness on blood donation are the reasons for not donating blood. Students gather information about voluntary blood donation from several sources mostly schools, colleges, family and friends.

Conclusions: This study showed that myths and misconceptions are leading the youngsters not to donate blood. Study also showed how increasing awareness and marketing through different ways can boost the culture of voluntary blood donation in society. Student population can be motivated to participate in different ways. There is a dire need to mobilize the electronic media for educating our youth about voluntary blood donation due to its access to masses.

PB 315 | Burden of Hemoglobinopathies on the Blood Bank of a Tertiary Care Setting

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Background: Hemoglobinopathies are an emerging global health burden. The true magnitude of the hemoglobinopathies is still unknown in our region because of lack of facilities for the diagnosis and management. Besides that, haemoglobinopathies impose additional burden to the blood transfusion services because of lack of availability of rare blood groups and blood components.Additionally they are also at high risk of transfusion transmissible diseases.

Aims: The aim of this study was to assess the burden of hemoglobinopathies on the blood bank of a tertiary care hospital.

Methods: In our study, the transfusion requirements of all the patients having various hemoglobinopathies were studied from Jan 2015-Jan 2016.The data of all the patients who were issued red cell concentrates during this period was recorded.Particulars of those patients who needed washed red cells were also recorded in the proforma Forward and reverse ABO grouping and Rh grouping was done on all samples along with cross match and all the findings were recorded in the proforma.

Results: A total of 4684 red cell concentrates were issued during the study period. Out of these,452 (9.64%) red cell concentrates were issued to the patients having various hemoglobinopathies.95% of these red cells were issued without replacement donations. Of these donations, the frequencies of A^+, B^+, $AB^{+,}$ and O^+blood groups were 22.44%, 33.61%, 6.58%, and 35.07%, respectively.

Conclusions: Extensive screening of the population for identification of carriers of hemoglobinopathies is essential and further it will be of assistance in taking adequate therapeutic and preventive measures.

PB 316 | Major Blood Groups and A2 Subgroup in Mixed Pakistani Population of Karachi

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Background: The Major blood groups are distributed in varying frequencies in different parts of world and in different ethnic groups. Information is scant in this aspect in mixed Pakistani population especially the frequency of weak subgroup A2 of blood group A.

Aims: To observe the frequency of major blood groups and sub-group A2 in a mixed Pakistani population in the catchment area of Dow international medical college, Karachi.

Methods: 536 healthy unrelated donors and their patients for whom they donated blood, of both sexes were selected for four months. Venous samples were taken and forward and reverse grouping were done by tube method in the blood bank of DDRRL affiliated with Dow international medical college and its affiliated Ojha hospital.

Results: The dominant group was "O" n 111(40 %) and B group was the second largest n 149 (39 %). The third largest was A group n 72 (20 %) and least was n 13 (4%). From A group, 20 were A2 group. This constituted 3 % of all samples and 18 % of A group individuals.

Conclusions: The blood group frequency of a mixed Pakistani population had differences with the studies done in other countries and for the first time, the frequency of A2 sub-group was estimated in mixed Pakistani population.

PB 317 | New Tools for the Delivery of RNA to Platelets

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Background: Platelets are an integral part of hemostasis and commonly used in the clinic to reduce bleeding. The ability to directly genetically manipulate platelets would be a useful tool for modifying and studying platelet function, and potentially for modifying and improving platelets which are used for transfusion. While a number of technologies have been used to deliver genetic material to cells throughout the body, few are well suited for use with platelets, which are anucleate and prone to activation.

Aims: Previous work has shown that platelets can be transfected with synthetic miRNA using Lipofectamine, however it is unknown whether lipid- based nanotechnologies suited for in vivo applications could also be used with platelets. Using these technologies, this project aims to develop a method that would allow for direct delivery of RNA to transfusable platelets with high efficiency, without inducing significant platelet activation.

Methods: Here, we developed a way to directly introduce mRNA to platelets, using lipid nanoparticles (LNPs) to deliver *in vitro* transcribed mRNA to platelets *ex vivo*. Flow cytometry, immunofluorescence confocal microscopy, and quantitative PCR were used to measure the endocytosis of LNPs and encapsulated mRNA into platelets. Platelet activation was determined by P-selectin expression and the extent of platelet aggregation.

Results: Using this approach, we have shown that exogenous mRNA encapsulated within LNPs is rapidly endocytosed by platelets while causing minimal platelet activation and aggregation. Components of the LNPs required to prevent platelet activation and promote uptake have been identified. Delivered mRNA can be detected in platelets for several hours following uptake, however regulation of protein expression has not yet been detected.

Conclusions: This is one step toward developing new tools for transfecting platelets. In the long term, these tools may lead to modified platelet products with a longer shelf-life or improved hemostatic ability.

PB 318 | Effects of Activated Human Platelet Releasates on Wound Healing of Immunodeficient Mice: Comparison with Basic FGF

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Background: Activated platelets release cytokines and growth factors, which are involved in tissue repair. Recent evidence supports the clinical application of platelets to accelerate wound healing. Recombinant basic fibroblast growth factor (rbFGF) is also in clinical use for this purpose.

Aims: The present study compared the effect of human platelets with that of rbFGF on wound healing of immunodeficient NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice.

Methods: Human platelets were washed and stimulated with 10 mM calcium/PBS for 15 min, and the sample was prepared as 4x10^7 platelets/20 µL (PLT). The cytokine and growth factor levels in the supernatants of PLT were measured by ELISA. Full-thickness excisional wound (0.5 cm^2) were treated with either PLT or 6 µg/20 µL rbFGF. These dosages were determined based on the therapeutic dose currently used in the clinical settings. The wound size was measured chronologically, and the histological examinations of wounded skin were performed with hematoxylin-eosin staining. This study was approved by the ethics review committee for the use of human samples and animal experiments of Keio University.

Results: PLT released 32±1 pg/mL of bFGF, 59±6 pg/mL of platelet derived growth factor, 78±4 pg/mL of vascular endothelial growth factor, 4±0.4 µg/mL of transforming growth factor beta 1, and 8±1 pg/mL of epidermal growth factor. The wound area (% of Day 0) on Day 5 was 87±6% in PLT group and 122±0% in rbFGF group, indicating that the healing process was faster in the PLT group. The histological examination indicated that there were differences in the patterns of wound healing. The wounds treated with PLT showed increased newly organized collagen, and relatively advanced growth of the epithelium and layer construction as compared to those treated with rbFGF.

Conclusions: While bFGF concentration in supernatant of activated platelets was low, they released various cytokines and growth factors related tissue repair and showed more beneficial effects on wound healing than rbFGF.

PB 320 | SAG Mediated Therapy Leads to a Predominant Th1 during Visceral Leishmaniasis on Triggering CD2 Epitope

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Background: Visceral Leishmaniasis is a macrophage associated disorder for the treatment of which antimony based drugs like SAG and SSG were the first choice in the recent past. The clinical value of antimony therapy is now declined against VL because increasing cases of Sodium Antimony Gluconate (SAG) resistance have reached outstanding proportion in Bihar, India.

Aims: We have evaluated the effect of combining CD2 with conventional antimonial (sb) therapy in protection in BALB/c mice infected with either drug sensitive or resistant strain of Leishmania donovani with 3 million parasites via-intra-cardiac route.

Methods: Mice were treated with anti CD2 adjunct SAG subcutaneously twice a week for 4 weeks. Assessment for measurement of weight, spleen size, anti-Leishmania antibody titer, T cell and anti-leishmanial macrophage function was carried out day 0, 10, 22 and 34 post treatments.

Results: The combination therapy was shown boosting significant proportion of T cells to express CD25 compared to SAG monotherapy. Although, the level of IFN-γ was not statistically different between combination vs monotherapy ($p = 0.298$) but CD2 treatment even alone significantly influenced IFN- γ production than either SAG treatment ($p = 0.045$) or with CD2 adjunct SAG treatment ($p = 0.005$) in Ld-S strain as well as in Ld-R strain. The influence of CD2 adjunct treatment was also documented in anti-leishmanial functions in macrophages. As shown, the super-oxide generation began enhancing very early on day 10 after SAG treatment with CD2 during which SAG action was at minimum. Interestingly, the super-oxide generation ability remained intact in macrophage after treatment with immunochemotherapy even in mice infected with Leishmania resistant strain.

Conclusions: Our results indicate that CD2, which can boost up a protective Th1 response, might also be beneficial to enable SAG to induce macrophages to produce Leishmanicidal molecules and hence control the infection in clinical situation like Kala-azar.

PB 321 | Characterization of a Universal Reversal Agent for Anticoagulants

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Background: Anticoagulant medicines are widely used. Until now, there are no universal reversal agents for all anticoagulants. Cyclodextrins are macromolecular cyclic sugars. In this study, we describe the discovery of a novel class of prohemostatic cyclodextrins.

Aims: To explore in vitro and in vivo prohemostatic effects of cyclodextrins.

Methods: Fibrin polymerization assay, calibrated automated thrombography and rotation thromboelastometry in normal plasma and whole blood were used to assess the effect of cyclodextrins on coagulation in the absence or presence of various anticoagulants. In vivo hemostatic effects of a potent prohemostatic cyclodextrin was tested in mice treated with rivaroxaban using a vena saphena bleeding model.

Results: A number of potent prohemostatic cyclodextrins were discovered that concentration-dependently increased thrombin generation in normal plasma. In addition, thrombin generation was markedly enhanced in plasma of patients treated with vitamin K antagonists, and normal plasma spiked with dabigatran, rivaroxaban, apixaban, edoxaban, unfractionated and low molecular weight heparin, pentasaccharide and hirudin. Also, the procoagulant cyclodextrins shortened the clotting time in rivaroxaban-spiked whole blood. One of the procoagulant cyclodextrins fully restored the bleeding tendency in rivaroxaban-treated mice.

Conclusions: A novel class of procoagulant molecules was identified. Prohemostatic cyclodextrins act as broad spectrum anticoagulant reversal agents.

PB 322 | Antiaggregant Effect of Rubia tinctorum Butanolic Extract on Human Platelets

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Background: *Rubia Tinctorum* is a plant belonging to Rubiaceae family that historically originated from Caucasus and Near East. This plant is widely distributed in southern and southeastern Europe, in central Asia, and in the Mediterranean including the north of Africa. It is commonly known as "El foua" in Morocco. Extracts from *Rubia Tinctorum*'s root have been used as a traditional medicine to cure various ailments. For example, studies have shown that the dried roots are useful in alleviating dropsy, paralysis, jaundice, amenorrhea and visceral obstructions. Platelets aggregation is an important reaction to stop bleeding but it is considered that excessive platelets aggregation causes thrombosis and ateriosclerosis. Recently, the antiaggregant activies of some spices were investigated.

Aims: The present investigation was undertaken to study the antiaggregatory effect of *Rubia Tinctorum* extracts on human platelets.

Methods: Ethanol extract and its n-hexane, ethyl acetate, butanol and aqueous fractions of *Rubia Tinctorum* (Rubiacea) were tested *in vitro* for their aggregant activitiy. Platelet aggregation was evaluated in platelet rich plasma (PRP) obtained from blood of healthy human volunteers. Human platelets were subjected to stimulation with collagen (0.2µg/mL) in the presence and/or absence of each extract at the concentrations 250, 500 and 1000 mg/ml respectively.

Results: A significant inhibition was observed only with the butanolic extract at 500 and 1000 mg/mL with 8% of inhibition.

Conclusions: Our findings suggest that the butanolic extract of *Rubia Tinctorum* may have component(s), which prevent platelet aggregation.

PB 1032 | Intraoperative Patient Blood Management: A Role for Very-low-Dose Recombinant Activated Factor VII

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Background: Recombinant activated factor VII (rFVIIa) is widely used for treatment of major bleeding beyond its labelled indications. However, conflicting data have been presented with respect to efficacy.

Aims: To evaluate the efficacy of single shot very-low-dose rFVIIa (vld-rFVIIa) in cardio-surgical patients with unabated bleeding.

Methods: Real-time monitoring of hemostatic parameters was conducted in a prospective cohort study of cardiac-surgery patients (n=281). Informed consent was obtained from all patients. The study was approved by the local medical ethical committee.

First-step bleeding therapy was carried out by goal-directed substitution of hemostatic compounds. In case of unabated bleeding single shot vld-rFVIIa (14 ± 3 micrograms/kg) was administered (n=167). Hemostatic efficacy in the vld-rFVIIa group was compared to patients without rFVIIa administration (n=114). The efficacy of vld-rFVIIa as compared to substitution therapy was assessed using prothrombin times and extrinsic clotting times by thromboelastometry as well as platelet function by impedance aggregometry.

Results: Hemostatis was achieved by single shot vld-rFVIIa in 61.3%-88.6% of patients without need for further hemostatic treatment. Clinical efficacy could be objectified by specific laboratory testing in a subgroup of 27 patients: clotting times of the extrinsic pathway were significantly shortened by vld-rFVIIa administration (prothrombin time (Quick test) +58.7 ± 13.8%, p< 0.0001; extrinsic clotting time by thrombelastometry -12.7 ± 11.9 sec, p< 0.0001). An increase of TRAP-induced platelet aggregation was observed in 18/27 patients after vld-rFVIIa administration however did not reach significance.

Conclusions: vld-rFVIIa is effective for the treatment of unabated bleeding in cardiosurgical patients. Substitutional restoration of the hemostatic potential with subsequent vld-rFVIIa-triggering of a momentary thrombin burst may represent a novel paradigm in major bleeding therapy.

PB 1033 | Preoperative Assessment of Bleeding Risk - Implementing Strategies in the Pursuit of Effective Patient Blood Management

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Background: Guidelines advise that a bleeding history should be taken and that routine coagulation screening is inappropriate in patients having surgery.

Aims: A project to optimise bleeding risk assessment in patients undergoing elective surgery at a single hospital was undertaken. This underpins effective patient blood management.

Methods: 121 medical records of patients attending surgical pre-admission clinic (PAC) in a single month were audited. Demographic and clinical data were recorded including number of days between PAC attendance and surgery, patient self-completion of health questionnaire, documentation of bleeding history, record of anti-platelet and anti-coagulant therapy, and coagulation test results. Following dissemination of initial audit results, a standardized preoperative bleeding risk questionnaire was developed and introduced into routine practice in PAC and a post-intervention audit was performed.

Results: Initial audit found that 54% patients were seen in PAC less than 1 week prior to surgery and that 19% patients did not self-complete their health questionnaire, No patients had a standardised bleeding risk assessment performed and 11% patients had limited documentation of bleeding history. Coagulation testing was requested in 67% patients and 41% patients were taking anti-platelet and/or anti-coagulant therapy. Audit results were disseminated and a structured bleeding questionnaire was introduced into PAC. No change to practice of ordering coagulation tests was made at the request of anaesthetists. Post-intervention audit of 109 medical records found that 62% patients had a structured bleeding history performed. Coagulation testing was ordered in only 49% patients, a significant reduction, even though a change in this practice had not been advocated.

Conclusions: Current practice indicates that there is an over-reliance on coagulation screening in lieu of appropriate history taking in assessing bleeding risk, however introduction of structured bleeding risk questionnaires can overcome this.

PB 1034 | Indications for the Use of Fresh Frozen Plasma in Neonatal Intensive Care Unit in a Tertiary Care Hospital

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Background: Utilization of fresh frozen plasma(FFP) is high in neonatal intensive care unit and the indications for the use of FFP in neonates are limited.

Aims: To evaluate the indications for the use of fresh frozen plasma (FFP) in a tertiary care neonatal intensive care unit (NICU), specifically to examine compliance with guidelines for the use of fresh frozen plasma in neonates.

Methods: It was a prospective observational study conducted over a period of 12 months from March 2015 to March 2016 at Fauji Foundation Hospital Rawalpindi Pakistan.Data of all the neonates who received FFP transfusion was recorded taking into account the indications, pretransfusion and post-transfusion laboratory test of haemostasis, and detailed history of any bleeding episodes.

Results: A total of 192 FFP units were issued to the neonatal intensive care unit over the peroid of 12 months.Of these, 21% FFP were issued because of active bleeding and 79% neonates received FFP prophylactically with the intention of preventing haemorrhage,generally in the setting of neonatal sepsis and necrotizing enterocolitis (NEC).The most common determining factor for FFP transfusion were an abnormal activated partial thromboplastin time or prothrombin time.Using the guidelines for FFP usage in neonatal setting,it was analysed that 60% of the 192 FFP transfusions were not compliant with the standard guidelines. Using linear regression analysis, abnormalities in the prothrombin time, activated partial thromboplastin time, were not independently associated with the hemorrhagic episodes.

Conclusions: FFP transfusions are frequently used in the NICU. In the present study, a remarkably high proportion of FFP transfusions were given to non-bleeding neonates for indications not compliant with guidelines for FFP usage in neonates. Coagulation studies e.g prothrombin time and activated partial thromboplastin time were poor predictors of clinical bleeding.

PB 1035 | Intermittent Intravenous Deferoxamine for the Treatment of Transfusional Iron Overload

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Background: Transfusional iron overload (TIO) is associated with reduced survival in thalassemia and in myelodysplasia. Deferoxamine (DFO), an iron chelator, improves survival in thalassemia and TIO. It is administered as a continuous subcutaneous or intravenous infusion over 8-12 hours, 5-7 days per week. Compliance is poor (59-78%). Deferasirox is an oral iron chelator. Gastrointestinal toxicities arise in 15% of patients. Effect on survival has not been studied. We have treated seven patients with TIO, who were poorly responsive to, had contraindications to, had toxicities with, or declined continuous subcutaneous DFO and/or deferasirox, with intermittent intravenous DFO (IID).

Aims: To determine in this cohort:

1. If IID led to a significant reduction in serum ferritin concentration
2. The percentage of patients who achieved serum ferritin < 1000 ng/mL, the goal of chelation.

Methods: Institutional review board approval was obtained. The charts of this cohort followed between 07/01/03 and 12/31/15 were reviewed.

Results: Seven patients (2 thalassemia, 1 myelodysplasia, 1 aplastic anemia, 1 HIV hypoplasia, and 2 sickle cell anemia) were studied. Median age was 37 years, range 24-61 years; M:F ratio was 1:6. DFO dosing regimens ranged from 2 to 5 grams over 4 to 7 hours weekly to monthly. Median duration of therapy was 61 weeks, range 17-213 weeks. Median pre-treatment ferritin was 2364.4 ng/mL, range 1106-3299.5 ng/mL. Median post-treatment ferritin was 1435.2 ng/mL, range 803.7-2012.5 ng/mL. Paired student´s t-test showed a median reduction in ferritin of 715 ng/mL, range 181-1736 ng/mL, p=0.009. The patients with thalassemia received higher maximal doses (4.5 to 5 grams over 7 hours weekly) than did those with other disorders (2 to 3 grams over 4 hours weekly). Two patients (1 myelodysplasia, 1 sickle cell anemia) achieved ferritin < 1000 ng/mL.

Conclusions: IID in TIO is associated with a significant reduction in serum ferritin levels, and may decrease the ferritin to < 1000 ng/mL in non-thalassemic TIO.

PB 1036 | Reasons for Blood Donor Deferral among Voluntary Blood Donors in a Tertiary Care Hospital in Kathmandu, Nepal

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Background: Ensuring the wellness of the voluntary blood donor is the main purpose of the donor deferral. It also minimizes the unwanted symptoms that may appear among blood donor recipient.

Aims: To find **Reasons For Blood Donor Deferral Among Voluntary Blood Donors In A Tertiary Care Hospital.**

Methods: This is the retrospective study carried out among voluntary blood donors at Grande International Hospital, a tertiary care hospital in Kathmandu, Nepal from January 2013 to January 2015.

Results: The data were collected from previous records of the blood donor history forms. From a total of 8,550 blood donations, 302(3.53%) blood donor were deferred due to various reasons. Among all the deferred blood donors 189(62.58%) were female where as 113(37.42%) were male. Furthermore, 289(95.69%) were temporarily deferred and 13(4.31%) were permanently deferred. The mean age of deferred blood donor was 35 years.

Out of total blood donor deferral; 101(33.48%) donor were rejected because of bed side hypertension

(i.e. blood pressure- systolic > 140 and diastolic >90 mm hg) which was followed by anaemia

(i.e. haemoglobin < 12 gm/dl) 94(31.12%), vaccinations history 43(14.23%), hypotension (i.e. Systolic < 90mm hg and diastolic < 60 mm hg) 35(11.58%), dental examination 10(3.32%) and medication history 6(1.98%). Permanent deferral namely, risk factor involving transfusion transmitted infections and chronic disease were 5(1.65%) and 8(2.64%) respectively. The prime cause of permanent deferral was risk factor involving transfusion transmitted infections while the temporary deferral was bed side hypertension. Gender wise, the leading cause of donor deferral in male was bed side hypertension and anaemia was the major cause in female.

Conclusions: The findings of the survey aid to evaluate the significant causes of blood donor deferral. This study suggests that the restrictive criteria can be used for blood donor selection. This will in turn increase the blood supply of tertiary care hospital.

PB 1037 | Haemovigilance Reports of Adverse Blood Donor Reaction among Voluntary Blood Donors in Tertiary Care Hospital in Kathmandu, Nepal

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Background: Voluntary blood donation is widely considered to be safe with very minimum chance of adverse reaction, which may occur during or after the end of phlebotomy procedure.

Aims: To access the **adverse Blood Donor Reaction Among Voluntary Blood Donors In Tertiary Care Hospital.**

Methods: This is a prospective study done among voluntary blood donors at Grande International Hospital, Kathmandu, Nepal from February 2013 to March 2015. The outlines of reported and communicated adverse donor reaction were also collected after the blood donation from voluntary blood donors in different locations including outdoor and in-house blood donation drive.

Results: In the present study 6,955 whole blood donors were included, during the period of 2 years, 105 (1.50%) adverse donor reactions were reported. Majority 89(84.76%) of adverse donor reactions were mild in nature such as, sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 8(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylaxis;11(10.49%), loss of consciousness; 3(2.85%) and convulsive syncope;2(1.90%). Markers of the adverse donor reaction were age, sex, pulse, weight, blood pressure and donation status. Age and first time status were related with significantly higher risk of adverse reaction with 18-23 years old at higher risk compared to 24-55 years old. First time donors were at higher risk compared to repeated volunteer donors.

Conclusions: The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donors is lower than in other studies. Donor age and donation status were strong possibilities of complications.

PB 1038 | Re-infusion of Heparinized Shed Blood in Aortic Surgery to Maintain Hemostatic Levels of Fibrinogen

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Background: Aortic surgery is associated with large volume blood loss. Blood salvage strategies primarily focus on red blood cell salvage and replacement withput plasma contributes to hemodilution. Fibrinogen is a critical procoagulant that must be maintained at adequate levels to preserve hemostasis.

Aims: We report early data on the impact of whole blood salvage and re-infusion in aortic surgery with a focus on impact to fibrinogen levels during whole blood salvage.

Methods: Patients (n - 9) undergoing thoraco-abdominal aortic aneurysm repair had heparinized salvaged blood collected and reinfused using a filtered cardiotomy reservoir and modular roller pump. Filtered blood was returned directly to a Belmont FMS rapid infuser with rate of return controlled by the anesthesiologist. Average volume of salvaged blood processed in this manner was 7600 ml (range 3155 ml - 13939 ml.) Fibrinogen concentration was measured by Clauss and viscoelastic methods at the following time points: pre-bypass, post-proximal anastamosis, post distal anastamosis, and post-bypass.

Results: Heparin used for bypass did not interfere with either fibrinogen assay. Fibrinogen concentration was preserved at adequate hemostatic levels in all but 1 patient. Early availability of data allowed treatment to restore adequate hemostatic activity at the post-CPB time point.

Conclusions: Salvage and re-infusion of heparinized shed blood during aortic surgery preserves hemostatic content despite large volume blood loss. Measurement of fibrinogen concentration early in the operative procedure allows for prompt restoration of fibrinogen to levels critical for hemostasis.

PB 1039 | Use of Molecular Biology to Reveal the Mechanism of Non-hemolytic Febrile Transfusion Reaction to Enhance the Transfusion Safety

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Background: Blood transfusion reactions occur frequently even after performing complete pre-transfusion tests. Among these reactions, febrile non-hemolytic transfusion reaction (FNHTR) is the most common type of reaction. Leukocyte-poor blood (LPR) can reduce the number of FNHTR, but FNHTR still occur in certain patients who are transfused with leukocyte-poor blood (LPR).

Aims: Hence, we proposed that the human platelet antigen (HPA), in particular HPA-3, is a potential membrane antigen that plays a role in ineffective transfusion in addition to the human leukocyte antigen (HLA) and human neutrophil antigen (HNA). In this study, we investigated whether the difference of HPA-3 genotype between the donors and recipients of blood transfusion contributes to the occurrence of FNHTR.

Methods: A total of 87 transfused LPR patients with (n=43) or without (n=44) FNHTR were collected to determine the HPA-3 gene genotype of the donors and recipients by PCR using sequence-specific primers.

Results: Our data indicated that the HPA-3 genotype was different between donors and recipients in 80.0% and 52.3% of the transfused patients with and without FNHTR, respectively (P=0.0412). The donors with heterozygous HPA-3a/3b genotype caused FNHTR more frequently than the donors with homozygous HPA-3a/3a or HPA-3b/3b genotype ($\chi 2$ = 7.86, P-value = 0.0197).

Conclusions: In conclusion, HPA-3 is a contributing factor for the occurrence of FNHTR after blood transfusion. Avoiding the use of blood from the donor with heterozygous HPA-3a/3b genotype is likely to enhance the safety of transfusion in the clinical setting.

PB 1041 | Pseudomyxoma Peritonei Surgery: Potential for Predicting Fibrinogen Use using Thromboelastometry

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Background: Pseudomyxoma peritonei is a rare malignancy treated using peritonectomy and hyperthermic intra-peritoneal chemotherapy which is associated with bleeding characterised by low fibrinogen. A subgroup of patients who required extensive diathermy from an early stage in surgery due to bulk and distribution of disease have been noted to receive more fibrinogen replacement therapy.

Aims: This is a retrospective pilot study to:

1. Assess correlation of fibrinogen concentration between thromboelastometry using ROTEMâ Sigma A20 FIBTEM (clot firmness (mm) at 20 minutes post clot time), EXTEM CT (clot time from start of test to 2mm amplitude) and Clauss fibrinogen measurements.
2. Assess potential of thromboelastometry to characterise a subgroup of "high risk" bleeding patients.

Methods: Coagulation screens (Prothrombin Time (PT), Activated partial thromboplastin time (APTT), Clauss fibrinogen (FIB),) thromboelastometry (ROTEMâ Sigma analyser and reagent cartridges) were performed during surgery at multiple time points on 6 patients according to standard care.

Results: Study identified significant correlation between Clauss FIB and FIBTEM A20 results at 14 time points (r^2=0.90) using the

Mann-Whitney U test. FIBTEM A20 mean = 18.9mm (normal range 6 - 21) and Clauss FIB mean = 3.21 (normal range 1.6 - 4.2g/L).

With respect to extensive diathermy sub-group significantly lower FIBTEM A20 (p= 0.028) and Clauss FIB (=0.026) was detected. Mean volume of cryoprecipitate in the diathermy sub-group was 4 units versus 2 units for other patients.

Conclusions: Pilot study has demonstrated utility of FIBTEM A20 measurement in assessing fibrinogen status in patients as risk of developing hypofibrinogenaemia. There also appears to be a difference in the haemostatic profile for patients requiring early and extensive diathermy. Further investigation to identify patients at high risk of bleeding, underlying mechanisms and application of FIBTEM A20 measurement to optimize fibrinogen replacement therapy are planned.

PB 1042 | Comparison of Arterial and Venous Coagulation Parameters Measured with the QuantraTM System in Cardiac Surgery

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Background: Coagulation testing is often performed in cardiac surgery and other major procedures to aid in the management of perioperative bleeding. Although reference ranges are obtained in venous samples, perioperative testing is often performed with arterial samples. Limited studies have been published on the effects of sampling site on coagulation testing.

Aims: To compare coagulation parameters determined by the Quantra System (HemoSonics LLC) for venous and arterial blood samples.

Methods: The Quantra is a novel cartridge-based viscoelastic analyzer that measures changes in clot stiffness during coagulation using ultrasound detection of resonance. A cohort of 25 patients undergoing cardiopulmonary bypass surgery were enrolled in this study. For each patient, arterial and venous samples were obtained in citrated tubes in close temporal sequence. Sampling was randomized at two of the following three time points: baseline, during bypass, or after protamine administration. Measurements were performed on a research use only (RUO) version of the Quantra and included Clot Time (CT), Heparinase Clot Time (CTH), Clot Stiffness (CS), Fibrinogen (FCS) and Platelet (PCS) Contributions to clot stiffness.

Results: Paired t-tests were performed on matched arterial vs venous samples. Data is summarized in Table I. CS, FCS and PCS were similar between arterial and venous samples. CT and CTH demonstrated a statistically significant difference. Venous clot times were prolonged relative to the arterial ones with an average bias of 0.24 min and 0.21 min, respectively.

Conclusions: This pilot study demonstrates that Quantra clot stiffness based parameters (CS, FCS, PCS) are unaffected by sampling site, whereas the clot time based parameters (CT and CTH) show good correlation in the presence of a small bias.

TABLE I

Output Parameter	Arterial (mean +/-1 SD)	Venous (mean +/-1 SD)	P-value
Clot Stiffness (CS)	19.9±9.2 hPa	20.2±9.7 hPa	P = 0.32
Fibrinogen Contribution (FCS)	2.94±2.4 hPa	2.87±2.14 hPa	P = 0.29
Platelet Contribution (PCS)	17.48±7.25 hPa	17.71±7.97 hPa	P = 0.43
Clot Time (CT)	2.11±0.64 min	2.35±0.49 min	P < 0.001*
Heparinase Clot Time (CTH)	2.15±0.41 min	2.36±0.31 min	P < 0.001*

PB 1043 | The Quantra Surgical Cartridge: A Novel Assay for the Rapid Assessment of Coagulation Function from Whole Blood

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Background: Previous studies have shown the benefit of rapid information to guide treatment of excessive bleeding, including targeted transfusion and better outcomes. The Quantra™ Hemostatic Analyzer (HemoSonics LLC) is a novel cartridge-based viscoelastic analyzer designed for rapid and comprehensive assessment of coagulation at the point-of-care (POC).

Aims: To introduce a novel and patented cartridge design for fully automated, rapid, and comprehensive measurements of the coagulation system to aid in the management of patient bleeding at the POC.

Methods: The Surgical Cartridge, shown in Figure 1 along with its output parameters, is a multi-channel consumable containing lyophilized reagents. The cartridge includes an interface for evacuated tubes so that no sample handling procedures are required. Cartridge performance was assessed using contrived samples that were spiked with heparin or with varying concentration of fibrinogen or platelets. Sample analysis was performed in a Research Use Only version of the Quantra by measuring changes in clot stiffness during coagulation using ultrasound detection of resonance.

Results: Clot Time (CT) demonstrated a linear response to heparin in the range 0-1U/ml, whereas Heparinase Clot Time (CTH) neutralized heparin up to 6U/ml. Fibrinogen Contribution (FCS) increased 23X over the range 90-1100mg/dl of fibrinogen, showing strong correlation with Clauss fibrinogen (r^2=0.94) and ROTEM FIBTEM (r^2=0.97). Clot Stiffness (CS) varied linearly (3X increase) over platelet count from 130-450K/ul. We also demonstrate that the reagents are stable at room temperature for over 6 months and that complete results are reported within 15 minutes of test initiation.

Conclusions: The Quantra Surgical Cartridge provides quantitative measures of coagulation function within 15 minutes. The cartridge was designed for automation, rapid results, and stability at room temperature to satisfy critical care requirements.

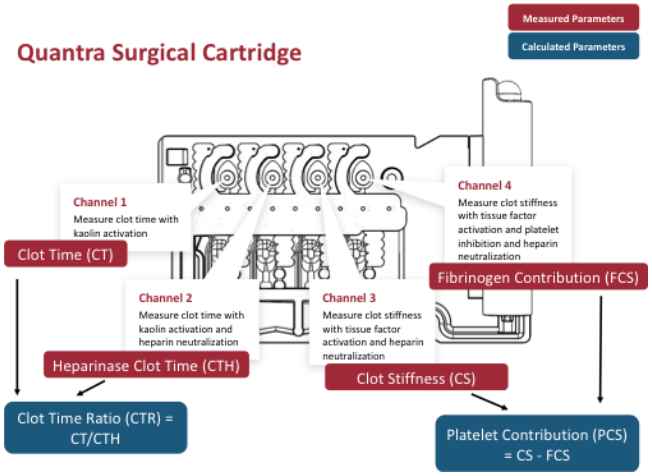

FIGURE 1 Schematic representation of Quantra Surgical Cartridge

PB 1044 | Five Years of Hemovigilance Reports of Complications of the Blood Donation Reported at a Tertiary Care Centre in Karachi

B. Nepal

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Background: There is a minor chance of risk among blood donors. Even though blood donors are usually screened for the presence of risk factors, sometimes blood donations can put a person at panic.

Aims: The safety of the blood supply depends on the actions to protect both; blood transfusion recipient and the blood donor. Hemovigilance practice of learning of complications of blood donation and protecting them from such complications is the best way to minimize the risk to blood donor.

Methods: Comprehensive blood donor hemovigilance program was studied at Dr. Ishratul Ebad Khan Institute of blood diseases, Karachi from 2010 to 2015. Outlines of reported and communicated complications were collected after whole blood donation. Analysis was done by general logistic regression.

Results: Complications after 30,000 Whole blood donation procedures calculated1620total .(54 per 1,000 donations). The majority of the complications were faint and pre-faint reaction with light headedness (58.6 %), Sore arm (24 %), Bruises and hematoma (14.4 %). Minor complications were Agitation/sweating (2 %) and arterial puncture(1 %). Markers of the complications were age, sex, race, weight, blood pressure and donation status. All associated independently after whole blood donation. Age and first-time status were associated with a significantly higher risk of complications with 18-22 years old at higher risk compared to 23 to 50 years old. First-time donor were at higher risk compared to repeat donor.

Conclusions: The results of this study are helpful in identifying and understanding the promoter to complication of blood donation. Donor age and status were strong predictors of complications. The remedies and specific areas of care should be provided.

PB 1046 | Performance Evaluation of the Quantra Surgical Cartridge for Rapid Point-of-Care (POC) Assessment of Coagulation Function

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Hemosonics LLC, Durham, United States

Background: The Quantra™ Hemostasis Analyzer and Quantra Surgical Cartridge (HemoSonics LLC) are components of a compact POC system for rapid measurement of key coagulation parameters. The system uses ultrasound to detect changes in clot stiffness for four separate assays tested in a single disposable cartridge using citrated whole blood. The four assays measure

1) kaolin clot time;

2) kaolin clot with added heparinase (to detect heparin);

3) overall clot stiffness from platelets and fibrinogen combined; and

4) clot stiffness from fibrinogen only.

Aims: Evaluate analytical performance of the Quantra Surgical Cartridge.

Methods: The Quantra Surgical Cartridge (Research Use Only version) was evaluated for precision, sample stability and sensitivity to therapeutic agents.

Results: ≤ Precision for normal whole blood samples, including variation due to instrument and cartridge lots, showed coefficients of variation (CV) of 3-6%. Precision for samples treated with 0.6 U/mL added heparin, samples containing 100-700 mg/dL fibrinogen and other abnormal samples gave CV=3-11%, except for samples with very low fibrinogen (≤ 100 mg/dL) where estimated precision was based on standard deviation. Within-device precision for quality control results was 4-11% CV. Between-lot and between-instrument variability based on QC controls were estimated as 1-4% CV. Samples were stable for ±4 hours at ambient temperature. Anticoagulants such as dabigatran and rivaroxaban prolonged kaolin clot time in presence and absence of heparinase. Heparin prolonged clot time, but prolongation was reversed by heparinase. Aspirin and tranexamic acid had little or no effect on results.

Conclusions: Results demonstrate the Quantra Surgical Cartridge provides precise assessment of four key coagulation functions with processing time of 15 min, requiring minimal operator intervention. The Quantra design is targeted to surgical settings, critical care, or other applications requiring compact size, speed and simple operation.

PB 1047 | ABO Discrepancies and Errors in a Routine Blood Bank

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Background: ABO discrepancy exist when red cell antigen grouping result do not correlate with serum grouping result.

Aims: To identify the incidence and type of ABO discrepancies and errors leading to wrong results.

Methods: All the data of ABO grouping from June 2012 till June 2013 was collected at Ishrat ul Ebad Khan institute of blood disease, Dow university of health sciences,Karachi,Pakistan.

ABO discrepancies, pre-analytical, analytical & post-analytical errors in routine blood grouping were noted and recorded on a Performa designed for the study and results were tabulated.

Results: 30 discrepancies (0.3 %), 18 (0.18 %) pre & post analytical errors were identified from a total of 10,000 blood group tests performed during the study period. The commonest of all was ABO discrepancy due to sub groups n 26 (86.6 %) followed by auto-antibodies n 4 (13.3 %).

Conclusions: ABO discrepancy is not an infrequent finding in routine blood banking while pre-analytical, analytical & post-analytical errors are also seen in routine practice and are small but very important source of mistakes which can lead to serious hazards of transfusion. Measures should be taken to identify and resolve these discrepancies while ensuring proper collection, labeling, analysis and reporting of results for safe blood transfusion.

PB 1048 | Is the Thrombin Generation Test, a Useful Research Tool, Suitable to Characterize Therapeutic Plasmas?

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Background: The regulatory hemostatic assays required to validate therapeutic plasmas probe their FVIII activity and fibrinogen concentration but not their overall hemostatic capacity. Current coagulation assays measure only the time necessary to generate the first traces of thrombin, whereas the majority of thrombin is produced in the heart of the clot. In contrast, the thrombin generation test (TGT), which investigates the total thrombin concentration using a fluorescent substrate, is a global test of plasma coagulability integrating the entire sequence of the hemostatic process. The TGT is rarely employed in routine practice, although it is useful to detect hemophilia (deficiency in FVIII or FIX) and monitor its treatment.

Aims: The aim of this study was to determine the suitability of the TGT to assess the global hemostatic quality of therapeutic plasma derived from whole blood (WB) after pathogen inactivation and freezing.

Methods: The TGT was performed on 40 plasma units inactivated with Intercept (Cerus) and frozen within 19 h of collection. The tests were conducted 2 weeks and 6 and 12 months after Intercept treatment. Coagulation was triggered with $CaCl_2$, phospholipids and different concentrations of tissue factor (TF).

Results: The thrombin generation capacity of WB derived plasma was not markedly impaired following photochemical treatment, even in the presence of suboptimal concentrations of TF, despite a moderate decrease (30%) in FVIII. Moreover, the hemostatic properties of plasma were not degraded by storage at -25°C.

Conclusions: The TGT could represent a surrogate or additional assay to investigate the hemostatic quality of therapeutic plasma preparations. Its results enable evaluation of the impact of the different steps of plasma preparation on the hemostatic efficiency. Nevertheless, standardized inter-laboratory protocols, with normalization of the results and well defined reference values, remain to be established to ensure widespread application of the TGT in the field of therapeutic plasma.

PB 1049 | Viral Inactivation and Enrichment of Factor VIII, Factor XIII, Fibrinogen and von Willebrand Factor (VWF) Multimers from Fresh Frozen Plasma (FFP)using, "VIPS Plasma, Virus Inactivation Treatment System"

B. Nepal

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Background: Human blood is the source of a wide range of medicinal products used for the prevention and treatment of a variety

TABLE 1 Effect of Intercept treatment and storage on thrombin generation using tissue factor at 20 pM or 1 pM (N=40)

		Average ± Standard Deviation (N=40)				Normal range (N=30)
	TF(pM)	T1	T2	T3	T4	
Lag time (min)	20	1.43 ± 0.27	1.39 ± 0.21[a]	1.43 ± 0.23	1.46 ± 0.26	1.32 - 2.33
Lag time (min)	1	4.82 ± 0.56	4.69 ± 0.60[a]	4.79 ± 0.63	4.79 ± 0.59	3.77 - 6.78
Peak (nM)	20	356.0 ± 27.9	356.3 ± 28.5	351.5 ± 25.7	346.5 ± 29.0[c]	331.0 - 545.6
Peak (nM)	1	310.6 ± 40.2	237.0 ± 42.2[a]	234.1 ± 36.8[b]	236.5 ± 39.3[c]	229.8 - 423.9
ETP - thrombin generated (nM x min)	20	1,581.1 ± 153.7	1,662.6 ± 127.3[a]	1,636.0 ± 143.7[b]	1,608.6 ± 141.4	1477 - 2618
ETP - thrombin generated (nM x min)	1	1,543.6 ± 117.1	1,527.8 ± 122.8	1,557.0 ± 182.7	1,509.5 ± 118.6[c]	1170 - 2182

T1: reference value in pooled plasma before treatment - T2: after treatment and 2 weeks of frozen storage - T3: after 6 months - T4: after 12 months - Two-sided paired t-Test (alpha 0.05) a, b or c indicate a significant difference ($p<0.05$) when comparing respectively T2-T1, T3-T1 or T4-T1

of life-threatening injuries and diseases. Despite measures such as donor selection, testing of donations and of plasma pools, the transmission of blood-borne viruses by plasma and purified plasma products is at risk

Aims: To assess viral inactivation and, Factor VIII, Factor XIII, Fibrinogen and von Willebrand factor (VWF) multimers enrichment capacity of, "VIPS Plasma, Virus Inactivation Treatment System".

Methods: VIPS Plasma, Virus Inactivation Treatment System" comprise of interconnected bag system where the S/D reagents are removed by filtration and the final products subjected to bacterial (0·2 µm) filtration. Cryoprecipitate mini-pools (400 ± 20 mL) were subjected to double-stage S/D viral inactivation, followed by one oil extraction and a filtration on a S/D and phthalate [di(2-ethylhexyl) phthalate (DEHP)] adsorption device and a 0·2 µm filter. The initial and the final products were compared for visual appearance, blood cell count, factor VIII, Factor XIII, Fibrinogen and Von Willebrand factor (VWF) multimers. Initial and final products were also checked for HIV, HBV, HCV, dengue, malaria and bacterial contaminations.

Results: Our analysis showed that the treated cryoprecipitate were very clear, with negative blood count and the protein content of factor VIII, Factor XIII, Fibrinogen and von Willebrand factor (VWF) multimers were well conserved (Table 1)

TABLE 1 (Test performed at Aga Khan Labs)

Sr.No	Factor	Start%	Final%
1.	Von Willebrand factor	418%	435%
2.	Factor VIII	183%	251%
3.	Fibrinogen Level	754.1mg/dl	672 mg/dl
4.	Factor XIII (quantative)	93.9%	----

Kit ensured bacterial sterility (Table 3)

TABLE 2 (Molecular Biology: Performed at Molecular lab,DDRRL)

Sr. No	Test(By PCR)	Pre-analyasis	Post-analysia
1.	Hepatitis B Virus	Negative	Negative
2.	Hepatitis C Virus	Negative	Negative
3.	HIV Virus	Negative	Negative

TABLE 3 (Microbiology: Performed at Microbiology Lab, DDRRL)

Sr. No	Test	Pre-analysis Results	Post-analysis Results
1.	Malarial Parasite(Slide)	Negative	Negative
2.	VDRL (ICT)	Negative	Negative
3.	Dengue IgG (ICT)	Positive	Positive
4.	Bacterial Culture	Staph. Species Coagulase negative	No Growth

and most importantly, final product was free of HBV, HCV and HIV (Table 2).

Conclusions: further investigation is needed to characterize functional activity of the enrich component. Irrespective of that the process may offer one additional option to blood establishments for the production of virally inactivated plasma components especially in low income countries.

PB 1050 | Prolonged Platelet Storage Associated with Increased Incidence of Platelet Transfusion Adverse Events

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Background: Platelet (PLT) transfusion is associated with a higher incidence of adverse evens (AEs) than any other blood product. Most of PLT associated AEs are inflammatory responses. Multiple factors have been investigated for their roles in inducing PLT associated AEs. However, clinical data supporting PLT storage lesion has been largely lacking especially in the era of universal prestorage leukoreduction. Actually, recent studies failed to show the association between PLT age and PLT associated AEs.

Aims: To better understand whether prolonged storage causes PLT related AEs.

Methods: In this retrospective study, 48,212 PLT products including about 80% apheresis PLT units and 20% pooled whole blood derived (WBD) PLTs were transfused to 8726 patients with a median age of 58 years old and 1.4 to 1 male to female ratio from July 2011 to September 2016. All the PLTs were leukoreduced prior to storage. A total of 241 AEs were reported in 229 patients, 96% of whom had advanced cancer, sepsis or cirrhosis. This study was approved by the institutional review board at The Ohio State University.

Results: There was a significant increase in the incidence of transfusion related AEs with the prolongation of PLT storage ($p < 0.001$). (Fig 1) The oldest PLTs (day 5) associated with a higher incidence in most of the symptoms/signs, except for itching, hives, and rash. (Fig 2)

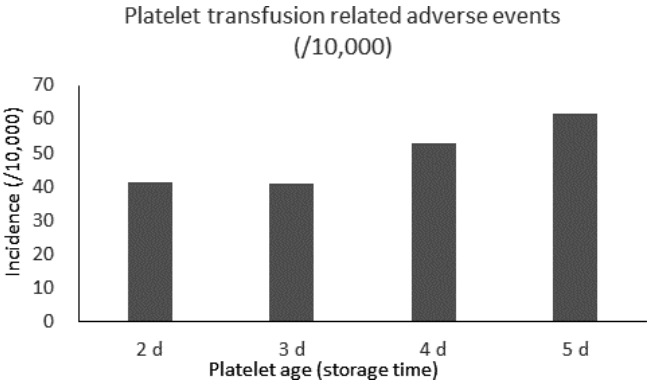

FIGURE 1 Prolonged platelet storage time associated with more transfusion related adverse events

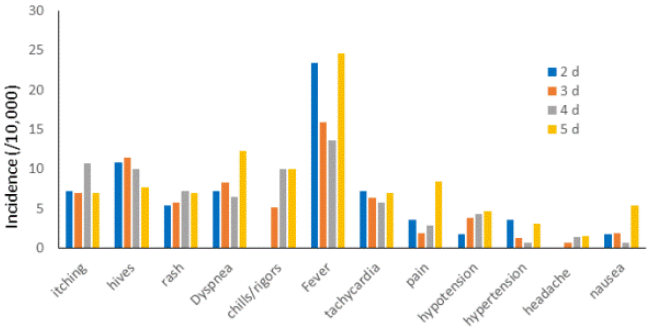

FIGURE 2 Symptoms and signs in platelet transfusion associated adverse events]

The strongest association with PLT age was seen in rigors/chills and dyspnea. Moreover, the incidences of apheresis PLT associated AEs (64.5/10,000) were significantly higher than that of pooled WBD PLTs (23.1/10,000) ($p < 0.001$).

Conclusions: Prolonged PLT storage associated with an increased incidence of transfusion AEs. Symptoms with a clear inflammation etiology had the strongest association with PLT age. Symptoms that did not appear to be associated with PLT age might be related to recipient's unstable vital signs or allergic reactions. Apheresis PLT caused more AEs than WBD platelets.

VASCULAR BIOLOGY & ANGIOGENESIS

PB 1605 | Role of the Extracellular Matrix Protein Biglycan for Platelet Adhesion and Thrombus Formation

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Background: Biglycan (BGN) is part of the small leucine-rich proteoglycans and modulates collagen fibrils to increase the stability of the collagen network. After myocardial infarction BGN is needed for tissue remodeling and deficiency of BGN leads to an increased amount of aortic aneurysms.

Aims: The aim of this project is to determine the role of BGN as an ECM protein of the vessel wall in platelet adhesion and thrombus formation.

Methods: *In vitro* and *in vivo* analysis of BGN deficient platelets and mice.

Results: ELISA and qRT-PCR data provided strong evidence that platelets are not a source of BGN. Moreover, the addition of soluble recombinant biglycan was not sufficient to activate platelets. However, we observed increased platelet adhesion under static conditions as well as increased thrombus formation on a collagen-biglycan matrix compared to collagen alone under flow *ex vivo*. In line with these results we found reduced platelet adhesion at the ligated carotid artery in *bgn*$^{-/0}$ mice *in vivo*. Furthermore *bgn*$^{-/0}$ mice displayed significantly prolonged tail bleeding times and significantly prolonged occlusion times of the carotid artery after $FeCl_3$-induced vessel injury. Accordingly, the analysis of bone marrow chimeric mice showed that loss of biglycan in the vessel wall is responsible for prolonged bleeding times, reduced platelet adhesion and prolonged occlusion times after injury of the carotid artery. Mechanistically, reduced platelet adhesion to immobilized recombinant biglycan after inhibition of the collagen receptor glycoprotein (GP)VI suggests that GPVI might serve as a receptor for biglycan.

Conclusions: This study reveals an important role of the extracellular matrix protein biglycan for platelet adhesion and thrombus formation in hemostasis and thrombosis.

PB 1606 | Platelet Inhibition Augments Rupture-induced Death in a Mouse Model of Abdominal Aortic Aneurysm

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Background: Platelets are critical in both maintaining hemostasis and propagating thrombosis. While platelets and platelet-specific cytokines are elevated in patients with abdominal aortic aneurysm (AAA), their role in the pathogenesis of aneurysm disease remains unclear.

Aims: The purpose of this study was to determine the effects of platelet inhibition or depletion in a mouse model of angiotensin II (AngII)-induced AAA.

Methods: Accumulation of platelets and platelet-derived cytokines were quantified in low-density lipoprotein deficient (*Ldlr*$^{-/-}$) mice fed a high fat and cholesterol diet (HFD) and infused with AngII (1,000 ng/kg/min) through 28 days. The effect of a genetic deficiency of platelet receptors (protease-activated receptor 4 and $P2Y_{12}$), anti-platelet therapies (clopidogrel or aspirin), thrombin inhibition (dabigatran) and platelet depletion (anti-CD42b antibody, compared to an irrelevant IgG control) were examined in *Ldlr*$^{-/-}$ mice fed a HFD and infused with AngII for 28 days.

Results: Platelets accumulated in the abdominal aorta prior to macrophages with the platelet-derived cytokine platelet factor 4 (PF4) highly correlated to increasing abdominal diameter ($R^2 = 0.914$, $P < 0.001$). PF4 was increased in human AAA patients (n = 169) versus age and gender matched healthy controls (n = 115; $P < 0.0001$) and highly correlated with both abdominal diameter and AAA growth rate. Infusion of AngII into mice with genetic deficiency of platelet receptors or mice given anti-platelet therapies resulted in no difference in abdominal aortic diameter but augmented rupture-induced death versus littermate and placebo controls, respectively ($P < 0.01$). Platelet depleted mice died of rupture-induced death by day 12 of infusion ($P < 0.001$) in *Ldlr*$^{-/-}$, apolipoprotein E deficient, and *C57BL/6J* mouse strains infused with AngII.

Conclusions: Platelets are the first cell to accumulate in the abdominal aorta during AAA formation. Inhibition of platelet function may be detrimental in the early stages of an expanding aortic lumen.

PB 1607 | Protein Disulfide Isomerase Is Regulated by S-nitrosylation: Implications for Vascular Quiescence and Thrombus Formation

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Background: Protein disulfide isomerase (PDI) serves an essential role in thrombus formation and PDI inhibition is being evaluated clinically as a strategy for anticoagulation management. Yet little is known about endogenous regulation of PDI in the vasculature.
Aims: To assess the effect of S-nitrosylation on the role of PDI and other thiol isomerases in the vasculature.
Methods: Platelets and endothelial cells were assessed for endogenous PDI S-nitrosylation. The effect of S-nitrosylated PDI on platelet aggregation, granule release and thrombus formation was determined.
Results: Thiols within the catalytic CGHC motif of PDI perform disulfide shuffling that is essential for its role in thrombosis. These same thiols bind nitric oxide (NO), forming S-nitrosylated PDI. We have evaluated the effect of NO on PDI function in endothelial cells and platelets. Incubation of endothelium with NO donors resulted in S-nitrosylated PDI and inhibition of its function. Elevation of endogenous NO levels by induction of endothelial NO synthase also resulted in increased S-nitrosylated PDI and decreased thiol isomerase activity at the endothelial cell surface. Inhibition of PDI by NO blocked LPS-induced thrombin generation on endothelium, which we show to be PDI-dependent. Platelet PDI is also S-nitrosylated and S-nitrosylation inhibits the reductase activity of PDI on platelets. Furthermore, PDI mediates the transfer of NO into platelets. S-nitrosylated PDI inhibited both platelet aggregation and granule release. Inhibition of PDI or exposure to NO also inhibited thrombin generation on platelets. Using a model of laser-induced vascular injury, infusion of S-nitrosylated PDI into mice blocks both platelet accumulation and fibrin formation at sites of vascular injury.
Conclusions: These studies identify NO as a critical regulator of vascular PDI, demonstrating that NO converts prothrombotic PDI into antithrombotic S-nitrosylated PDI. Thus, regulation of PDI functions as a new mechanism by which NO maintains vascular quiescence.

PB 1608 | Ultra-sensitive Molecular Magnetic Resonance Imaging of Blood Cells - Endothelium Interactions *In vivo*

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Background: Endothelial activation is a key pathophysiological mechanism in a number of diseases, acting either as a cause or a consequence of organ injury. After activation, endothelial expression of adhesion molecule leads to leucocytes adhesion and subsequent diapedesis. In vivo imaging of these interactions may have significant diagnostic applications.
Aims: To develop a molecular imaging tool to detect endothelial activation in vivo, in a noninvasive manner.
Methods: Magnetic resonance imaging (MRI) enhanced by new formulations of microparticles of iron oxide (MPIO) targeted to activated endothelial cells either in the brain, kidney, heart or intestines in mice.
Results: Thanks to the use of micron-sized superparamagnetic particles targeted to activated endothelial cells and the optimizations performed, we were able to develop a new generation of contrast agent with very high sensitivity. Using MPIOs targeted to VCAM-1, P-Selectin or MAdCAM-1, we were able to detect endothelial activation at its early and late stages in clinically relevant contexts in mice. For instance, we detected cerebral transient ischemic attack using P-selectin targeted MPIOs in a noninvasive manner, a notoriously difficult diagnosis using currently available methods. Moreover, we detected intestinal inflammation in models of colitis and intestinal ischemia-reperfusion injury using MAdCAM-1 targeted MPIOs. If successfully translated to clinical use, this method could be used instead of colonoscopy for the diagnosis of these intestinal disorders.
Conclusions: This new platform of contrast agent for magnetic resonance imaging allows ultra-sensitive detection of the molecular effectors of blood cells - endothelium activation, which could have numerous applications for medical diagnostic.

PB 1609 | Contribution of the P2X1 Receptor Expressed by Lung Macrophages to Experimental TRALI

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Background: Transfusion Related Acute Lung Injury (TRALI) is a non-cardiogenic pulmonary edema that may occur within 6 h after blood transfusion. Most often, this syndrome results from the presence of anti-HLA or anti-neutrophil allogeneic antibodies (Ab) in donor's

blood. We have shown previously that NF449, an antagonist of the ATP-gated $P2X_1$ receptor, markedly reduced mortality at 2 h in experimental immunological TRALI in mice.

Aims: Our objective is to investigate which cell type expressing $P2X_1$, i.e. platelets, neutrophils and monocytes/macrophages, is responsible for TRALI.

Methods: To induce TRALI, Balb/c mice were primed with lipopolysaccharides (0.1 mg/kg) 24 h before the injection of a cognate anti-MHC-I Ab (0.5 mg/kg). TRALI was assessed by the i) protein content in bronchoalveolar liquid, ii) partial pressure of oxygen in arterial blood, iii) histology. The role of the $P2X_1$ receptor was evaluated using its antagonist NF449 (10 mg/kg) administered intravenously before LPS and before anti-MHC-I Ab injection.

Results: Within the first 10 min following the injection of anti-MHC-I Ab, the severity of lung edema and the drop in aortic blood oxygenation were profoundly inhibited in mice treated with NF449. Since platelets are dispensable for TRALI, the role of neutrophils or inflammatory monocytes was evaluated by specific depletion with an anti-Ly6G mAb or an anti-CCR2 mAb, respectively. Both depletions did not inhibit TRALI, indicating that neither $P2X_1$ expressed by neutrophils nor by these monocytes is crucial for TRALI. In contrast, depletion of monocytes/macrophages by injection of clodronate liposomes, 6 h before anti-MHC-I Ab, abolished TRALI. This treatment led to the elimination of 50% of interstitial macrophages and dendritic cells in the lungs and most of Kupffer cells as shown by flow cytometry analysis or histology.

Conclusions: Macrophages play an important role in immunological TRALI which could be driven by $P2X_1$ receptor activation.

PB 1610 | The Role of von Willebrand Factor in Experimental Malaria-associated Acute Respiratory Distress Syndrome

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Background: Malaria is a global health burden resulting in 429,000 deaths in 2015. Recent clinical studies have demonstrated that severe malaria is associated with acute endothelial cell activation, accumulation of highly active von Willebrand factor (VWF) multimers, and a significant reduction in ADAMTS13 activity.

Aims: To investigate the role of VWF in malaria using a murine model of malaria-associated acute respiratory distress syndrome.

Methods: Wild-type (WT) and VWF knock out ($Vwf^{-/-}$) mice on a C57BL/6J background were inoculated with 10^4*Plasmodiumberghei* (*Pb*) NK65-infected erythrocytes. Blood samples were taken to assess platelet count as well as levels and activities of VWF and ADAMTS13. Giemsa-stained blood smears were made to determine parasitemia. Pulmonary edema was assessed by measuring protein levels in bronchoalveolar lavage fluid.

Results: Plasma VWF levels in infected WT mice significantly increased 3 days after infection (2-fold increase; p< 0.0001), but normalized afterwards. No change in VWF multimer patterns was observed until the end stage (day 8/9) at which high molecular weight VWF multimers were markedly decreased (p< 0.0001). This was accompanied by a reduction of the ADAMTS13 activity/antigen ratio (p< 0.0001). Interestingly, severe thrombocytopenia was observed in both WT and $Vwf^{-/-}$ mice, indicating a VWF-independent mechanism. $Vwf^{-/-}$ mice died more rapidly with higher parasitemia compared to WT (p=0.003). Alveolar leakage in lungs was significantly lower in $Vwf^{-/-}$ mice ($Vwf^{-/-}$: 1.8±0.4, WT: 3.6±0.5 mg/mL; p=0.02).

Conclusions: Our data demonstrate that *Pb*NK65-mediated murine malaria infection is associated with early elevated levels of plasma VWF, which is indicative for endothelial cell activation and in accordance with human malaria. Our findings also show that VWF does not contribute to malaria-associated thrombocytopenia. Furthermore, VWF might influence the development of parasitemia and lung pathology, potentially by interfering with the sequestration of infected erythrocytes.

PB 1611 | Blood Platelets Bind Aβ Peptides via Integrin αIIbβ3 and Induce Amyloid-β Aggregation in Cerebral Vessels

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Background: Cerebral amyloid angiopathy (CAA) is a critical factor in the pathogenesis of Alzheimer`s disease (AD). It is characterized by the deposition of the Aβ peptide, mainly Aβ40, in the walls of cerebral vessels, which induces the degeneration of vessels wall components, reduces cerebral blood flow and aggravates cognitive decline. Moreover, Aβ was shown to activate platelets and to enhance platelet aggregation. Activated platelets contribute to more than 90% circulating Aβ. Recently, we have shown that platelets modulate soluble Aβ peptides into fibrillar Aβ structures in platelet cell culture.

Aims: Investigation of the molecular mechanisms mediated by platelets in the development of CAA and its implication for the progression of AD.

Methods: Analysis of amyloid and platelet interaction.

Results: The binding of Aβ with its RHDS sequence (N-Terminal region) to platelet integrin αIIbβ3 induces outside-in signaling and the secretion of clusterin from platelets. Chaperon protein clusterin promotes the modulation of soluble Aβ40 peptides into fibrillar Aβ aggregates. We found that not only the N-terminal region but also the C-terminal region of Aβ is important for the aggregation of Aβ peptides and the induction of signaling pathways in platelets. Moreover, the antiplatelet therapy with Clopidogrel reduces vascular Aβ plaques in cerebral vessels of APP23

transgenic mice, which develop CAA. The effects of long-term platelet inhibition for the reduction of parenchymal plaques are currently on going.
Conclusions: The interaction of platelets with Aβ peptides directly contribute to Aβ plaque formation. Thus, AD patients may benefit from anti-platelet therapy.

PB 1612 | Platelet Extracellular Vesicles Induce a Proinflammatory Smooth Muscle Cell Phenotype

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Background: Extracellular vesicles are gaining increasing attention as mediators of cell communication during health and disease. Platelets are a major source of extracellular vesicles, releasing them upon activation or during aging. Platelet extracellular vesicles (platelet EV) have been shown to exert modulatory effects on immune as well as vascular cells.
Aims: We hypothesize that platelet EV modulate the function of vascular smooth muscle cells (SMC).
Methods: In this study, platelet EV were isolated from aging platelet concentrates by differential centrifugation, quantified and characterized by flow cytometry, nanoparticle tracking analysis (NTA) and cryo-transmission electron microscopy (cryo TEM). Platelet EV were incubated with SMC for up to 96 hours in order to assess binding, proliferation, migration and pro-inflammatory phenotype of the cells.
Results: Under resting conditions, platelet EV firmly bound to SMC through the platelet integrin $\alpha_{IIb}\beta_3$, while binding also occurred upon cytokine stimulation in a CX3CL1-CX3CR1-dependent manner. In chemotaxis experiments, platelet EV increased SMC migration to a similar extent as did platelet derived growth factor (PDGF) or platelet factor 4 (CXCL4). Additionally, platelet EV induced SMC proliferation, which relied on CD40- and P-selectin-interactions. Adhesion of monocytic cells to platelet EV-treated SMC under flow conditions was significantly increased in comparison to adhesion to untreated SMC. Again, this adhesion was dependent on integrin $\alpha_{IIb}\beta_3$ and P-selectin, and to a lesser extent on CD40 and CX3CR1. Treatment of SMC with platelet EV induced the production of the cytokine interleukin 6 (IL-6). Finally, platelet EV induced a synthetic SMC morphology and decreased the expression of the contractile protein calponin.
Conclusions: These findings indicate that platelet EV exert a strong immunomodulatory activity on SMC. In particular, platelet EV induce a switch towards a pro-inflammatory phenotype, stimulating vascular remodeling.

PB 1613 | Conditional Knockout of Heme Oxygenase-1 in Lysozyme M Positive Cells Attenuates Angiotensin II Induced Vascular Inflammation

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Background: Heme oxygenase-1 (HO-1) protects vascular function in hypertension, presumably by anti-inflammatory effect on monocytes.
Aims: The purpose of this study was to explore the role of HO-1 specifically in monocytes in the development of angiotensin II (AngII) induced hypertension.
Methods: Lysozyme M ($LysM^+$) specific HO-1 knockout mice ($LysM^{Cre/wt}Hmox\text{-}1^{flox/flox}$ referred below as MHOKO) and controls ($LysM^{Cre/wt}$) were infused with AngII (1 mg× kg^{-1}× d^{-1} for 7 days using osmotic minipump) or sham-treated. Blood pressure was assessed with tail cuff plethysmography. Vascular inflammation and reactivity were explored with oxidative fluorescent microtopography and isometric tension studies. Leukocytes activation and infiltration were assessed with flow cytometry of the aortic tissue and intravital videomicroscopy Imaging.
Results: AngII infusion induced increased systolic blood pressure in both strain and aortic reactive oxygen species formation was reduced in AngII-infused MHOKO mice. AngII-infused MHOKO presented reduced vascular reactivity impairment with smooth muscle cells-dependent relaxation. Flow cytometry revealed, that vascular accumulation of $Ly6G^+$ neutrophils and both $Lys6G^-/Ly6C^{hi}$ and $Ly6G^-/Ly6C^{lo}$ monocytes was attenuated in AngII-infused MHOKO mice compared to controls. This was paralleled by reduced aortic mRNA expression of C-C chemokine receptor type 2, reduced ROS formation in circulating blood as well as attenuated leukocyte rolling in carotids of AngII-infused MHOKO mice.
Conclusions: In 1-week AngII-infusion model, conditional deletion of HO-1 in $LysM^+$ cells unexpectedly reduced vascular recruitment and protected from ROS formation in blood and infiltration of inflammatory cells, but not from arterial hypertension. Further investigations are needed to test the impact of myelomonocyte-specific deficiency of HO-1 on the phenotype and transcriptome of monocytes since very recent studies suggest a specific role of HO-1 in the maturation and trafficking of these cells.

PB 1614 | Defective Fibrin Accumulation and Thrombus Stability in Endothelial Cell-specific BAMBI Knockout-mice

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Background: BAMBI is a TGFβ superfamily transmembrane protein that is highly expressed in platelets and endothelial cells. *Bambi*-deficient mice exhibit decreased thrombus stability. Chimeric mice revealed that endothelial, rather than platelet BAMBI confers this phenotype.

Aims: We aimed to delineate the mechanisms by which BAMBI exerts its function on the endothelium and how it influences thrombus stability.

Methods: *Bambi*-deficient mice were subjected to the laser-induced thrombosis model. Platelets, neutrophils and fibrin(ogen) accumulation were assessed. Mouse lung endothelial cells (MLEC) were isolated from $Bambi^{+/+}$ and $Bambi^{-/-}$ mice. Expression of adhesion molecules and thrombomodulin was assessed by flow cytometry.

Results: No difference in the levels of plasma prostacyclin or nitric oxide could be observed between $Bambi^{-/-}$ and $Bambi^{+/+}$ littermates (WT). Incorporation of neutrophils in thrombi after laser injury of the endothelium was similar in $Bambi^{+/+}$ and $Bambi^{-/-}$ mice. Consistent with this, no difference in expression levels of PECAM-1, ICAM-1 or ICAM-2 was detected between $Bambi^{-/-}$ and WT MLEC. The increased number of emboli during thrombus formation present in $Bambi^{-/-}$ mice was accompanied with a defect in fibrin accumulation. Injection of hirudin prior to thrombus formation in WT mice recapitulated the $Bambi^{-/-}$ thrombus instability phenotype while it had no effect in $Bambi^{-/-}$ mice. Finally, EC *Bambi*-deficient mice ($Bambi^{EC-/-}$) exhibited normal haemostasis but defective thrombus stability with decreased fibrinogen accumulation.

Conclusions: $Bambi^{EC-/-}$ similar to $Bambi^{-/-}$ mice display increased thrombus instability accompanied by a markedly diminished ability to accumulate fibrin during thrombus formation, confirming the important role of endothelial BAMBI. Preliminary data suggest $Bambi^{-/-}$ MLEC display increased levels of thrombomodulin compared to WT MLEC. We are currently investigating how altered anticoagulant pathways may cause thrombus instability in *Bambi* deficient mice.

PB 1615 | FXI Inhibition Protects from Vascular Inflammation and Endothelial Dysfunction in Arterial Hypertension

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Background: Interactions of platelets, leukocytes and the vessel wall play pivotal roles in activating coagulation and precipitating thrombosis, but how they promote vascular inflammation in arterial hypertension is unclear.

Aims: The aim of this work was to explore the roles of FXI on inflammatory driven vascular dysfunction in experimental arterial hypertension and in patients with uncontrolled hypertension.

Methods: C57BL/6 mice infused with angiotensin II (AngII) (1 $mg \times kg^{-1} \times d^{-1}$ for 7 d) and 5/6 nephrectomized (Nx) rats were used. FXI synthesis was inhibited by a FXI antisense oligonucleotide (FXI ASO). Blood pressure, endothelial function, leukocyte adhesion, vascular inflammation and thrombin generation (TG) in platelet rich plasma were measured.

Results: In mice, AngII-induced vascular dysfunction involved the activation of FXI but not of FXII. Blockade of TF or thrombin or depletion of platelets during AngII administration attenuated both endothelial dysfunction and vascular inflammation.

Inhibition of FXI synthesis was sufficient to prevent AngII-induced thrombin formation on platelets, leukocyte adhesion to the endothelium, vascular inflammation, endothelial dysfunction as well as arterial hypertension. AngII-infused mice lacking the extracellular binding domains of GP1bα were protected from AngII-induced vascular injury, identifying platelet GPIbα as a relevant platelet receptor for AngII-induced endothelial dysfunction and vascular inflammation.

FXI ASO treatment also reduced the elevated blood pressure and attenuated vascular and kidney dysfunction in Nx rats with established arterial hypertension. Further, platelet-localized TG was amplified in a platelet-dependent manner and normalized with FXI inhibition in patients with uncontrolled arterial hypertension.

Conclusions: Our results outline a new coagulation-inflammation circuit that promotes vascular dysfunction and points to the possible utility of FXI-targeted anticoagulants beyond their application as antithrombotic agents in cardiovascular disease.

PB 1616 | Function of an Endothelial-specific GPCR in Thrombosis

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Background: Venous thromboembolism (VTE) affects roughly 1 in 1000 individuals annually. The role of the endothelium in VTE is not fully understood. We recently characterized the human ‚endothelial-enriched transcriptome', which contained a number of orphan g-protein coupled receptors (GPCRs). We identified an association between a single nucleotide polymorphism (SNP) in the gene locus of one of these GPCRs and the risk of VTE in the genome wide association study ‚MARTHA'. Expression quantitative trait loci analysis in human endothelial cells (EC) revealed this SNP was associated with reduced transcription of the GPCR.

Aims: The aim was to investigate the role of this endothelial-enriched orphan GPCR in VTE. Specifically, how this GPCR is regulated and its role in coagulation.

Methods: We used siRNA to deplete the GPCR (GPCR-siRNA) in EC and assessed global protein expression by quantitative mass spectrometry proteomics. Findings were confirmed by real-time pcr and protein immunoblotting. Tissue factor (TF) activity was measured using a chromogenic activity assay. The effect of GPCR-siRNA on coagulation was tested in a thrombin generation assay adapted to incorporate an EC monolayer. In addition, we developed a flow assay exposing an EC monolayer to non-coagulated human blood at venous wall shear rate and measured fibrin deposition by immunofluorescence.

Results: GPCR-siRNA significantly increased EC TF expression and activity. This effect was amplified by pre-treatment of EC with tumor necrosis factor alpha. GPCR-siRNA increased EC-induced thrombin generation in human plasma and pre-treatment with a TF inhibitory antibody abolished this effect. In addition, fibrin formation from flowing whole blood was significantly enhanced on GPCR-siRNA EC.

Conclusions: Our results suggest that this endothelial-enriched GPCR could play a role in VTE development, though the regulation of tissue factor expression.

PB 1617 | Factor VII Activating Protease (FSAP) Regulates the Expression of Inflammatory Genes through Protease Activated Receptor (PAR1) in Vascular Smooth Muscle and Endothelial Cells

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Background: Factor VII activating protease (FSAP) knockout mice have a bigger neointima after vascular injury and a larger infarct volume after stroke. In humans, the loss of function Marburg I (MI) single nucleotide polymorphism (SNP) in the FSAP-encoding gene is associated with increased risk of stroke and carotid stenosis.

Aims: In order to understand how FSAP modifies vascular cells in man and mice, further information at a molecular level is needed.

Methods: Vascular smooth muscle cells (VSMC) and endothelial cells (EC) were stimulated with FSAP and a microarray-based expression analysis was performed. Selected genes were further analysed by qPCR and proteins by Western blotting, flow cytometry or ELISA. Phosphorylation of signal transduction proteins as well as receptor- and pathway-inhibitors were used to elucidate the mechanisms involved.

Results: Pathway analysis showed that expression levels of apoptosis/proliferation and inflammation related genes were influenced by FSAP. Following stimulation with FSAP, we identified *AREG*, *IL6* and *PTGS2* to be up-regulated in VSMC and *VCAM1*, *SELE* and *IL8* in EC. These observations were confirmed by qPCR and an elevated secretion of IL6 and IL8 was observed. These effects of FSAP were inhibited by aprotinin. Recombinant wild type protease domain of FSAP, but not the MI-isoform, could recapitulate this effect. Cellular activation by FSAP was impaired by blocking PAR1. Moreover, FSAP activated the ERK1/2 and CREB pathways, but EGFR transactivation was not detected. Besides, FSAP increased DNA synthesis and protected VSMC against serum starvation-induced apoptosis.

Conclusions: FSAP promotes an inflammation and proliferative/apoptosis-related gene expression pattern in VSMC and EC via a PAR1-dependent pathway. Also, cytokine expression, proliferation and apoptosis were altered in vascular cells. These novel cellular effects of FSAP provide a fundamental basis for understanding the role of FSAP in vascular diseases.

PB 1618 | Regulation of Platelet String Formation on Endothelial Cells in the Presence of Coagulation under Shear

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Background: Activated vascular endothelial cells secrete von Willebrand factor (VWF), which has been shown to polymerize and capture platelets (PLT) to form PLT-VWF strings. In purified systems, the metalloprotease ADAMTS-13 has been shown to rapidly degrade VWF-strings. Coagulation factors including thrombin are known to proteolytically inactivate ADAMTS-13. Thrombin generation is promoted by feedback activation by coagulation factor XI.

Aims: Study how activation and propagation of the coagulation cascade regulates PLT-VWF string formation on endothelial cells in whole blood under shear.

Methods: Human umbilical vein endothelial cells (HUVECs) were grown to confluence in an ibidi microfluidic flow chamber. VWF secretion was induced by stimulation of HUVECs with TNFα for 4 h. Recalcified human whole blood was perfused through the chambers at a venous shear rate of 2.5 dyne/cm2 for 10 min. PLT-VWF string and fibrin formation was visualized by labeling with anti-CD41 and anti-fibrinogen antibodies, respectively, and Alexa Fluor-labeled secondary antibodies. Platelet adhesion, mean aggregate size, and fibrin deposition were quantified using ImageJ.

Results: TNFα stimulation dramatically induced ultra large (UL)-VWF secretion from HUVECs. In whole blood in the presence of coagulation, Platelets were observed to form strings on VWF. Concomitant with platelet deposition was the formation of fibrin, resulting in localized fibrin-rich platelet aggregates. The presence of a function-blocking antibody to ADAMTS13 promoted VWF-string formation, Platelet adhesion, mean aggregate size, and fibrin deposition as compared to vehicle. Interestingly, inhibition of thrombin generation either by blocking tissue factor, directly inhibiting thrombin with hirudin, or through inhibition of the intrinsic pathway of coagulation with a function-blocking FXI mAbs promoted PLT-VWF string formation.

Conclusions: Our findings suggest that activation of the coagulation cascade to generate thrombin negatively regulates PLT-VWF string formation.

PB 1619 | Recombinant Adeno-associated Virus Vector Carrying the Thrombomodulin Lectin-like Domain Suppresses the Development of Abdominal Aortic Aneurysm in Mice

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Background: The lectin-like domain (D1) of thrombomodulin (TM) suppressed the receptor for advanced glycation end product (RAGE)-mediated inflammation by sequestering proinflammatory high-mobility group box 1 (HMGB1). Our previous study showed that short-term treatment with recombinant TM containing all the extracellular domains (rTMD123) attenuates HMGB1-RAGE signaling and confers protection against $CaCl_2$-induced abdominal aortic aneurysm (AAA) formation in mice.

Aims: Herein, we attempted to investigate the potential therapeutic benefits of TM domains for AAA using the recombinant adeno-associated virus (AAV) vector.

Methods: The therapeutic effects of recombinant TMD1 (rTMD1) and recombinant AAV vectors carrying the lectin-like domain of TM (rAAV-TMD1) were evaluated in two AAA mouse models, $CaCl_2$-induced AAA model and angiotensin II-infused AAA model, respectively. One-way analysis of variance followed by post hoc Bonferroni test was used in data passing tests for Kolmogorov-Smirnov test and Bartlett´s test; otherwise, a non-parametric Kruskal-Wallis test was used.

Results: In the $CaCl_2$-induced model, treatment with rTMD1 reduced the tissue levels of HMGB1 and RAGE, macrophage accumulation, elastin destruction and AAA formation, and the effects were comparable to a mole-equivalent dosage of rTMD123. In the angiotensin II-infused model, a single intravenous injection of rAAV-TMD1 (10^{11} genome copies) effectively attenuated AAA formation, accompanied by a reduction of HMGB1 and RAGE levels and suppression of proinflammatory cytokine production, macrophage accumulation, matrix metalloproteinase activities and oxidative stress in the aortic wall.

Conclusions: These findings imply the therapeutic potential of the TM lectin-like domain in AAA. The attenuation of angiotensin II-infused AAA by single injection of rAAV-TMD1 provides a proof-of-concept validation of its application as a potential gene therapeutic agent for aneurysm development.

PB 1620 | A2 Domain of von Willebrand Factor (VWF) Mediates Human Vascular Smooth Muscle Cells (VSMCs) Proliferation and Migration

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Background: Vascular Smooth Muscle Cells (VSMCs) proliferation and migration are crucial events in vascular ageing and atherosclerosis. Von Willebrand factor (VWF) is a multimeric glycoprotein produced in endothelial cells and platelets, and known for its contribution to platelet-adhesion/aggregation during vascular injury. VWF deposition in the vessel wall during intimal thickening has been reported.

Aims: We hypothesized that VWF could induce human VSMCs proliferation and migration.

Methods: Human aortic VSMCs between passages 3-6 were used. After synchronization in serum free medium, cells were treated for 24h with VWF (50 - 2000 ng/mL) or PDGF (2 - 100 ng/mL) and cell proliferation was evaluated by cell counting. Cell migration was performed by wound-healing assay and evaluated at t=0,16, 24 and 48h after wounding. ERK1/2 and Akt pathways activation, and VSMCs differentiation markers were analyzed by Western blot.

Results: Our results show that VWF induces substantial VSMCs proliferation with an optimal concentration of 250 ng/ml (1.7±0.2 fold

vs. 1.8±0.2 fold for PDGF at 5 ng/mL). VWF also promotes cell migration, with a coverage up to 70% of wounded area at 48h. Moreover, VSMCs cultured with VWF showed a decrease in the protein expression of smooth muscle α-actin and serum response factor (SRF) and an increase in matrix metalloproteinase 2, indicating some degree of dedifferentiation. In search for the molecular mechanisms, we observed that VWF exposure led to an upregulation of phospho-ERK1/2 (3.1±0.1fold) and phospho-Akt (1.5±0.1 fold), suggesting that these signaling pathways contribute to VWF-mediated VSMCs proliferation. Using recombinant VWF-fragments, we next demonstrated that the proliferative and migratory effect of VWF on VSMCs was mediated by its A2 domain.

Conclusions: In conclusion, these findings provide evidence on VWF inducing VSMCs proliferation and migration. These effects involve the activation of ERK1/2 and Akt signaling pathways and are at least partially mediated by the A2 domain of VWF.

PB 1621 | Dietary Alpha-linolenic Acid Reduces Platelet Activation and *ex vivo* Thrombus Formation in Sickle Cell Disease Mice

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Background: Sickle cell disease (SCD) is a genetic hemoglobinopathy associated with high morbidity and mortality.

Aims: We investigated the potential therapeutic use of the plant-derived omega-3 ALA in SCD.

Methods: 8-week-old male Berkeley mice were fed for 4 weeks a low (0.03%) or a high (7.3%) ALA diet (n=14). We analysed reticulocytes and reticulated platelets, neutrophils/RBC/platelet aggregates, neutrophils PSGL-1, platelet activation (P-selectin exposure and $a_{2b}b_3$ activation), thrombus formation on collagen under flow, and aortic VCAM-1, ICAM-1 and vWF expression.

Results: Four weeks feeding with ALA significantly decreased the number of sickle cells on blood smears (3.1/100 cells low-ALA vs 2.6/100 cells high-ALA, p=0.009). Platelet/neutrophils aggregates were 28% lower in the high-ALA group (83.56% of tot neutrophils in low-ALA vs 69.03% in high-ALA, p=0.03), and neutrophil PSGL-1 was reduced by 32% by ALA feeding (mean fluorescence intensity (MFI) 2797±547 low-ALA vs 1927±373 high-ALA, p=0.05). Platelet basal activation was reduced upon high-ALA feeding, as measured by P-selectin exposure (MFI 208.42±74 low-ALA vs 59.23±79 high ALA, p=0.006, figure 1) and active $a_{2b}b_3$ integrin (MFI 233.08±29 low-ALA vs 79.4±37 high-ALA, p=0.03). Expression on aortic endothelium of ICAM-1 was reduced by 70% in the high-ALA group (positive area: 3599 mm^2 low-ALA vs 1170 mm^2 high-ALA, p=0.01), by 55% for VCAM-1 (1195 mm^2 low-ALA vs 560 mm^2 high-ALA, p=0.0006), and by 30% for vWF (5072 mm^2 low-ALA vs 3933 mm^2 high-ALA, p=0.001). Thrombus formation on collagen *ex vivo* was significantly reduced by dietary ALA (thrombus area: 43'783±2959 mm^2 low-ALA vs 10'028±724 high-ALA, p=0.04, figure 2).

Conclusions: In a mouse model of SCD, ALA reduces 1) platelet activation, 2) endothelial adhesion, 3) inflammatory responses of platelet-leucocyte interaction and vascular adhesion molecule expression. It could therefore represent a novel and cheaply available agent for the treatment of sickle cell disease.

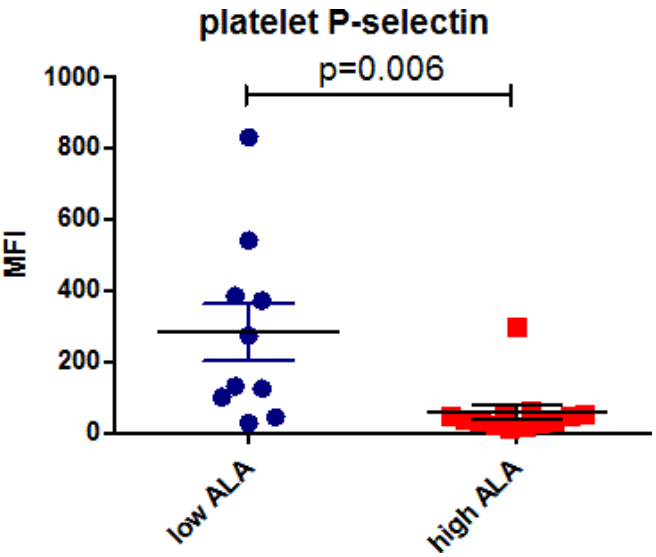

FIGURE 1 Platelets P-selectin exposure

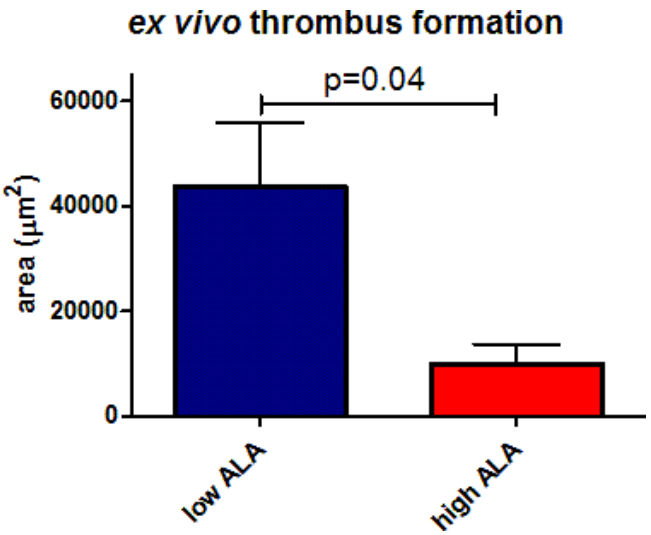

FIGURE 2 *Ex vivo* thrombus formation

PB 1622 | Podoplanin Overexpression on Endothelial Cells Promotes Superficial Erosive Injury and Thrombus Formation in Rat Carotid Artery

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Background: Atherosclerotic plaque erosion, a morphological pattern of plaque disruption, is characterized by a denuded plaque surface and thrombus formation. Podoplanin expression was significantly enhanced in atherosclerotic plaques. The precise mechanisms of plaque erosion and the roles of podoplanin in atherosclerotic lesions remain unclear.
Aims: To examine whether overexpression of podoplanin in arterial wall affects superficial arterial injury and thrombus formation in vivo, and factors which induce podoplanin expression in endothelial cells in vitro.
Methods: We evaluated superficial arterial injury and thrombosis formation induced by ligation in rat carotid artery. Recombinant adenoviruses expressing human podoplanin (Ad-podoplanin) or β-galactosidase (Ad-LacZ) were infected to the carotid arteries after distal ligation. We examined the effects of interleukin (IL)-3, 6, transforming growth factor (TGF)-β, and vascular endothelial growth factor (VEGF)-A (VEGF165, 500ng/mL) on podoplanin expression in cultured human aortic endothelial cells (HAOEC).
Results: Arterial endothelial cells were immunopositive for human podoplanin one day after the gene transfer. Four days after the gene transfer, occlusive thrombi were formed in all arteries (n=6) infected with Ad-Podoplanin, whereas small mural thrombi were developed in those infected with Ad-LacZ (n=6). Human podoplanin expressing cells were detached and involved in the occlusive thrombi. The administration of VEGF-A but not IL-3, 6, or TGF-β induced podoplanin mRNA expression 1.9-fold up to 24 hours, and increased the podoplanin protein level 4.0-fold at 24 hours.
Conclusions: Overexpression of podoplanin may enhance thrombus formation via detachment of endothelial cells, and VEGF-A could be a possible stimulant for podoplanin expression in endothelial cells. The results may provide novel insight into the mechanisms of plaque erosion.

PB 1623 | Evaluation a Novel Mouse Model of Abdominal Aortic Aneurysm to Study Platelet Mechanisms of the Proinflammatory Thrombus

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Background: Abdominal Aortic Aneurysm (AAA) is defined as a permanent localized dilation in the arterial wall with a diameter >50% and characterized by thinning and weakness of the vascular wall. In humans, aneurysms are associated with intramural thrombus and are prone to rupture and often result in death. Thrombi from abdominal aortic aneurysm (AAA) patients are highly enriched in leukocytes and in bacteria, eg. Porphyromonas gingivalis (Pg). This leukocyte-rich thrombus is considered the driving force in vessel wall rupture leading to death. Beyond their role in aneurysmal thrombus formation, platelets support leukocyte recruitment and interact with bacteria. This cross-talk is an important feature in thrombo-inflammatory vascular disease.
Aims: To study the role of platelets in the aneurysmal thrombus formation.
Methods: AAA was induced by applying an elastase-soaked filter paper on the infrarenal abdominal aorta of wild-type mice. Mice were then injected or not with *Pg* once a week for 2 weeks. Platelet adhesion and leukocyte recruitment to the vessel wall were analyzed by intravital microscopy and the presence of thrombi was quantified by immunohistology at early and late time points.
Results: In elastase-treated WT mice, we observed, by intravital microscopy, an early recruitment of platelets and leukocytes to the damaged vessel wall. At 14 days, abdominal aorta aneurysm was found in all treated mice. The diameter of the aorta was increased two-fold compared to sham mice and histological analysis did not reveal thrombus formation. In contrast, to elastase-treated WT mice injected with *Pg*, the dilatation was three-fold compared to untreated mice and importantly, immunohistology showed large thrombi in the dilated vessel wall which were enriched with platelets and leukocytes.
Conclusions: Here we establish a novel mouse model of abdominal aortic aneurysm allowing us (i) to study platelet activation mechanisms in the initiation and progression of AAA and (ii) test efficient anti-platelet therapies in AAA.

PB 1624 | Increased Inflammatory Properties of Neutrophils and Endothelial Cells (ECs) in Patients with Venous Thromboembolism (VTE), Long after the Acute Disease

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Background: Inflammatory cells, such as neutrophils, play a role in venous thromboembolism (VTE). Briefly, neutrophil-endothelial cell (EC) interactions consist of neutrophil chemotaxis, adhesive interactions with EC, including tethering and rolling, followed by firm attachment and migration to extravascular tissues.
Aims: Whether neutrophil-EC interactions are persistently activated after the acute phase of VTE remains a matter of debate and this is the purpose of this study.
Methods: The expression of activated adhesive molecules (CD11a/CD11b) of neutrophils was determined by flow cytometry, under basal

conditions and after TNF-α stimulus. Neutrophil chemotaxis assays were performed under basal conditions and with IL-8 stimulus. Vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule (ICAM-1) and the chemokine RANTES were measured by Multiplex analysis. Serum high sensitive CRP was analyzed by nephelometry.

Results: Thirty-seven spontaneous VTE patients and 37 healthy controls, matched according to age, gender and ethnicity, were included in the study (Table1). The median period of time since VTE diagnosis was 25 months (Table1). The expression of CD11a was higher in neutrophils from VTE patient, under basal conditions and after TNF-α stimulus. Higher CD11b expression was observed in VTE patient only after TNF-α stimulus (Table2). Neutrophil chemotaxis was increased in VTE patients under basal conditions and after IL-8 stimulus (Table2). Levels of circulating VCAM-1 and ICAM-1 were also increased in VTE patients (Table2), as well as the inflammatory biomarkers hsCRP and RANTES (Table1).

TABLE 1 Demographic and clinical data and comparative analysis of inflammatory markers in VTE patients and healthy controls

	Healthy Controls (N=37)	VTE patients (N=37)	P
Female/Male	24/13	24/13	1
Age (range)	43.56 (21- 66)	44 (19 - 65)	0.48
Caucasian/Non Caucasian	28/9	28/9	1
Time after VTE (range - months)	-	25 (12-42)	-
Spontaneous VTE episode (%)	-	51.35	-
chemokine RANTES (pg/ml)	63070 ± 23930	80020 ± 29420	0.03
hs¬CRP (mg/dl)	0.17 ± 0.12	0.59 ± 0.58	0.00

TABLE 2 Comparative analysis of neutrophil and endothelial cell activation in VTE patients and healthy controls

	Healthy Controls (N=37)	VTE patients (N=37)	P
Basal CD11a (MFI)	30.84 ± 6.82	38.72 ± 22.75	0.04
TNF-α stimulated CD11a (MFI)	34.09 ± 9.64	45.65 ± 33.06	0.01
Basal CD11b (MFI)	96.22 ± 32,53	120.9 ± 71.84	0.267
TNF-α stimulated CD11b (MFI)	149.10 ± 52.74	200.0 ± 100.5	0.02
Chemotaxis (basal) (%)	12.64 ± 4.78	17.55 ± 9.79	0.02
IL-8 stimulated Chemotaxis (%)	49.88 ± 19.48	63.48 ± 29.73	0.06
VCAM-1 (ng/ml)	468.7 ± 138.4	626.8 ± 285.8	0.01
ICAM-1 (ng/ml)	44.84 ± 9.758	90.87 ± 43.74	0.00

Conclusions: In conclusion, our findings demonstrated a profile of neutrophil and ECs activation in a group of VTE patients even long after the acute event. In addition, inflammatory markers were also increased in these patients. Our data suggest that increased inflammation may persist even after a long period since the acute episode of VTE, and neutrophils and ECs, possibly, play a role in this process.

PB 1625 | Analysis of Body-wide Unfractionated Tissue Data to Identify a Core Human Endothelial Transcriptome

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Background: Endothelial cells (EC) line blood vessels and regulate haemostasis, inflammation and blood pressure. Proteins critical for endothelial function tend to be enriched or specifically expressed in EC across vascular beds. Currently, there is no definitive description of the human pan EC enriched transcriptome.

Aims: To identify a comprehensive panel of human endothelial-enriched genes using global, body-wide transcriptomics.

Methods: We performed RNA-seq tissue transcript profiling of 124 samples from 32 human organs as part of the Human Protein Atlas Project (www.proteinatlas.org). We selected 3 transcripts that encode for proteins that are known to be EC enriched across vascular beds; c-type lectin domain family 14, member A (*CLEC14A*), von Willebrand factor (*vWF*) and CD34 (*CD34*) and calculated correlation coefficient values between the FKPM values of these transcripts and those of the other >20,000 mapped protein-coding genes. A high correlation value with all 3 EC reference genes indicated tissue wide EC-enriched expression of the gene(s) in question. We used antibody-based profiling to confirm protein expression of identified transcripts across vascular beds and measured expression in cultured EC.

Results: We identified a panel of 232 human pan EC-enriched transcripts, which contained both well-described EC transcripts (e.g. *CDH5*, *ESAM*, *KDR*) and a number that encode for novel or uncharacterised EC proteins (e.g. *CXorf36*, *FAM110D*). Most identified transcripts could be detected in cultured EC from various vascular beds, and we observed maintenance of relative expression in early passage cells.

Conclusions: We describe a widely applicable method to determine cell type specific transcriptome profiles in a whole organism context, based on differential abundance across tissues. We identify potential vascular drug targets or endothelial biomarkers, and highlight candidates for functional studies to increase understanding of the endothelium in health and disease.

PB 1626 | Effects of Aspirin and Rivaroxaban on Murine Arterial Vessel Wall Remodelling after Vascular Injury

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Background: Dual therapy with aspirin and rivaroxaban is increasingly being prescribed to patients with acute coronary syndrome and after coronary bypass grafting, but little is known about the effects of this treatment on the vasculature.

Aims: To study the effects of aspirin and/or rivaroxaban on vascular remodelling in mice subjected to vascular injury.

Methods: C57BL/6 mice were treated with vehicle, aspirin and/or rivaroxaban during the entire experimental period (n=12/group). Vascular injury was induced by temporary ligation of the carotid artery. Vascular stiffening was monitored for two weeks using non-invasive ultrasound imaging. Two weeks after ligation, mice were sacrificed, after which platelet-leukocyte aggregates (PLA) and vascular changes were assessed.

Results: Aspirin-treatment, calculated at 5 mg aspirin/kg/day, led to complete inhibition of arachidonic acid-induced platelet aggregation. Mean levels of plasma rivaroxaban were in the therapeutic clinical range with 344±35 and 552±101 ng/mL for the rivaroxaban and aspirin/rivaroxaban group, respectively. In all animals, temporary ligation resulted in the formation of nonocclusive thrombi. Treatment with aspirin with(out) rivaroxaban decreased the number of adhered platelets per PLA (P< 0.05) in both unstimulated and 2-MeSADP (ADP receptor) or AYPGKF (PAR4 receptor)-stimulated whole blood. Interestingly, treatment with aspirin with(out) rivaroxaban protected the arteries against ligationinduced stiffening (P< 0.05), while rivaroxaban alone showed a trend herein (P=0.08). Histological analysis indicated that ligation of the carotid artery resulted in local intima-media thickening, which was reduced with aspirin and rivaroxaban individually (P< 0.01).

Conclusions: This study provides new insight into the vasculardirected effects of aspirin and/or rivaroxaban treatment after thrombotic injury. Treatment with aspirin significantly antagonised, and rivaroxaban showed potential to inhibit, vascular stiffening, intimamedia thickening and PLA formation.

PB 1627 | Plasminogen Activator Inhibitor Type 1 in Platelets Evokes Thrombogenicity and Increases Thrombus Size by Elevating Thrombolysis Resistance under Shear Stress

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Background: Tissue plasminogen activator (t-PA) has been considered responsible for quick dissolution of unnecessary and aged thrombi.

Aims: We evaluated how tPA as well as PA inhibitor type 1 (PAI-1) affect thrombus formation under flow using a microchip-based flow chamber system equipped with a videomicroscope (Total Thrombus-formation Analysis System [T-TAS]; Fujimori Kogyo Co., Ltd., Tokyo, Japan).

Methods: t-PA-treated human whole-blood samples (n=6) were perfused over a microchip coated with collagen and tissue thromboplastin at different shear rates, and thrombus formation was quantified by measuring flow pressure changes during the perfusion experiments. The time required to reach 80 kPa was defined as complete capillary occlusion time. Rotational thromboelastometry (ROTEM) was used to evaluate fibrinolytic activity under static conditions.

Results: At a shear rate of 240 s^{-1}, t-PA (200-800 IU/mL) concentration-dependently delayed capillary occlusion, whereas at 600 s^{-1}, capillary occlusion was significantly faster and t-PA had limited effects. Thrombus formed inside the microchip after treatment by t-PA (400 and 800 IU/ml) at the shear rate of 240 s^{-1} showed reduced firmness, as observed by the frequent collapse of thrombi. The combined treatment of blood with a specific PAI-1 inhibitor (PAI-039) moderately enhanced the efficacy of t-PA, only under flow conditions. In addition, 1:1-diluted blood samples of PAI-1 deficient (-/-) mice showed a significant delay of capillary occlusion at 240 s^{-1}, compared with those from wild-type mice (1.55 fold; P< 0.001). This delayed occlusion was reproduced in samples containing platelets from PAI-1-/- and plasma from wild type, but was not observed by the opposite combination of blood components.

Conclusions: The anti-thrombotic efficacy of t-PA appeared sensitive to arterial shear flow, and that PAI-1 secreted from activated platelets plays an essential role in thrombolysis resistance.

PB 1628 | Tissue Engineered Small Vessel Conduits - The Anti-thrombotic Effect of Re-endothelialisation of Decellularised Baboon Arteries

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Background: The use of decellularised biological scaffolds for the reconstruction of small-diameter vascular grafts remains a challenge

in tissue engineering. Thrombogenicity is an important cause of obstruction in these vessels as a result of the decellularisation process. Seeding of the decellularised vascular constructs with endothelial cells is therefore a pre-requisite for the prevention of early thrombosis.

Aims: The aim of this study was to seed the internal surfaces of decellularised baboon arteries with endothelial cells and to compare the thrombogenicity of these conduits with that of decellularised arteries and also to that of a freshly harvested arteries in an *in vitro* circulating blood thrombotic model.

Methods: Carotid, radial and femoral arteries were harvested from two Papio ursinus baboons. Normal morphology was confirmed in the two control vessels and after decellularising the remaining arteries the effect of de-endotheliliasation were studied in the vessel scaffolds using scanning electron microscopy and transmission electron microscopy. Six of the decellularised vessel scaffolds were seeded with human umbilical vein endothelial cells. The luminal endothelialisation was established after seven days in a bioreactor and SEM confirmed confluency. Two fresh baboon arteries, four decellularised vessel scaffolds and six decellularised re-endothelialised vessel scaffolds were studied in an *in vitro* flow chamber using fresh citrated baboon blood.

Results: The decellularisation protocol resulted in a vessel scaffold with a well-preserved extracellular matrix and intact basal membrane. No thrombi could be demonstrated in the fresh control arteries and in the re-endothelialised vessel scaffolds. In the decellularised vessel scaffolds widespread platelet adhesion and activation occurred despite a relatively intact basal membrane.

Conclusions: This study supports the development of re-endothelialised tissue engineered small vessel conduits for clinical use.

PB 1629 | APAC, a Dual Antiplatelet and Anticoagulant Heparin Proteoglycan Mimetic, Integrates with Extravascular Matrix during Vascular Injury

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Background: APAC, a semisynthetic mimetic of mast cell-derived heparin proteoglycans inhibits both collagen-induced platelet aggregation (ED50 3-10 µg/ml) and coagulation *in vitro* and *vivo* (Lassila&Jouppila STH 2014). In two baboon arterial thrombosis models APAC locally reduced platelet thrombus and fibrin formation, maintaining vessel patency. Systemic APAC prevented ischemic kidney injury in rats (Tuuminen et al. 2016).

Aims: To assess APAC's binding and localization to denuded porcine iliac and femoral artery (IA, FA) after local exposure *in vitro* and *in vivo*. APAC co-localization with von Willebrand factor (VWF), laminin, podocalyxin, and PECAM was studied.

Methods: We used 3 models of injury: 1) *in vitro*, 2) *in vivo* repeated balloon angioplasty of IA, and 3) *in vivo* arterio-venous fistula (AVF) between FA and vein. In all models biotinylated APAC (0.5 mg/ml) reacted with vessel injury site for 2 min, prior to release of blood flow. APAC was detected using streptavidin conjugated to eFluor660, and VWF, Laminin, PECAM and podocalyxin by Immunofluorescence using a confocal microscope.

Results: APAC bound *in vitro and vivo* both to balloon-injured arteries and AVF at anastomosis and the adjacent artery and vein co-localizing with VWF, and laminin. APAC binding was limited when PECAM or podocalyxin was present. APAC-VWF and APAC-Laminin Manders' co-localization coefficients (MCC) were > 0.7 indicating strong co-localization. APAC-PECAM MCC was < 0.3 indicating weak co-localization. APAC penetrated the injured vessel wall and in AVF, leakage of APAC in the venous extravascular tissue was depicted.

Conclusions: APAC, in contrast to heparins, strongly inhibits collagen-platelet aggregation and subsequent coagulation and rapidly targets the injured vessel wall. APAC co-localized with multiple matrix components, excluding PECAM and podocalyxin. Cardiovascular interventions benefit from APAC's antithrombotic potential and local integration to vascular injury sites.

PB 1630 | Protein Kinase C-Dependent Loss of Tissue Factor by Human Pericytes Results from Altered Synthesis, Trafficking and Degradation

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Background: Tissue factor (TF) exerts procoagulant and signaling activities that contribute to hemostasis, angiogenesis and immune responses. Conversely, increased TF expression is linked to the progression of malignancy, vascular disease and thrombosis. Many mediators of TF up-regulation have been described. However, we reported the first example of active downregulation of TF around angiogenic vessels near healing cutaneous wounds.

Aims: The aim of the current study was to characterize mechanisms that down-regulate pericyte TF, with the ultimate goal of therapeutically modulating TF expression.

Methods: Similar to dermal pericytes *in vivo*, cultured pericytes express high levels of TF. We previously reported that TF antigen is lost within 8 hours of exposure to phorbol-12 myristate 13-acetate (PMA), and remains low for at least 24 hrs. Cell surface TF activity does not decline as rapidly as total TF protein. This model recapitulates the loss of TF antigen following wounding *in vivo*. We assessed changes in TF mRNA by qRT-PCR and TF protein expression by western blotting in cultured human pericytes.

Results: TF mRNA decreased 4- and 6-fold at 8 and 12 hours after addition of PMA ($p < 0.01$) and remained low for 24 hrs. Inhibitors of

Protein Kinase C (PKC), Go6983 and GFX, inhibited PMA-induced reduction of TF mRNA ($p< 0.001$) and protein ($p< 0.001$). Since the decrease in TF mRNA did not precede the loss of protein, we also examined the role of protein degradation. PMA triggered internalization of surface TF, and shortened the half-life from 11 to 5 hours ($p< 0.001$). Inhibitors of lysosome and proteasome activity each reduced the decrease in TF in response to PMA. Confocal microscopy confirmed the pattern of TF trafficking suggested by biochemical data.

Conclusions: We propose a model in which activation of a PKC-dependent pathway triggers internalization of surface TF, which is degraded in lysosomes. Surface TF is replaced from internal stores until reduced synthesis and increased degradation deplete cellular TF protein.

PB 1631 | Design and Utility of Extracellular Matrix-embedded Microvessels to Study the Platelet-endothelium Interface

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Background: Under normal conditions, endothelial cells (ECs) maintain vessel patency by providing a barrier between the blood and tissue space and by pacifying platelet activation and thrombin generation in the bloodstream. The breakdown of this EC barrier function is a hallmark of vascular diseases including inflammation, atherosclerosis and cancer. In sepsis, for example, the dysfunction of vascular ECs has been correlated with poorer outcomes due to hemorrhage and multi-organ failure associated with consumption of platelets and coagulation factors into clots within the microcirculation, a condition termed disseminated intravascular coagulation (DIC).

Aims: Develop an endothelialized flow chamber to study the platelet-endothelium interface.

Methods: We developed a 3D-chamber with a perfusable cylindrical microvessel embedded in an extracellular matrix (ECM) material. This platform allows for the study of the role of thrombin generation and platelet aggregation in maintaining endothelial barrier function in healthy as compared to inflamed microvessels. Incorporation of sub-endothelial matrix proteins in these 3D-microvessel devices expands the capacity of the microfluidic studies to investigate blood cell extravasation and enables the control of physical parameters such as transmural pressure and interstitial flow through the ECM.

Results: Our initial findings show that stimulation of ECs with TNFα induced platelet deposition, VWF-string formation and increased vessel permeability and vascular leak.

Conclusions: This platform may provide insight into the pathophysiology of different disease states and serve as an expedient platform for therapy design and testing.

PB 1632 | Micropatterning Induced Alterations of Endothelial Outgrowth Cells

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Background: Small diameter artificial vascular grafts are a critical unmet need. *In vitro* endothelialization may be a solution, however the invasiveness of endothelial cell (EC) harvest makes this impractical. In

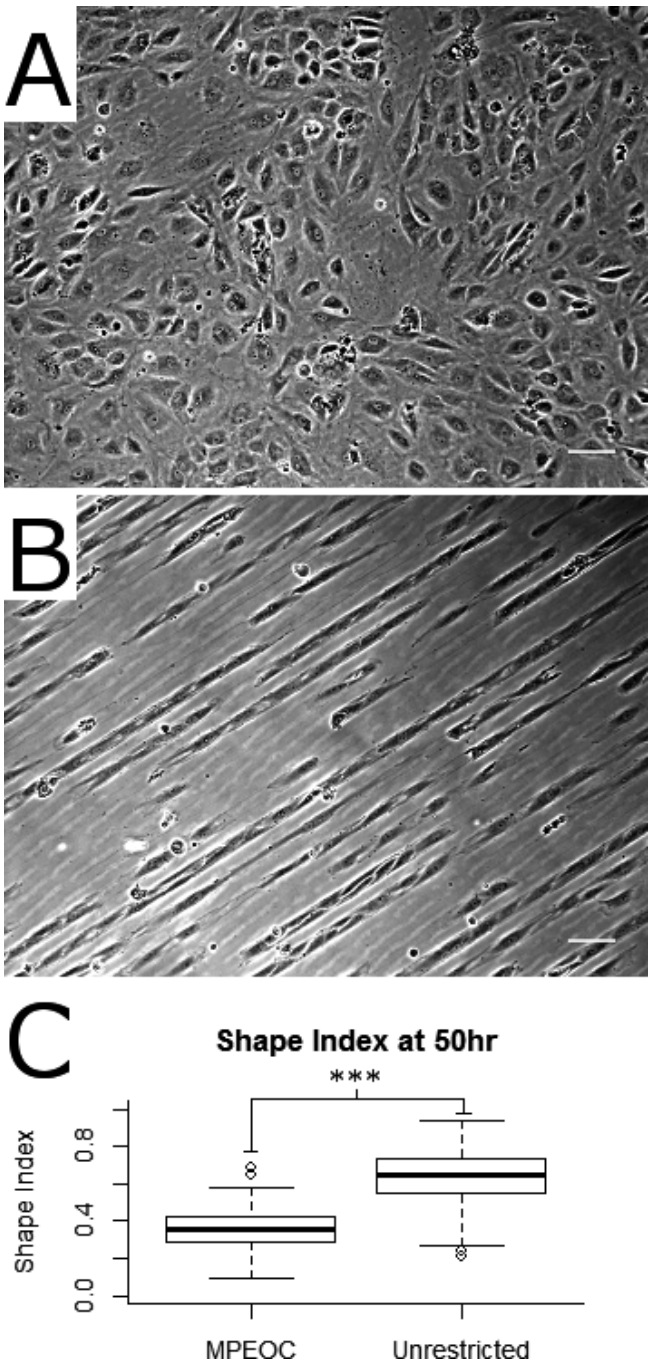

FIGURE 1 EOC patterning after 50 hrs. A) Unrestricted EOC. B) MPEOC. C) MPEOC have lower shape index than unrestricted EOC $p<.0001$. Scale bars 100μm

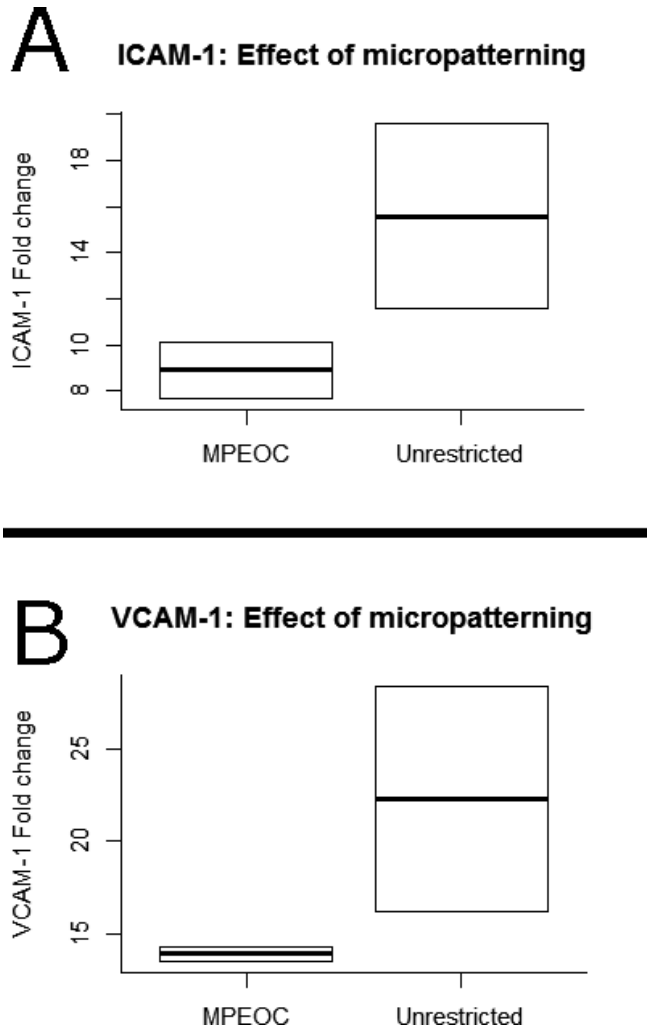

FIGURE 2 MPEOC trend toward reduced inflammatory response to TNF relative to unrestricted shown by A) ICAM-1 gene expression; B) VCAM-1 gene expression.

contrast, endothelial outgrowth cells (EOCs) are isolated with a simple venous blood draw. Like ECs, EOCs respond to flow. Static micropatterning of ECs drives elongation and immunoresistance without flow. Hence, micropatterning is considered a promising tool to alter EC phenotype. To date, attempts at EOC micropatterning have failed, thus it is unclear whether they respond similarly.

Aims:

1. Validate a novel EOC micropatterning procedure.
2. Define micropatterned EOC (MPEOC) immunogenicity to address the hypothesis that MP confers immunoresistance.

Methods: Surfaces were patterned with 25μm lanes of collagen-I and Pluronic. EOCs were seeded on patterned or unrestricted surfaces for 48 hrs. Cultures were treated with tumor necrosis factor α (TNF) for 3 hrs and collected for qPCR. EOCs were imaged at 22 and 50 hrs after seeding. A shape index (SI) quantifying cell roundness (1= circle; 0= line) was calculated. qPCR data were ΔΔ-ct calculated. SI data were analyzed using repeated measures ANOVA, while qPCR data were analyzed using two-way ANOVA.

Results: MPEOCs remained in lanes for 50 hours (Fig. 1B). SI showed significant elongation (Figure 1C, $p < .0001$), and increasing elongation over time ($p < .0001$). Preliminary qPCR showed no significant changes as a result of micropatterning, however a trend toward reduced expression of the adhesion molecules ICAM-1 and VCAM-1 was seen in TNF-treated MPEOCs relative to TNF-treated unrestricted EOCs (Figure 2).

Conclusions: EOC patterning was robust with our novel procedure. There was a trend toward decreased EOC immunogenicity with micropatterning which will be clarified in further work. EOC patterning is feasible and may be a promising tool for graft development.

PB 1633 | Endothelial Dysfunction in Idiopathic Thromboembolism Investigated through Gene Expression Profiling of Endothelial Colony Forming Cells

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Background: Endothelial integrity is essential to prevent thrombosis. Endothelial colony-forming cells (ECFCs) are bone-marrow-derived cells involved in homeostasis.ECFCs isolated and cultured from peripheral blood allow an endothelial functional assessment.ECFCs from patients with idiopathic venous thrombosis (ITE) present premature cell senescence.We analyzed the gene expression profiling of ECFCs in ITE patients.

Aims: To assess if endothelial abnormalities could have a role on the pathogenesis of ITE.

Methods: ECFCs isolated and cultured from the peripheral blood of 8 patients and 5 matched controls. ECFCs were used to analyze gene expression profiling.Total RNA was isolated and reverse-transcribed cRNA was hybridized onto Illumina HumanHT-12v4 Expression BeadChip arrays.Gene Set Enrichment Analysis (GSEA) was used to interpret gene expression data.

Results: More than 4000 gene sets collected in GSEA database were analyzed: 8 gene sets resulted significantly enriched in patients, 200 gene sets resulted significantly down-regulated (false discovery rate < 10%, nominal p value < 0.01).The gene sets enriched in patients comprised the gene set composed by genes typically up-regulated by enhancer of zeste homolog 2 (Ezh2),a methyltransferase involved in the epigenetic regulation of endothelial cells functions.The gene set composed by genes typically repressed by Ezh2 resulted down-regulated in ITE patients,thus indicating an higher activity of Ezh2 in patients.The gene sets down-regulated in patients also included the Weigel oxidative stress response gene set,involved in cell response to oxidative stress.

Conclusions: Our results demonstrate ECFCs from ITE patients differ from control ECFCs in their gene expression profiling confirming that ECFC characterization can be a valid non-invasive tool to evaluate endothelial compartment.Other experiments clarifying the role of Ezh2 are needed to characterize the epigenetic dysregulation that may possibly hinder the physiologic antithrombotic function of endothelium in ITE patients.

PB 1634 | Role of Lysozyme M+ Cells in the Development of Angiotensin II-induced Vascular Inflammation

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Background: Multicellular interactions of platelets, leukocytes and the vessel wall play pivotal roles in activating coagulation and precipitating thrombosis, but also in promoting vascular dysfunction. We uncovered a coagulation-inflammation circuit through factor XI (FXI)-thrombin amplification loop that promotes vascular dysfunction highlighting the possible utility of FXI-targeted anticoagulant in treating hypertension. The role of $LysM^+$ cells (myelomonocytic cells) and their interaction with platelets during adhesion to the vascular endothelium is not completely understood.

Aims: We wanted to explore the role the interaction of $LysM^+$ cells and platelets in the development of angiotensin II (AngII)-induced vascular dysfunction.

Methods: IRG transgenic double-fluorescent mice with widespread expression of red fluorescence and GFP expression in $LysM^+$ cells were infused with AngII (1 mg× kg^{-1}× d^{-1} for 7 days using osmotic minipumps) and $LysM^+$ cells rolling in carotids were quantified using intravital videomicroscopy imaging.

Results: We were able to confirm that rolling and adhering cells were only $LysM^+$ cells with injection of acridine orange thought jugular catheter in order to mark nucleated cells, but were not able to see platelet-Leukocyte-complex formation during the time of recording. It is also interesting to note the catheter implantation increases the number of rolling and adhering $LysM^+$ cells showing here the importance of having available double transgenic mice model with expression of red fluorescence and GFP to not interfere with *in vivo* processes during experimentation.

Conclusions: We showed here for the first time that AngII-activated cells rolling and adhering to the endothelium are LysM+ cells. Further investigations are needed to explore the exact role of platelets in these processes.

PB 1635 | The Response of HUVEC to Prolonged Shear Stress for Use in in vitro Studies

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Background: Endothelial cell (EC) activation and leukocyte recruitment are key events in inflammation and thrombosis. Studying EC *in vitro* has led to important insights into these processes. Many experiments have used EC cultured under static conditions. However, EC experience continuous shear stress *in vivo*. Subjecting EC to shear stress for up to 24h has been shown to modulate the expression of cell adhesion molecules involved in leukocyte adhesion and migration, including intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), in response to inflammatory stimuli. The effect of more prolonged culture under shear stress has been less well characterised.

Aims: We aimed to establish the optimal time for exposure of EC to arteriolar shear stress prior to experimentation, and examine the response of these EC to TNF-α.

Methods: Human umbilical vein EC (HUVEC) were cultured under unidirectional laminar flow at a shear stress of 12dyn/cm^2 for 24h or 72h, followed by treatment with 100U/ml TNF-α for 4h. EC were stained for f-actin and vascular endothelial (VE)-cadherin, and morphology was assessed using fluorescence microscopy. ICAM-1 and VCAM-1 surface expression was measured by flow cytometry.

Results: EC cultured at 12dyn/cm^2 demonstrated cellular alignment and f-actin organisation in the direction of flow after 72h. This was not readily apparent after 24h at 12dyn/cm^2, suggesting that prolonged culture under shear stress induced further phenotypic changes. TNF-α increased ICAM-1 and VCAM-1 expression following static culture or 24h at 12dyn/cm^2. The TNF-α-induced increase in VCAM-1 expression was suppressed following 24h culture under shear, although no differences were observed in ICAM-1 expression between static and shear conditions.

Conclusions: These data suggest that prolonged exposure of EC to shear stress promotes further changes in phenotype, as despite suppressing VCAM-1 expression in response to TNF-α, 24h preconditioning was insufficient to induce complete EC alignment.

PB 1636 | Serum Sphingosine-1-phosphate is Not Associated with Age and Gender but with Blood Cell-related Parameters: Analysis of the Study of Health in Pomerania Cohort

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Background: The platelet-derived sphingosine-1-phosphate (S1P) is a bioactive signaling lipid involved in numerous biological processes such as inflammatory and immunomodulatory responses as well as vascular homeostasis. The secretion of S1P from platelets is dependent on the formation of thromboxane and the subsequent activation of the thromboxane receptor. S1P does not only play a role as mediator but also as a biomarker for cardiovascular pathologies as altered concentrations in serum are associated with coronary disease, peripheral artery disease and carotid stenosis.

Aims: To date, no reference intervals for S1P have been defined in a population-based and well characterized study cohort. The present study aimed to determine a reference range for serum S1P. We also investigated associations between individual S1P concentrations and age, gender as well as blood cell-related parameters.

Methods: We determined reference intervals for serum S1P levels according to age and gender in a sample of 1,339 healthy participants of the Study of Health in Pomerania (SHIP)-TREND cohort. Subjects with cardiovascular disease, diabetes mellitus, hypertension, metabolic syndrome, elevated liver enzymes, chronic kidney disease stadium III or IV, or body mass index > 30 kg/m^2 were excluded. S1P was measured using a liquid chromatography-tandem mass spectrometry method.

Results: The median age of the participants was 41 (25th; 75th percentile 32; 51) years and 65% were women. The median concentration of serum S1P was 0.804 (0.694; 0.920) µmol/L. No association of age and gender with S1P was observed. The overall reference interval was 0.534-1.242 µmol/L (2.5th; 97.5th percentile). S1P levels were significantly correlated with platelet numbers (R=0.28) but not with red (0.09) or white blood cell counts (0.11).

Conclusions: This study provides reference intervals for serum S1P in healthy individuals. Total serum S1P concentrations vary among healthy individuals irrespectively of age and gender but they correlate with platelet numbers.

PB 1637 | Plasma Modification of Poly(Vinyl Alcohol) for Enhanced Endothelialization and Anti-thrombogenicity

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Background: The interaction between a biomaterial and the constituents of blood flow are critical to the long-term patency and integrity of vascular grafts. Poly(vinyl alcohol) (PVA) is under investigation as a material for vascular grafts due to its tunable mechanical properties, non-thrombogenic surface, and amenability to luminal surface modifications. However PVA, by virtue of its inherent surface composition, discourages the migration of endothelial cells and ultimately prevents the considerable long-term benefits of endothelialization *in vivo*. Plasma treatment of the luminal surface of PVA grafts offers the opportunity to promote cell migration while maintaining or enhancing the desirable mechanical properties and non-thrombogenicity of PVA as a vascular graft.

Aims:

- Systematically evaluate plasma treatments of PVA and characterize elemental changes in surface composition in order to establish critical guidelines toward the creation of a long-term, small diameter, artificial graft.
- Determine the relationship between amide density and cell migration.

Methods: Dry PVA films were placed in a plasma chamber with either 0_2, N_2, or Ar gas to treat the films for 0.5, 1, and 5 min. The composition of the films was interrogated using x-ray photoelectron spectroscopy (XPS). Surface topography was investigated using AFM, SEM, and ESEM. Contact angle measurements were also performed to quantify the change in hydrophobicity of the films.

Results: Figure 1 shows the percent composition of PVA films without plasma treatment and with exposure to 0_2 and N_2 plasma. The results show that with this plasma treatment, nitrogen present on the surface of the film (Figure 1 insert) increases from a negligible percentage to 5.2%.

Conclusions: The increase in nitrogen content of the plasma-treated PVA surface indicates the presence of amine and amide groups critical for integrin binding. An increase in binding sites should increase cell migration and thus native endothelialization of PVA vascular grafts improving their long-term patency.

PB 2415 | Thioredoxin Interacting Protein Endothelial Deletion Protects from Alterations of Angiogenic Process in Adult Mice Submitted to Vascular Accelerated Aging

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Background: Endothelium plays a central role in angiogenesis and the ability to limit ischemic complications.Yet, aging endothelial cells become pro-oxidative, pro-inflammatory leading to endothelial dysfunction that can impair angiogenesis.

Aims: We have developed a model of vascular accelerated aging consisting in a 3 month High protein-Low carbohydrate (HP-LC) diet given to 6 month old mice. This model induced endothelial dysfunction. We also generated EC-TXNIP KO mice on the Cdh5-cre background and demonstrated that they were protected from endothelial dysfunction induced by 3-month HP-LC diet.

In this work, we hypothesized that HP-LC diet would induce a defect of angiogenesis and that endothelial TXNIP deletion might provide protection against aged-impaired angiogenesis.

Methods: EC-TXNIP KO mice and littermate controls were submitted or not to3 month HPLC diet. Angiogenic process was studied *in vivo* with the hindlimb ischemia model using near infrared Laser Doppler Imager; microCT scans and in *vitro* assays. The aortic ring assay was used for an ex *vivo* approach.

Results: After surgery, the perfusion was measured at days 0, 3, 7, 14 and 21 and showed a lower decrease of blood flow after 3 month of HP-LC diet in EC-TXNIP KO mice compared with littermate controls. Blood flow recovery was correlated with collateral formation *in vivo*. These results were associated with a protection against inflammation cells and adipocytes infiltration, myofibers necrosis. Sprouting angiogenic responses on Matrigel were assessed with the aortic ring assay. Endothelial TXNIP deletion prevents from vessel area and number of endothelial microvessel sprouts HP-LC-induced-decrease. A lowering of decreased VEGF pathway in gastrocnemius and aorta was observed in EC-TXNIP KO mice compared with littermate controls.

Conclusions: This work shows a defect in angiogenic process with ischemic damage in a vascular aging model and demonstrate the key role of TXNIP in these impairments, making TXNIP a potential therapeutic target in aging context.

PB 2416 | FHL2 is Important for Angiogenesis and Regulates TF Expression in Normal Vasculature

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Background: Four and a Half LIM domain 2 (FHL2) is a multifunctional adaptor protein with numerous binding partners. FHL2 is abundantly expressed in the cardiovascular system and has been proposed to modulate angiogenesis. Since Tissue Factor (TF) and Factor VIIa (FVIIa) also regulate angiogenesis, we hypothesized that FHL2, TF and FVIIa cooperate in the formation of new blood vessels.

Aims: To investigate the effects of FHL2 silencing on TF, FVIIa and angiogenesis.

Methods: We performed *ex vivo* angiogenesis assays with aortic explants from *Fhl2*-deficient ($Fhl2^{-/-}$) and C57BL/6 wild-type (WT) mice. Embedded in collagen, explants were fed with growth medium containing vascular endothelial growth factor (VEGF) and treated with mouse FVIIa (mFVIIa). *In vitro* angiogenesis assays were performed on stably transduced immortalized human endothelial cells (ECRFs). Levels of TF and several angiogenic markers were measured by qPCR and western blot.

Results: Sprout formation was significantly reduced in $Fhl2^{-/-}$ explants. Addition of mFVIIa increased the mean number of sprouts for both $Fhl2^{-/-}$ and WT. Knockdown of FHL2 in ECRFs reduced tube formation, cell migration and angiogenic markers *in vitro*. Paradoxically, silencing of FHL2 also upregulated expression of TF both *in vivo* and *in vitro*. Overexpression of FHL2 increased formation of tubes and angiogenic markers, while it had no impact on migration, nor did it change TF levels in ECRFs.

Conclusions: Silencing of FHL2 led to impaired angiogenesis and increased TF levels. Despite the abundance of endogenous TF in $Fhl2^{-/-}$ explants, addition of mFVIIa did not rescue the antiangiogenic phenotype.

PB 2417 | MiRNA-21 Mediates the Antiangiogenic Activity of Metformin through Targeting PTEN and SMAD7 Expression and PI3K/AKT Pathway

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Background: Metformin, an anti-diabetic drug commonly used for type 2 diabetes therapy, is associated with anti-angiogenic effects in conditions beyond diabetes.

Aims: miR-21 has been reported to be involved in the process of angiogenesis. However, the precise regulatory mechanisms by which the metformin-induced endothelial suppression and its effects on miR-21-dependent pathways are still unclear.

Methods: Bioinformatic analysis and identification of miR-21 and its targets and their effects on metformin-induced antiangiogenic activity were assessed using luciferase assays, quantitative real-time PCR, western blots, scratch assays, CCK-8 assays and tubule formation assays.

Results: In this study, miR-21 was strikingly downregulated by metformin in a time- and dose-dependent manner. miR-21 directly

targeted the 3'-UTR of PTEN and SMAD7, and negatively regulated their expression. Overexpression of miR-21 abrogated the metformin-mediated inhibition of endothelial cells proliferation, migration, tubule formation and the TGF-β-induced AKT, SMAD- and ERK-dependent phosphorylations, and conversely, down-regulation of miR-21 aggravated metformin's action and revealed significant promotion effects.

Conclusions: Our study broadens our understanding of the regulatory mechanism of miR-21 mediating metformin-induced anti-angiogenic effects, providing important implications regarding the design of novel miRNA-based therapeutic strategies against angiogenesis.

PB 2418 | Role of SerpinE2 (PN-1) in Diabetic Microangiopathy

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Background: Angiogenesis deregulation is involved in many diabetic complications, in particular retinopathy and nephropathy, in which it is excessive and deleterious. Angiogenesis is highly regulated by a balance between pro- and anti- angiogenic factors, including proteases and protease inhibitors involved in vascular remodelling. SerpinE2, or protease nexin-1 (PN-1), is a protease inhibitor belonging to the serpin family, expressed by vascular and inflammatory cells. We recently demonstrated a significant anti-angiogenic effect of PN-1. In particular, data obtained in vivo using PN-1 deficient mice (PN-$1^{-/-}$) have shown that PN-1 is involved in the regulation of retinal vascular development.

Aims: We therefore hypothesize that PN-1 could modulate diabetic microangiopathies through regulation of angiogenic responses.

Methods: In this study, we used the mouse model of streptozotocin-induced type 1 diabetes to assess the role of PN-1 in the development of diabetic retinopathy and nephropathy.

Results: Neither WT, nor PN-$1^{-/-}$ diabetic mice showed any sign of retinopathy, even after 6 months of hyperglycemia. However, our results show that PN-1 may play a protective role in diabetic nephropathy. Indeed, PN-$1^{-/-}$ diabetic mice presented histological features of an aggravated nephropathy as well as an increased albuminuria, compared to WT mice. Using transgenic mice expressing beta-galactosidase under PN-1 promoter, we observed by X-Gal staining that PN-1 was essentially expressed in kidney vessels and glomeruli and that PN-1 expression was greatly increased during diabetes.

Isolectin labelling of kidney sections indicated that diabetes induced an increase of glomerular vascularisation that was greatly delayed in PN-$1^{-/-}$- compared with WT- diabetic mice, suggesting that PN-1 play a protective role in kidney.

Conclusions: In conclusion, our results indicate that PN-1 play a role in the development of diabetic nephropathy by a mechanism that remains to be established.

PB 2419 | Human Aortic Valvular Interstitial Cells: Evidence of Vasculogenic Potential during Aortic Valve Stenosis

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Background: Calcific Aortic valve disease (CAVD) is the most common heart valve disease in the world. Normal adult aortic valve (AV) is thin and avascular, however during CAVD a neoangiogenesis process is observed. Valvular interstitial cells (VICs) are the main cells found in AV and are responsible of valve integrity.

Aims: The purpose of this study was to examine whether VICs are progenitor cells able to modulate newly formed vessels in CAVD.

Methods: VICs were isolated from human aortic valves obtained after surgery for AVD (Pathological VICs: VICp) and normal aortic valve unsuitable for grafting and without any detected cardiac pathology (Control VICs: VICc).

Results: VIC's mesenchymal phenotype was confirmed by a $CD90^+$/$CD44^+$ expression and a multipotential differentiation potential. We injected VICp *in vivo*, in combination with endothelial progenitor cells (EPCs) in a Matrigel implant mouse model and found that VICp formed microvessels by differentiating in perivascular cells. VIC_p perivascular differentiation was confirmed *in vitro*. VIC_p are not able to undergo endothelial differentiation *in vivo* and *in vitro*. We found that conditioned media from VICp *vs* VICc were able to induce EPC proliferation. We then applied a comparative whole genomic transcriptomic approach of human VICp and VICc. Among angiogenic genes differentially expressed in VICp *vs* VICc, we observed an imbalance between ANG-2 gene (-3.51 fold VICp vs VICc) and VEGF-A (2.02 fold VICp *vs* VICc). Only VEGF-A modulation has been confirmed at the protein level in conditioned media from VICp *vs* VICc. VEGF-A blocking abolished proliferative effect of VICp.

Conclusions: In conclusion, VICs are vasculogenic progenitor cells with pericyte differentiation ability. We shown that VICp release VEGF-A and promote neovascularization during CAVD. Our findings provide evidence that blocking VEGF-A could have a pivotal role in maintaining valvular normal function by preventing angiogenesis that may lead to CAVD.

PB 2420 | Nestin Is an Endothelial Enriched Protein in the Nonproliferative Vasculature with Potential Role in Endothelial Apoptosis

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Background:

- Known endothelial cell (EC) markers were used to seed a correlation analysis of RNAseq data from 124 individuals to search for novel endothelial genes
- One of the novel EC specific genes identified was the cytoskeletal protein nestin
- Nestin is an intermediate filament protein thought to only be expressed in proliferating cells, such as stem cells and angiogenic endothelium

Aims: To characterise nestin expression and function in nonproliferative human adult endothelial cells

Methods:

- Correlation analysis using RNAseq data from 124 healthy individuals
- Immunohistochemistry (IHC) and immunofluorescence (IF) staining alongside siRNA and qPCR to confirm gene and protein expression
- Live time lapse microscopy and annexin V/PI staining

Results:

- Nestin RNA transcripts from human tissues are positively correlated with endothelial markers such as CD34 and von Willibrand factor(VWF), and have negative correlation with proliferative markers such as PCNA and Ki-67
- IHC staining of human adult tissues shows endothelial specific nestin expression, with no concurrent endothelial staining of proliferative markers such as PCNA, indicating a nonproliferative state
- IF staining of *in vitro* cultured EC shows nestin forming a large perinuclear clump when cultured under static conditions, but spreading out to form a cell-wide filamentous network under flow conditions
- Annexin V/PI assays indicate that EC with siRNA inhibited nestin expression have increased rates of apoptosis after TNF stimulation

Conclusions:

- In contrast to previous assumptions, endothelial nestin expression is not confined solely to angiogenesis as previously thought, but is expressed in the majority of EC throughout the human adult

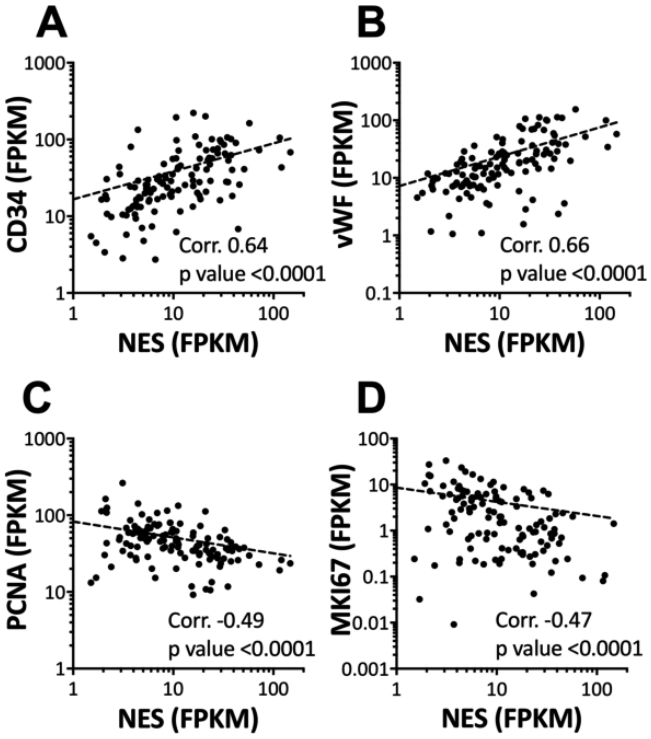

FIGURE 1 Correlation of nestin FPKM values with endothelial markers (A)CD34 and (B)VWF, and with proliferation markers (C) PCNA and (D)Ki-67

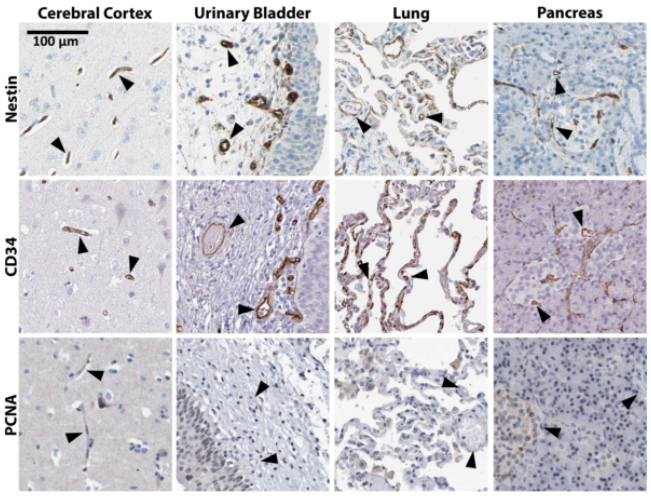

FIGURE 2 IHC stained tissue samples showing EC staining of nestin and the endothelial marker CD34, but not the proliferation marker PCNA

- Nestin expression is regulated by flow conditions, perhaps implying an important function in EC function under flow. This requires further study to elucidate the function of nestin in human EC
- EC with reduced nestin expression have increased rates of apoptosis after exposure to apoptotic stimuli

PB 2421 | Shiga Toxin Type 2, a Causal Agent of Hemolytic Uremic Syndrome, Improved Angiogenic and Repair Abilities of Late Outgrowth Endothelial Progenitor Cells

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Background: Hemolytic Uremic Syndrome (HUS), the main cause of pediatric acute renal failure, is caused by *E. coli* producing Shiga toxins (Stx) and characterized by massive endothelial damage, which is worsened by inflammation, especially in the presence of bacterial lipopolysaccharides (LPS). Endogenous regeneration of the vessel wall involves local and bone marrow-derived endothelial progenitor cells (EPC) as well as nearby mature endothelial cells (EC). Although Stx toxic effects on mature EC have been widely studied, its action on EPC and angiogenesis remain to be elucidated.

Aims: We aimed to analyze the effect of Stx alone or in combination with LPS on survival and angiogenic properties of EPC.

Methods: Human cord blood-derived late-outgrowth EPC and umbilical vein EC were cultured in cytokine-rich growth medium EGM2. Cells were sensitized with LPS for 18 h and then Stx type 2 was added for another 18 h.

Results: Nuclear morphology analysis revealed that Stx, at concentrations that are known to induce mature EC death, had no effect on EPC survival cultured in basic medium (EBM2+ 2% FBS), which undergo apoptosis only after LPS sensitization (Fig.1). Moreover, no Stx effect was observed when EPC were cultured on EGM2 (Fig.1). Stx receptor Gb3 was expressed on EPC surface and upregulated by LPS+Stx (180±12*% of control, n=3, *p< 0.05, ANOVA, flow cytometry). While EPC proliferation was not affected, ICAM-1 and E-selectin expression

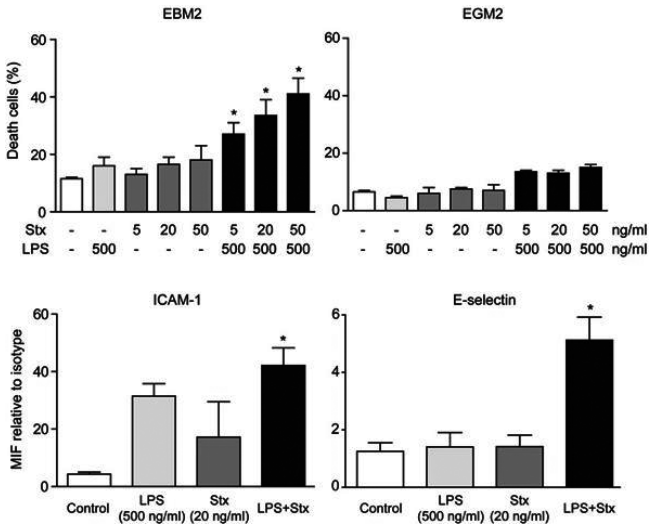

FIGURE 1 Toxicity and activation of EPC. n=3-5, *p<0.05 vs control, ANOVA

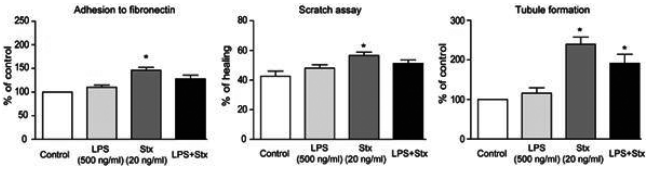

FIGURE 2 Angiogenic responses of EPC. n=4-5, *p<0.05 vs control, ANOVA

was significantly augmented by Stx on LPS-sensitized EPC (flow cytometry, Fig.1). Surprisingly and opposite to its well-known toxic effect on mature EC, Stx alone triggered EPC angiogenic functions like adhesion (pNPP assay), self-repair abilities (scratch assay) and new vessel formation (matrigel), while LPS seems to have no effect (Fig.2).

Conclusions: Our results showed that EPC not only were more resistant to Stx-mediated toxicity than mature EC, but also displayed improved angiogenic abilities, suggesting that EPC could be considered a therapeutic target to promote repair in HUS.

PB 2422 | Osteoprotegerin Promotes Tumour Development by Increasing Neovascularization

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Background: OPG is involved in ischemic tissue neovascularization through the secretion of SDF-1 by pretreated-OPG endothelial colony-forming cells (ECFCs).

Aims: As the vascularization is one of the key factor influencing the tumour growth and cancer cell dissemination, we investigated whether OPG was able to modulate the invasion of human MNNG-HOS osteosarcoma and DU145 prostate cancer cell lines *in vitro* and *in vivo*.

Methods: Cell motility was analysed *in vitro* by using Boyden chambers. Human GFP-labelled MMNG-HOS cells were inoculated in immunodeficient mice and the tumour nodules formed were then injected with OPG and/or FGF-2, AMD3100 or 0.9% NaCl (control group). Tumour growth was manually followed and angiogenesis was assessed by immunohistochemistry

Results: .*In vitro*, SDF-1 released by OPG-pretreated ECFCs markedly attracted both MNNG-HOS and DU145 cells and induced spontaneous migration of cancer cells. *In vivo*, tumour volumes were significantly increased in OPG-treated group compared to the control group and OPG potentiated the effect of FGF-2. Concomitantly, OPG alone or combined with FGF-2 increased the number of new vasculature compared to the control group. Interestingly AMD3100, an inhibitor of SDF-1, prevented the *in vivo* effects of OPG induced by SDF-1.

Conclusions: This study provides experimental evidence that OPG promotes tumour development trough SDF-1/CXCR4 axis

PB 2423 | Platelet Endothelial Aggregation Receptor-1 (PEAR1) Modulates Neoangiogenesis Via the Notch Pathway

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Background: Tissue ischemia triggers the formation of new blood vessels from preexisting ones, a process called *sprouting angiogenesis*. This process involves endothelial cell (EC) migration, proliferation and maturation, which are tightly controlled by the Notch pathway. Recently, PEAR1 was found to modulate sprouting angiogenesis via expression regulation of PTEN, a negative regulator of EC proliferation. The molecular link between PEAR1 and PTEN is unknown, but in ECs the expression of PTEN is controlled by Notch. We hypothesized that PEAR1 indirectly influences the expression of PTEN via interaction with the Notch pathway.

Aims: This study aims to demonstrate that PEAR1 controls the expression of PTEN indirectly via the Notch pathway.

Methods: Using lentiviral vectors, PEAR1 knockdown/overexpression was introduced in human umbilical vein ECs. Next, we investigated the effect of PEAR1 knockdown/overexpression on the mRNA levels of different Notch pathway genes (HES1, HEY1, Notch1, DLL4, JAG1) and PTEN. We validated the DLL4 expression during wound healing in *Pear1*$^{-/-}$ mice with western blot.

Results: PEAR1 knockdown resulted in reduced expression of Notch1, HEY1 and mainly DLL4 (Figure 1A), genes that were less affected after PEAR1 overexpression (Figure 1B). Interestingly, the expression of PTEN followed the expression of PEAR1 both in the knockdown and overexpressing ECs (Figure 1). Moreover, during wound healing *Pear1*$^{-/-}$ mice had lower DLL4 protein levels (corrected for CD31) in the forming granuloma compared to wild type mice.

Conclusions: We identified a new link between PEAR1 and DLL4 expression in EC cultures and in a wound healing model. Further research is needed to demonstrate if expression regulation of PTEN, via the interaction of DLL4 with its receptor (Notch), is PEAR1 dependant.

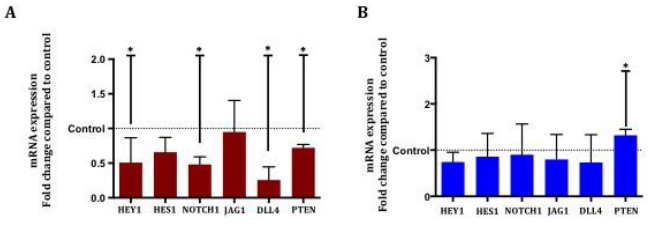

FIGURE 1 Relative mRNA expression in PEAR1 (A) knockdown and (B) overexpressing endothelial cells compared to control, for the indicated genes.

PB 2424 | Angiostatin Demonstrates Differential Hypoxic-dependent Anti-angiogenic Effects on Cardiac Endothelial Cells Derived from Non-vs Type 2 Diabetics

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Background: Angiostatin is a platelet-generated anti-angiogenic factor. During hypoxia, angiostatin inhibits endothelial cell (EC) matrix metalloproteinase (MMP)-2 expression and EC migration, an important early angiogenic step. Angiostatin may also impair angiogenesis by inhibiting endothelial nitric oxide synthase (eNOS) expression causing reduced NO biosynthesis and EC survival, as NO-signaling has been reported to protect ECs from apoptosis/necrosis. Angiostatin has been reported to be elevated in Type II diabetics (T2D) and NO-signaling to be reduced by T2D ECs. However, little is known about the effects of angiostatin on T2D endothelial cells during hypoxia.

Aims: To investigate the effects of angiostatin and hypoxia on cell death and the expression of angiogenic mediators by control and T2D ECs. To investigate the effects of angiostatin on angiogenesis in an *in vivo* hind limb ischemia model.

Methods: Human cardiac microvascular ECs (HMVEC-C) from non- and Type II diabetics were treated with 600nM angiostatin and incubated under hypoxic conditions. MMP-2 and eNOS levels were determined by immunoblot. HMVEC-C apoptosis/necrosis was measured via flow cytometry. Transgenic eNOS-GFP mice were injected with angiostatin (30μg) and underwent femoral artery ligation.

Results: Compared to control, angiostatin reduced MMP-2 (38±9% reduction) and eNOS (62±18% reduction) expression by hypoxic non-T2D ECs. Additionally, angiostatin induced necrosis of hypoxic T2D ECs, but not non-T2D ECs (6.98±2.1% vs non-T2D 3.03±0.3%). Administration of angiostatin reduced blood flow recovery to the ischemic limb on day 14 (30±0.08% reduction vs PBS control) along with reduced eNOS expression.

Conclusions: Our preliminary data suggests that during hypoxia angiostatin inhibits expression of angiogenesis mediating proteins by non-T2D ECs; whereas, it induces necrosis of T2D ECs. Moreover, elevated angiostatin levels reduce angiogenesis in hypoxic/ischemic tissues *in vivo*.

PB 2425 | Structural and Functional Study of Hypochlorous Acid Modified Human Antithrombin: Possible Role in Cancer

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Background: Antithrombin (AT) is involved in many critical bio-processes like blood coagulation and inflammation. Native AT is non-antiangiogenic, however, cleaved and latent conformers of AT have been shown

to have antiangiogenic and hence, anticancer property. Any alteration in their structures and functions may cause cancer development/progression. During respiratory burst, activated phagocytes initiate the production of hypochlorous acid (HOCl) which reacts with a wide variety of bio-molecules including proteins. Excessive production of HOCl can cause tissue damage which may progress to diseases like cancer.

Aims: Effects of HOCl on structures and functions of various conformers of AT.

Methods: The purified native AT was used to make cleaved and latent forms. Various concentrations of HOCl were used to modify AT and structural/functional alterations were detected by absorbance, fluorescence and circular dichroism (CD)–spectroscopy; transmission electron microscopy (TEM), polyacrylamide gel electrophoresis (PAGE) and factor Xa inhibition assay.

Results: In PAGE, the modified native AT showed polymer formation and fragmentation, while only fragmentation took place in cleaved and latent conformers. Polymerization was further confirmed by TEM. Compared to unmodified AT, modified counterparts exhibited hyperchromicities at 278 nm. At higher concentrations of HOCl, AT showed fragmentation. A significant gradual decrease in fluorescence intensity of native AT was seen at 340 nm with increasing concentration of HOCl, while cleaved and latent conformers showed first increase and then gradual decrease in intensity. Far-UV CD results, in all the cases, showed alterations in secondary structures of AT after modification. Inhibitory activity of modified AT against protease was reduced at a concentration of HOCl that didn't initiate polymerization or fragmentation.

Conclusions: The results showed profound effects of HOCl on various conformers of AT that may have implications in cancer.

PB 2426 | Frequency of Post Thrombotic Syndrome (PTS) in Patients with Proximal Deep Venous Thrombosis (DVT) - A Prospective Observational Study from a Tertiary Care Hospital in North India

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Background: The post thrombotic syndrome (PTS) is estimated to develop within 1 to 2 years in 20% to 50% of patients of DVT.

Aims: To investigate the frequency of PTS and the clinical indicators of PTS.

Methods: In a prospective, observational studystudy,adult adult patients with DVT of the proximal veins of lower limbs (Iliac, femoral and popliteal veins) presenting to PGIMER over 10 years (January 2005 to June 2016) who had been anticoagulated for at least six months were enrolled.Post-thrombotic syndrome (PTS) was defined in any patient with DVT with the pain and swelling of a chronic duration (occurring daily for at least 1 month) occurring 6 months or more after an episode of DVT.Patients were evaluated for symptoms and signs of PTS by using CEAP clinical scale and Villalta's scale at 0, 3 and 6 months.

Results: 210 patients (230 limbs) were enrolled in our study.198(210 limbs) and 150(162 limbs) patients were re-evaluated at 3 and 6 months.The frequency of PTS at 0, 3 and 6 months was 80.1%, 73.6% and 62.7% (CEAP clinical scale) and 51.9%, 23.5% and 25.6%(Villalta's scale).Common signs and symptoms observed among patients with PTS were pain (89.3%), cramps (35.6%), heaviness (66.8%), pruritus (22.5%), edema (80%), induration (50.4%), hyperpigmentation (35.4%), telengectesias (48.2%). Active ulceration was seen in 15 limbs (7.1%) and varicose veins were present in 35 limbs (16.6%) at enrollment. It was observed that the above signs and symptoms of PTS decreased with time with lower frequencies observed at the 3 and 6 months follow-up.Previous history of recurrent ipsilateral DVT, a BMI OF > 25 kg/m^2 ,right sided DVT at enrollment were the predisposing factors significantly associated with significantly higher frequency of PTS.

Conclusions: We observed a difference between CEAP clinical scale and Villalta's scale in diagnosing and categorizing PTS. The question is which one is better will require a gold standard against which both could be compared.

PB 2428 | Intracellular von Willebrand Factor Predominately Influences Angiogenesis

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Background: Bleeding associated with angiodysplasia is a recognized complication in many patients with acquired and inherited abnormalities in von Willebrand factor (VWF). Deficiency of the largest VWF multimers has been suggested to play a role in the development of angiodysplasia but the mechanism has been unclear.

Aims: This study examines the impact of extracellular and intracellular VWF on angiogenesis using blood outgrowth endothelial cells (BOECs).

Methods: The study was approved by Queen's University REB and one healthy control gave consent for BOEC isolation. Following transfection of BOECs with Control siRNA (siCTL) or VWF siRNA (siVWF), knockdown efficiency was confirmed using confocal IF, qRT-PCR, ELISA, and western blot analysis. Recombinant VWF (rVWF; Vonvendi®) was proteolyzed by recombinant human ADAMTS13 to 75% and 50% of high molecular weight multimers (HMWM). siVWF cells were cultured in the absence or presence of 10 µg/mL of proteolyzed or unproteolyzed rVWF. Angiogenic profiles of BOECs were then characterized by migration, proliferation, and tubule formation.

Results: Relative to siCTL, siVWF inhibition of VWF gene expression (5%) and protein synthesis (0.05 ng/mg) persisted up to four days after transfection (Fig 1). Compared to siCTL BOECs, siVWF BOECs had significantly increased cell migration velocity (0.42

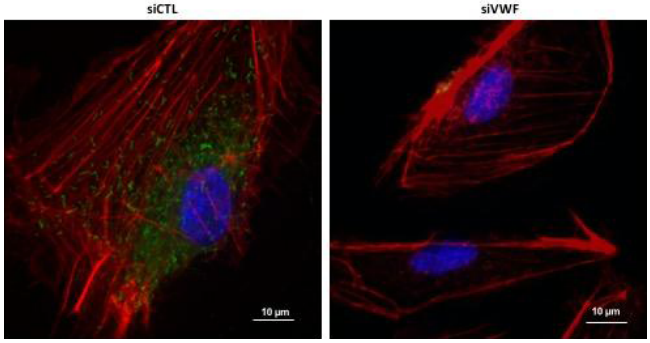

FIGURE 1 Confocal microscopy of siCTL or siVWF-treated BOECs stained for actin (red), VWF (green), and nuclei (blue)

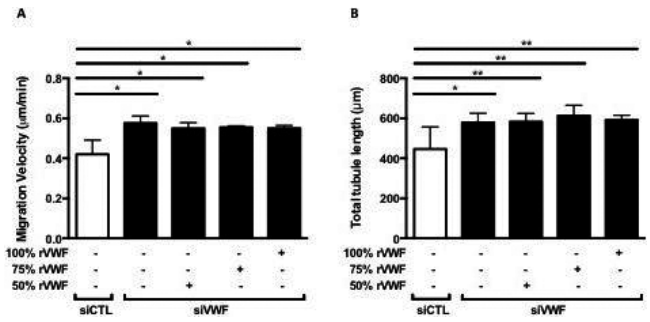

FIGURE 2 VWF-deficient BOECs display increased (A) cell migration velocity and (B) tubule formation. Error bars = mean ± SD. *P<0.05; **P<0.01

µm/min and 0.58 µm/min, respectively; P=0.04; Fig 2A), formed significantly more tubules in Matrigel (446 µm and 579 µm, respectively; P=0.04; Fig 2B), and were less proliferative (1511 cells and 1241 cells, respectively). The addition of unproteolyzed rVWF did not reverse the pro-angiogenic phenotype of siVWF BOECs. Notably, the addition of 75% and 50% HMWM rVWF to the culture media did not modify the angiogenic profile of siVWF BOECs (Fig 2).
Conclusions: Taken together, these findings highlight the importance of intracellular VWF in angiogenesis and suggest that in its absence, the multimer content of extracellular VWF does not appear to modulate the angiogenic capacity of the cell.

PB 2429 | Low Grade Inflammation Inhibits VEGF Induced HUVECs Migration in p53 Dependent Manner

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Background: In the course of studying crosstalk between inflammation and angiogenesis, high doses of pro-inflammatory factors have been reported to induce apoptosis in cells. Under normal circumstances also the pro-inflammatory cytokines are being released in low doses and are actively involved in cell signaling pathways.
Aims: Our aim is to study the effects of low grade inflammation in growth factor induced angiogenesis using tumor necrosis factor alfa (TNF*a*) and vascular endothelial growth factor A (VEGF) respectively.
Methods: Human umbilical vein endothelial cells (HUVECs) were cultured with EGM-2 media. Recombinant human TNF*a* and VEGF were used for the stimulation of HUVECs. Western blotting, ELISA assay, and immunostaining were performed as previously described. Wound healing assay and transmigration assay were used for quantifying migration of endothelial cells.
Results: We found that low dose of TNF*a* can inhibit VEGF induced angiogenesis in HUVECs. Low dose of TNF*a* induces mild upregulation and moreover nuclear localization of tumor suppressor protein 53 (P53) which causes decrease in inhibitor of DNA binding-1 (Id1) expression and shuttling to the cytoplasm. In absence of Id1, HUVECs fail to upregulate b_3-integrin and cell migration is decreased. Connecting low dose of TNF*a* induced p53 to b_3-integrin through Id1, we present additional link in cross talk between inflammation and angiogenesis.
Conclusions: Low dose TNF*a* and VEGF work in concert to upregulate P53 but the exact mechanism of how TNF*a* and VEGF together upregulate P53 has to be further studied.

PB 2430 | Oroxin A Effectively Inhibits Breast Cancer Cell-mediated Tumor Vasculogenic Mimicry

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Background: Breast cancer is a major cause of cancer death among women. Increasing evidence has shown that breast cancer cells possess vasculogenic ability and directly form tumor blood vessel through the mechanism of vasculogenic mimicry which favors tumor growth and metastasis. Thus, vasculogenic tumor cells are sensible target for ant-tumor vasculogenic drug discovery. Although various anti-tumor angiogenic approaches have been used in the clinics to treat the disease these years, most current conventional treatment options are not well-effected and not effective to tumor cell-mediated vasculogenic mimicry.
Aims: Explored new anti-vasculogenic phytochemical derived from traditional Chinese medicinal herbs to cure breast cancer with vasculogenic ability and directly form tumor blood vessel, then figure out the mechanisms.
Methods: Using human breast cancer MDA-MB-231cells as a model, we detected the effect of Oroxin A by cell adhesion, migration, invasion, and tube-like formation in a concentration-dependent manner, followed by mRNA and protein detect to reveal the molecular mechanisms.
Results: We found that Oroxin A, an active component from the herb Oroxylum indicum, suppressed breast cancer cell-mediated vasculogenic mimicry through inhibiting breast cancer cell adhesion, migration, invasion, and tube-like formation in a concentration-dependent

manner. Mechanistic studies revealed that Oroxin A induced tumor cell mitotic catastrophe and senescence through inducing a tumor suppressive ER stress and up-regulation of P21 gene, resulting in cell cycle arrest at the G2/M transition and vasculogenic inhibition.

Conclusions: Taken together, Oroxin A effectively suppresses breast cancer cell-mediated tumor vasculogenic mimicry with low toxicity, suggesting that OA is a potential new anti-tumor neovascularization and anti-breast cancer drug candidate.

PB 2431 | Acidic preconditioning improves endothelial progenitor cell survival and angiogenesis under proinflammatory conditions and post-ischemic tissue regeneration in a murine model of type II diabetes

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Background: We have previously demonstrated that acidic preconditioning exacerbates angiogenic responses of human late outgrowth endothelial colony forming cells (ECFC).

Aims: We now aimed to analyze whether this strategy also improves ECFC survival and functionality in the presence of damage associated molecular patterns (DAMPs) highly augmented in ischemic milieu or in diabetes, where tissue regeneration is compromised.

Methods: Cord blood-derived ECFC in EGM2 were exposed at pH 6.6 for 6 h (preconditioned) or pH 7.4 (control) and then medium was replaced by fresh EGM2 at pH 7.4. N=4-6, *p< 0.05 vs pH 7.4, one-way ANOVA.

Results: Nuclear morphology analysis showed that several DAMPs like MSU crystals (150 mM), TNFα (50 ng/mL), histones (3 μM) or their combination induced ECFC death at pH 7.4 (30±4, 25±3, 24±3 or 52±5% of cell death), whereas a significantly lower effect was observed in preconditioned ECFC (15±3*, 5±3*, 7±2* or 18±6*%). TNFα triggered ECFC apoptosis in a concentration-dependent manner and was potentiated by high glucose, but this effect was significantly prevented by acidic preconditioning (Fig.1). When non-toxic concentrations were used, high glucose, TNFα or their combination reduced proliferation (cell count), SDF1-driven migration (transwells) and tubule formation (matrigel), whereas almost no inhibition was observed in preconditioned ECFC (Fig.1). In a murine model of type II diabetes, blood flow recovery (Doppler) after induction of hind limb ischemia was significantly improved by transplantation of preconditioned ECFC (0.79±0.04* perfusion index at 14 d post-ischemia), but not control ones (0.66±0.07), when compared with PBS-treated group (0.55±0.07).

Conclusions: Preconditioned ECFC showed improved survival and angiogenic activity in the presence of DAMPs and efficiently restored blood flow after ischemia in diabetic mice, suggesting that acidic preconditioning is an effective strategy to improve tissue regeneration despite the stressful conditions associated with inflammation and diabetes.

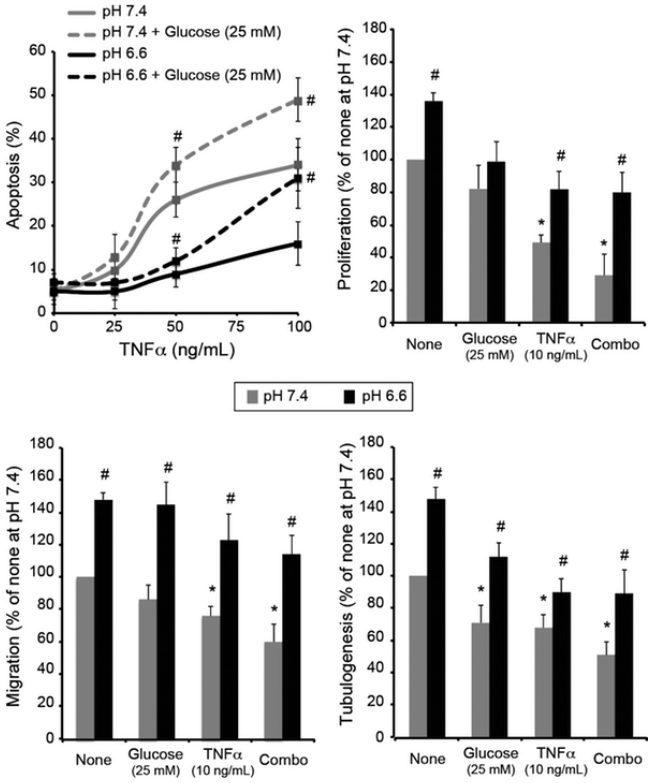

FIGURE 1 Preconditioned ECFC are more resistant to harmful effect of TNFα and high glucose. *p<0.05 vs none at pH 7.4, #p<0.05 vs same condition at 7.4

PB 2432 | Angiopoietin-2 Reduces Arteriogenesis Associated with the Suppression of the Infiltration of Macrophages in Mouse Ischemia Hindlimb Model

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Background: Angiopoietin-2 (Ang-2), a ligand of the Tie-2 receptor, plays an important role in maintaining endothelial cells and in destabilizing blood vessels. Collateral artery growth (arteriogenesis) is a key adaptive response to arterial occlusion.

Aims: It is unknown whether the destabilization of blood vessels by Ang-2 can affect arteriogenesis and modulate mononuclear cell function. This study aimed to investigate the effects of Ang-2 on collateral artery growth.

Methods: Hindlimb ischaemia model was produced in C57BL/6 mice by femoral artery ligation. Blood flow perfusion was measured using a Laser Doppler Perfusion Imager Quantitative RT-PCR analysis was applied to identify the level of angiogenic factors.

Results: After the induction of hindlimb ischaemia, blood flow recovery was impaired in mice treated with recombinant Ang-2 protein; this was accompanied by a reduction of peri-collateral macrophage infiltration. In addition, quantitative RT-PCR analysis revealed that Ang-2 treatment decreased monocyte chemotactic protein-1 (MCP-1), platelet-derived growth factor-BB (PDGF-BB) mRNA levels in

ischaemic adductor muscles. Ang-2 can lead to macrophage M1/M2 polarization shift inhibition in the ischaemic muscles. Furthermore, Ang-2 reduced the in vitro inflammatory response in macrophages and vascular cells involved in arteriogenesis.

Conclusions: Our results demonstrate that Ang-2 is essential for efficient arteriogenesis, which controls macrophage infiltration.

PB 2433 | Role of Circulating Endothelial Progenitor Cells at the Early-stage of Neovascularization in Post-menopausal Luminal-A-Type Breast-cancer Patients

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Background: Malignant diseases are the second, after heart disease, cause of morbidity and mortality worldwide.

Aims: The aim of the study has been to evaluate the number of circulating endothelial progenitor cells (CEPCs) in the blood of patients diagnosed with luminal A type breast cancer and to make an attempt at identifying associations with the number of CEPCs and selected clinicopathological factors: histological grading, hormonal status, the lump size and parity.

Methods: The study involved 74 Caucasian post-menopausal women. Forty-four women aged 48-65 (mean age of 56) with luminal A type breast cancer without distant metastasis (M0). The control group consisted of 30 healthy, non-smoking, post-menopausal women. The exclusion criteria for all the subjects were hypertension, hyperlipidaemia, hyperglycaemia, acute and chronic infection. In the whole blood samples the number of circulating endothelial progenitors was determined using flow cytometry. During the analysis the fluorescence of 100.000 cells was measured. CEPCs were identified with immunophenotype CD45-, CD31+, CD34+, CD133+.

Results: There was recorded a significantly higher number of circulating EPCs in the study group, as compared to the controls. Higher numbers of circulating endothelial progenitors were noted in the patients with luminal A-type breast cancer localised in the left breast. Additionally, there was identified a positive correlation between CEPCs and the age as well as between CEPCs and parity, as well as a negative correlation between CEPCs and the diameter of the lump as well as between CEPCs and histological breast cancer grading among luminal-A-type breast-cancer patients.

Conclusions: Based on the clinical and molecular determinants (histological grading and lump diameter), we can assume that circulating EPCs are necessary at the early-stage of breast cancer development and they can be used as a non-invasive biomarker to monitor the clinical state of patients. However, a more further study is required to confirm this thesis.

PB 2434 | Pro-Angiogenic Biomarkers are Upregulated in Patients with Advanced Osteoarthritis Undergoing Orthopedic Surgery

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Background: Angiogenesis is a central component of wound healing following surgery and may also contribute to the pathogenesis of degenerative joint disease (DJD) due to osteoarthritis (OA). OA may result in the upregulation of pro-angiogenic biomarkers from the vascular system.

Aims: We sought to examine pro- and anti-angiogenic biomarkers in patients with DJD due to OA undergoing orthopedic surgical repair.

Methods: We prospectively enrolled patients (n=25) undergoing elective total knee (TKA) or total hip (THA) arthroplasty secondary to end-stage DJD from OA. Matched healthy controls (n=48) free from DJD were included. Endogenous pro-angiogenic biomarkers (vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), and IL-8) and two anti-angiogenic factors (IFNg and IL-4) were measured using a high sensitivity biochip method.

Results: At baseline, patients with DJD had significantly higher levels of VEGF (10.5±1.2 pg/mL vs. 4.8±0.2 pg/mL, p< 0.001), MCP-1 (130.6±7.7 pg/mL vs. 88.6±3.9 pg/mL, p< 0.0001), and IL-8 (4.0±0.5 pg/mL vs. 2.6±0.1 pg/mL, p< 0.05). In patients with OA, baseline MCP-1 levels also significantly correlated with age (r^2=0.30, p=0.005). Compared to baseline values, in patients with OA, post-operative levels of VEGF (10.5±1.2 pg/mL vs. 18.8±1.5 pg/mL, p< 0.001) and MCP-1 (130.6±7.7 pg/mL vs. 153.1±11.5 pg/mL, p< 0.05) were significantly increased, with a trend towards increased IL-8 as well (4.0±0.5 pg/mL vs. 4.7±0.6 pg/mL, p=0.051). MCP-1 levels post-operatively also correlated with age in patients with OA (r^2=0.28, p=0.006).

Conclusions: In conclusion, systemic levels of the pro-angiogenic biomarkers were increased in patients with DJD at baseline compared to healthy controls and rose post-operatively while the anti-angiogenic factors IFNg and IL-4 remained unchanged. Baseline MCP-1 levels correlated positively and significantly with age, suggesting an interaction between aging and the pathophysiology of OA.

PB 2435 | VEGF-induced Angiogenesis Requires Autophagy

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Background: Endothelial cell functionality and integrity depends on metabolic homeostasis including the elimination of dysfunctional

organelles and proteins. Autophagy is a lysosomal degradation pathway known to regulate homeostasis in different cell types. However, its role in endothelial cells is incompletely understood.

Aims: This study was aimed at identifying the implication of autophagy in regulating endothelial functions and angiogenesis.

Methods: Experiments were performed in human umbilical vein endothelial cells with or without genetic or pharmacological impairment of autophagy (siRNA against ULK1 and Beclin1 or bafilomycin A1, respectively). Parameters of cellular stress (cytokine release, GSH levels) and function (proliferation, survival, *in vitro* angiogenesis) were monitored.

Results: Inhibition of autophagy by ULK1/Beclin1-siRNA led to cytokine release, GSH depletion and reduced basal proliferation and survival indicating the contribution of autophagy to endothelial homeostasis. Endothelial cells treated with ULK1/Beclin1-siRNA maintained sensitivity to vascular endothelial growth factor (VEGF) with respect to signalling, proliferation and survival but showed decreased angiogenic sprouting. The angiogenic response was also reduced upon a mild inhibition of autophagy with bafilomycin A1, which did not affect cell survival. These data suggest a specific role of autophagy in regulating angiogenesis in addition to its homeostatic function, which is currently being unravelled. VEGF itself was able to initiate autophagy via a pathway involving AMPKα1-mediated phosphorylation of ULK1 underlining the necessity of functional autophagy for an angiogenic response.

Conclusions: Autophagy has an important homeostatic function in endothelial cells by suppressing inflammatory and oxidative stress. It is activated by VEGF, an important angiogenic growth factor, via an AMPKα1-dependent pathway, and may promote angiogenesis by maintaining endothelial functionality and affecting angiogenic pathways.

PB 2436 | G-protein Coupled Receptor 15 Mediates Angiogenesis and Cytoprotective Function of Thrombomodulin

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Background: Thrombomodulin (TM) stimulates angiogenesis and protects vascular endothelial cells (ECs) via its fifth epidermal growth factor-like region (TME5); however, the cell surface receptor that mediates the pro-survival signaling activated by TM has remained unknown.

Aims: We aimed to identify the binding partner of TM expressed on the cell surface of ECs.

Methods: Immunoprecipitation followed by MALDI-TOF MS analysis was employed to identify the cell surface receptor for TME5. Bromodeoxyuridine incorporation and apoptosis assays with annexin V staining were used to assess the effect of TME5 on proliferation and apoptosis in vascular ECs.

Results: We identified G-protein coupled receptor 15 (GPR15) as a binding partner of TME5. TME5 rescued growth inhibition and apoptosis caused by calcineurin inhibitor FK506 in vascular ECs isolated from C57BL/6 wild type (WT) mice. On the other hand, TME5 failed to protect ECs isolated from GPR15 knockout (GPR15 KO) mice from FK506-caused vascular injury. In addition, in vivo Matrigel plug angiogenesis assay found that TME5 stimulated angiogenesis in WT mice but not in GPR15 KO mice.

Conclusions: GPR15 plays an important role in mediating cytoprotective function as well as angiogenesis of TM.

PB 2437 | CCL5 and CXCL4 Are Rapidly Internalized in Endothelial Cells

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Background: Platelet-endothelial communication is essential in the recruitment of lymphocytes to the vessel wall in thrombosis and haemostasis. Chemokines are released from the alpha granules by activated platelets and are deposited on the endothelium, facilitating subsequent leukocyte arrest. The chemokine CCL5 is essential in the recruitment of monocytes, while CXCL4 by heterophilic interactions increases the monocyte arrest-promoting properties of CCL5.

Aims: To further elucidate the role of CCL5 and CXCL4 in their leukocyte arresting properties, we investigated internalization and localization of extracellular CCL5 and CXCL4 to the endothelial cell.

Methods: HUVECs and EA.hy926 cells were incubated with recombinant human CCL5 or CXCL4 for 1 up to 120 minutes. Cells were stained against CCL5/CXCL4, DAPI and f-actin, and analysed with light-, confocal- or stimulated emission depletion (STED) microscopy. To quantify internalization cell lysates were fractionalized using ultracentrifugation and analysed by ELISA.

Results: Both CCL5 and CXCL4 are rapidly internalized in endothelial cells, whereas CXCL4 is partly presented on the cell surface, all CCL5 is internalized. The chemokines are endocytosed by a process dependent on dynamin and clathrin, as internalization was inhibited by inhibitors of these processes. Cell surface proteoglycans have a less definite role in the internalization process, as enzymatic cleavage of heparin sulphate and chondroitin sulphate did not result in a decreased internalization of CCL5 and CXCL4. Coincubation of CCL5 and CXCL4 did not influence the internalization or the localization of either of the chemokines. Localization studies by confocal and super-resolution microscopy suggested that both CCL5 and CXCL4 partly have a nuclear localization which, in some cells, seem to be confined to the nucleoli.

Conclusions: Endothelial cells rapidly internalize CCL5 and CXCL4 by clathrin and dynamin dependent endocytosis, where the chemokines seem to localize diffusely through the cytosol and nucleus.

PB 2438 | Biased Activation of PAR1: Dissecting Signal Transduction Networks that Regulate the Endothelial Barrier Function

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Background: The barrier integrity of endothelial cells (ECs) is tightly regulated. Two key players in this process are Protease Activated Receptor 1 (PAR1) and sphingosine-1-phosphate receptor 1 (S1PR1). Activation of S1PR1 leads to a barrier-protective response in ECs, while activation of PAR1 results in either a barrier-disruptive (thrombin) or a barrier-protective response (Activated Protein C (APC)). This so-called biased activation has been attributed to differential cleavage of the PAR1 N-terminus. It remains unknown how ECs transmit these external signals and how these translate into a coordinated response.
Aims: Dissect barrier-disruptive and barrier-protective phosphoregulation in ECs and unravel biased activation of PAR1 using quantitative phosphoproteomics.
Methods: Blood outgrowth endothelial cells were metabolically labeled using SILAC and stimulated for 2/10 min with thrombin (1U/ml), APC (20nM, in presence of 1U/ml hirudin), S1PR1 agonist SEW2871 (5μM) or TL-peptides that resemble PAR1 cleavage by Thrombin (SFLLRN-NH2), APC (NPNDKYEPF-NH2), Matrix Metalloprotease-1 (PRSFFLRN-NH2), Neutrophil Protease 3 (TLDPRSF-NH2) or Neutrophil Elastase (RNPNDKYEPF-NH2) (50μM). Phosphorylated peptides were enriched using TiO_2 precipitation, detected by Orbitrap Fusion MS and analyzed using MaxQuant software.
Results: We identified and reliably quantified >4,000 phosphosites of which ± 10% were regulated. Unlike thrombin and the canonical PAR1 TL-peptide, APC and all 4 non-canonical PAR1 TL-peptides failed to induce extensive regulation at the phosphorylation level. In contrast to barrier-disruption by thrombin, barrier-protection by SEW2871 was accompanied by limited, but distinct phosphoregulation.
Conclusions: These data indicate that only canonical PAR1 cleavage by thrombin generates a tethered ligand that potently induces signaling. In addition, in ECs barrier disruption is accompanied by extensive phosphoregulation, while this seems to play a less prominent role in protection of the endothelial barrier.

PB 2440 | Major Endothelial Cell Integrin, Alphavbeta3 as a Target in Gram-negative Sepsis Caused by Urinary Tract Infection

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Background: Urinary tract infections caused by *Escherichia coli* are one of the most common predisposing factors for sepsis and serves as the initial manifestation of the multisystem organ failure syndrome. The vascular endothelium is a major target of sepsis-induced events and endothelial activation accounts for much of the pathology of sepsis.
Aims: The aim of this study is to identify the mechanism by which *E. coli* binds to the endothelium and exerts its injurious effects.
Methods: Human endothelial cells were sheared at physiological rates. Fluorescently labelled *E. coli* were used to assess binding. Changes in vascular permeability were measured by transwell paracellular permeation and apoptosis was assessed by flow cytometry.
Results: *E. coli* from sepsis patients attached to endothelial cells under static (P< 0.01) and hemodynamic shear (P< 0.01) conditions. *E. coli* attachment resulted in disturbances in endothelial barrier integrity, as determined by loss of tight junction protein staining (VE-cadherin), permeability changes (P< 0.01) and apoptosis (P< 0.05). Bioinformatic analysis of the *E. coli* genome identified an interesting protein called outer membrane protein A (ompA) which contains an integrin recognition motif, (R)GD. A strain deficient in expression of ompA significantly reduced binding to endothelial cells under both static (P< 0.05) and shear (P< 0.05) conditions. Complementing the deficient strain with the *ompA* gene recovered binding back to wildtype levels (P=NS). αVβ3 is a major RGD dependent integrin expressed on the surface of human endothelial cells. Low concentrations of an αVβ3 specific peptide (5nM), significantly reduced *E. coli* binding to endothelial cells (P< 0.01), apoptosis (P< 0.05) and loss of barrier integrity P< 0.05) pre- and post-infection.
Conclusions: Inhibition of *E. coli* binding to endothelial cell αVβ3 prevents endothelial dysfunction and therefore presents a first in class novel drug candidate for prevention or treatment of sepsis following a UTI.

PB 2441 | Free Heme Triggers the Disruption of the Endothelial Barrier

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Background: Disruption of the endothelial barrier (EB) is observed during the inflammatory response to invading pathogens, and is thought to facilitate the access of leukocytes and phagocytes to infected or damaged tissues. Heme is an evolutionary conserved molecule present in organisms of all kingdoms, whose role as an activator of innate immunity has been recently demonstrated. Several conditions in which free heme escapes its natural scavenging systems such as sepsis and sickle cell disease are associated with increased microvascular permeability.

Aims: Here we investigated whether free heme could cause changes on EB function and morphology.

Methods: HUVEC were exposed to heme (30uM) or vehicle, and evaluated using an electric cell-substrate impedance sensing system (ECIS) that measures real-time changes in transendothelial electrical resistance (TEER), and by immunofluorescence (IF) of cell-cell junctions. The % of TEER decrease (which indicates the increase EB permeability) was monitored for at least 4 h. For IF, cells were incubated with heme for 30 minutes, and stained for VE-cadherin.

Results: Heme caused a significant increase in EB permeability that was evident as early as 30 minutes after stimulation (vehicle vs heme: at 30 minutes, 9.76±8.54 vs. 53.60±16.19, P= 0.014; at 1 hour, 8.77±6.82 vs. 38.59±6.84; P= 0.006), lasted 2 hours (at 2 hours, 9.47±10.10 vs. 33.07±3.59, P= 0.019), and was no longer significant after 4 hours (13.51±13.98 vs. 32.93±9.34, P= 0.116) and at later timepoints (Fig 1).

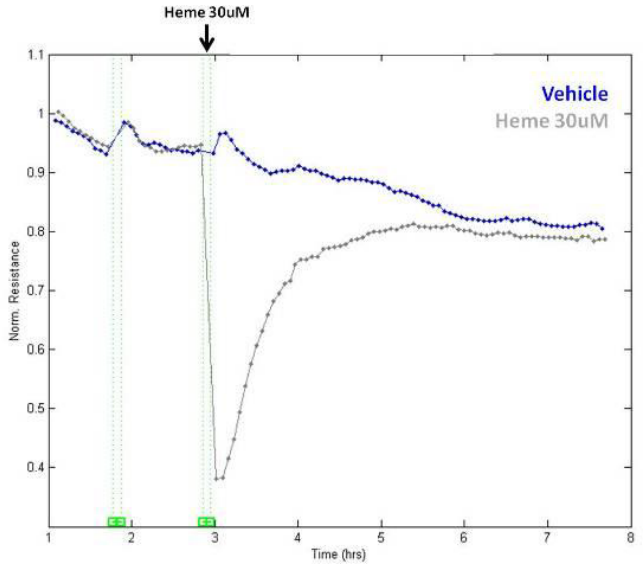

FIGURE 1 Heme stimulation decreases transendothelial electrical resistance (TEER)

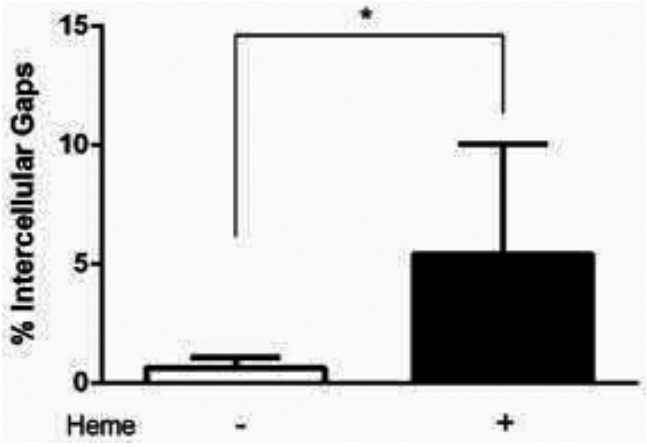

FIGURE 2 Heme induces disruption of adherens junction architecture leading an increase of Intercellular gaps (*P=0.05)

Consistent with these functional changes, the % of intercellular gaps in heme-treated cells was significantly higher (5.41%±4.639) when compared to vehicle-treated cells (0.64%±0.43; P= 0.05) (Fig 2).

Of note, higher levels of heme were observed in a cohort of patients with septic shock.

Conclusions: Heme induces a reversible increase of EB permeability which is associated with the disruption of the architecture of adherens junctions. Further studies are warranted to investigate the *in vivo* relevance of these findings.

PB 2442 | The Relevance Study of between miRNA Machinery Gene Polymorphisms (DICER rs3742330A>G, DROSHA rs10719T>C, RAN rs14035C>T, XPO5 rs11077A>C, DGCR8 rs417309G>A) and the Risk of Recurrent Implantation Failure (RIF) in Korean Women

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Background: Recurrent implantation failure (RIF) refers to unsuccessful implantation after recurrent transfers of morphologically good quality embryos into a normal uterus. The miRNAs are small non-coding RNA molecule containing about 22 nucleotides that found in plants, animals and some viruses. Also, the miRNAs are well known regulation of gene expression during implantation and crucial factor for successful pregnancy. In addition, the miRNA machinery gene polymorphisms were associated with implantation failure by regulation of miRNA biogenesis. So, we were selected miRNA five machinery gene polymorphisms that thought to be related implantation failure.

Aims: The aim of our study was associated with miRNA machinery gene polymorphisms (*DICER* rs3742330A>G, *DROSHA* rs10719T>C, *RAN* rs14035C>T, *XPO5* rs11077A>C, *DGCR8* rs417309G>A) in RIF risk.

Methods: We conducted a case-control study of 347 Korean women: 119 patients with at least two unexplained consecutive pregnancy losses and 228 controls with at least one live birth and no history of pregnancy loss. Genotyping was performed by polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) method.

Results: Consequently, the *DROSHA* rs10719TC type (AOR: 0.581, *P*=0.026) had significant association for decreasing RIF risk and dominant type (AOR: 0.641, *P*=0.051) shows tendency for decreasing RIF risk. The *RAN* rs14035CT type (AOR: 0.582, *P*=0.027) and dominant type (AOR: 0.606, *P*=0.034) had significant association for decreasing RIF risk. Also, the C-T (*DROSHA* rs10719/*RAN* rs14035; OR: 0.505, *P*=0.037) was decreased RIF risk in gene-gene interaction analysis and

the TC/CT (*DROSHA* rs10719/*RAN* rs14035; AOR: 0.326, *P*=0.004) was decreased RIF risk in combined genotype analysis.

Conclusions: In conclusion, our study demonstrated that *DROSHA* rs10719T>C and *RAN* rs14035C>T were associated with decreasing RIF risk.

PB 2443 | Plasma microRNA (miRNA) Profiling in Immune Thrombocytopenia (ITP) before and during Treatment with Thrombopoietin Receptor Agonists (TPO-RA)

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Background: Dysregulated expression of miRNAs has been associated with several autoimmune diseases. ITP is an autoimmune disease characterized by thrombocytopenia and increased bleeding risk. TPO-RA stimulate megakaryocyte differentiation and platelet production and are an effective treatment for ITP. The exact role of miRNAs in the pathogenesis of ITP and in relation to TPO-RA use has not been explored.

Aims: Determine the expression profile of circulating miRNAs in ITP patients before and during treatment with TPO-RA to identify miRNAs that might be associated with pathogenesis of ITP and help understand the molecular mechanisms of effect of TPO-RA.

Methods: Exiqon Serum/plasma Focus microRNA PCR panel was used to determine the expression profile of 179 miRNAs in plasma acquired from 8 ITP patients before and 2, 6 and 12 weeks after initiation of treatment with TPO-RA and 8 matched controls. Repeated measures one-way ANOVA was used to analyze sequential samples. Pathway analysis was performed using miRPath 3.0.

Results: Comparing the expression profiling before and after the initiation of TPO showed that 14 circulating miRNAs were significantly differentially expressed; eight of these were also significantly differentially expressed in controls compared to pre-treatment patients. Among those, miR-199a-5p was up-regulated during treatment with TPO, while 423-5p and miR-590-5p were down-regulated. miR-195-5p, previously shown to be associated with thrombosis, was not among the differentially expressed in ITP compared to controls but increased after treatment. Results from pathway analysis are shown in Table 1.

Conclusions: We identified several miRNAs that were differentially expressed in ITP patients before and during treatment with TPO. Further validation of these miRNAs in a larger patient cohort with defined clinical outcomes will clarify their potential as biomarkers. The role of these miRNAs in ITP pathophysiology deserves additional study given the potential biological pathways that they may regulate.

PB 2444 | The Role of miR-143 in Collateral Vessel Growth

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Background: Arteriogenesis denotes the growth of collateral arteries from existing small vessels, thereby bypassing major arterial obstructions. Several microRNAs (miRs) were shown to be highly expressed in growing collaterals including miR-143. Accordingly, knockdown of miR-143 inhibited the growth of collateral arteries in mice. Furthermore, the expression of collagen type V alpha 2 chain (COLVA2), the predicted target gene of miR-143, was downregulated in growing collaterals.

Aims: We aimed to examine the role of miR-143 in vascular cell proliferation and to prove that COLVA2 is a direct target of miR-143 in arteriogenesis.

Methods: Cell proliferation was measured by using the BrdU Cell Proliferation ELISA assay.

To confirm the direct interaction between miR-143 and COLVA2 mRNA, a Dual Luciferase Reporter Assay was performed by cloning

TABLE 1 miRPath 3.0 GO analysis with subcategories of biological process and Tarbase v7.0

GO Category	p-value	#genes	#miRNAs	miRNAs
Transcription initiation from RNA polymerase II promoter	<1E-325	60	3	miR-423-5p, miR-195-5p, miR-33a-5p
Immune system process	<1E-325	350	5	miR-199a-5p,miR-423-5p, miR-195-5p, miR-221-3p, miR-141-3p
Transcription, DNA-templated	<1E-325	528	5	miR-33a-5p,miR-423-5p, miR-92b-3p, miR-221-3p, miR-141-3p
Blood coagulation	<1E-325	147	5	miR-195-5p,miR-423-5p, miR-92b-3p, miR-221-3p, miR-141-3p
Fibroblast growth factor receptor signaling pathway	<1E-325	68	5	miR-195-5p,miR-423-5p, miR-33a-5p, miR-221-3p, miR-141-3p

the COLVA2-3'UTR-sequence into the miR-specific plasmid vector psiCHECK™-2.

Results: The transfection with anti-miR-143 (but not with a scrambled anti-miRNA) in murine vascular smooth muscle cells (MVSMC) or NIH/3T3-fibroblasts, which both express high amounts of miR-143, resulted in downregulation of miR-143 expression, and decreased cell proliferation, which became significant after 72 h. The cotransfection of the plasmid carrying the COLVA2-3'UTR-sequence together with anti-miR-143 (but not with scrambled anti-miRNA) in MVSMC or NIH/3T3 cells induced the knockdown of miR143 and upregulated the luciferase expression after 48 h. These data indicate that miR-143 modulates COLVA2 expression directly by targeting the 3´UTR-region of the COLVA2 mRNA.

Conclusions: These findings identify miR-143 as a direct regulator of the COLVA2 expression. Furthermore miR-143 induces vascular cell proliferation and collateral vessel growth. Data will be helpful to develop effective strategies for the treatment of vascular diseases.

PB 2445 | Circulating miRNA-24 as a Potential Biomarker for Coronary Heart Disease in Type 2 Diabetes Mellitus Patients

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Background: Type 2 diabetes mellitus (DM2) is associated with cardiovascular complications and is characterized by high levels of YKL-40, an inflammatory glycoprotein involved in endothelial dysfunction.

Aims: We investigated the predictive potential of circulating miR-24 in DM2 without chronic complications, DM2 patients with coronary heart diseases (CHD), and control subjects.

Methods: Blood samples were taken from 94 subjects of both genders, and divided over three groups as follows; patients with CHD, patients with DM2 and CHD, and control subjects. Both miR-24 (using real time PCR) and routine parameters were measured.

Results: Using bioinformatic analysis, we found that miR-24 has high complementarity and a high degree of species conservation with respect to the binding sites within the 3' UTR of the YKL-40 mRNA. The expression levels of circulating miR-24, determined by quantitative real time PCR, were significantly decreased in peripheral blood of DM2-CHD and CHD patients compared with controls. Furthermore, MiR-24 strongly associated with DM2-CHD, negatively correlated with YKL-40 in DM2-CHD and DM2 patients after conducting multiple regression analysis.

Conclusions: These results provide a novel regulatory mechanism of circulating miR-24 in regulating YKL-40 levels in DM2-CHD, may serve as a biomarker for predicting patients with DM2 with CHD.

PB 2446 | Circulating Extracellular Vesicles microRNA Profile in Transfusion Dependent β-Thalassemia

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Background: Circulating miRNAs are small non-coding RNAs, encased in extracellular vesicles (EVs), which regulate the expression of multiple genes. Circulating miRNAs emerged as promising disease biomarkers for diagnostic and therapeutic purposes in hematological diseases. In transfusion dependent β-thalassemia (TDT) a life-threatening anemia with multi-organ complications little is known about the features of circulating miRNAs.

Aims: Evaluation of EV-miRNA profile in TDT patients according to their clinical characteristics and spleen status and in comparison with EV-miRNAs from healthy controls (HC).

Methods: 32 TDT patients and 15 HC were included in the study. The TDT patients were divided into 3 groups:
(1) non-splenectomized,
(2) splenectomized (SP) and
(3) hypersplenic (HS).
Blood samples were collected prior to blood transfusions, in EDTA tubes. Total RNA was isolated from EV pellets using miRNeasy isolation kit (Qiagen). EV-miRNA samples from the SP, HS and HC study groups were screened for the expression level of 800 miRNAs by Nanostring and validated by quantitative real-time PCR (RT-qPCR).

Results: The expression of 30 miRNA was found to be higher (>150%) in both patient groups compared to HC; the expression of 14 miRNAs was found to be lower (< 50%) in SP and HS patients compared to HC. RT-qPCR confirmed the different expression of 6 miRNAs in TDT patients compared to HC. High levels of miR-155-5p, miR-195-5p, miR-144-3p, miR-210, miR-376a-3p, and miR-378a were evident. In patients with hypersplenism the miRNAs miR-451a, miR-144-3p and miR-210 were particularly elevated. In SP patients the miR-376-c was increased.

Conclusions: Important differences in EV-miRNA profile were found between patients and HC and between different patient groups. Increased levels of miRNAs are probably involved in erythropoiesis in patients with hypersplenism. The identified alterations may represent potential peripheral biomarkers for monitoring transfusion dependent β-thalassemia patients.

PB 2447 | Plasma miR-103b as a Novel Biomarker for Screening Pre-diabetes and Newly Diagnosis of Type 2 Diabetes

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Background: MicroRNAs (miRNAs) play a crucial role in the pathogenesis of type 2 diabetes (T2DM). The prevalence of pre-diabetes predisposes an individual to the development of T2DM. Recent studies suggested an association of circulating miR-103b with pre-diabetes and T2DM.

Aims: In the current study, we investigated whether plasma miR-103b plays a diagnostic role in the pathogenesis of T2DM and pre-diabetic syndrome.

Methods: Bioinformatic analysis and identification of miR-103b and its targets were assessed using quantitative real-time PCR and receiver-operating characteristic (ROC) analyses in 50 patients with pre-diabetic mellitus (pre-DM), 48 patients with non complicated diabetic group (NCDM), and 52 healthy individuals.

Results: miR-103b was especially gradually down-regulated in plasma from patients with pre-DM and NCDM. Dramatically, miR-103b level in pre-DM was decreased 10-fold comparing to healthy individuals. Further analysis found that the area under the curve (AUC) for the ROC analysis of miR-103b was 0.887 (95% CI 0.809-0.944). At a cut-off value of 1.633, the sensitivity was 89.8 % and the specificity was 73.5 % in discriminating T2D patients from healthy individuals. The bioinformatic analysis has demonstrated that miR-103b has a high degree of site conservation among different mammalian species i.e., *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Pongo pygmaeus*, *Sus scrofa* and etc. 53 potential targets of miR-103b were predicted; most of these putative target genes involved in MAPK, Wnt, insulin and RAS signaling pathways and enriched in various biological processes, moleculer functions, and cell component.

Conclusions: These results reveal that plasma miR-103b can be used as an objective complement to traditional diagnosis of pre-diabetes, indicating important implications regarding the distinguish of the undiagnosed cases between diabetes and pre-diabetes by circulating miRNA.

PB 2448 | Overexpression of p21Cip1 and p27Kip1 Regulates the Proliferation of Endothelial Colony Forming Cells from Patients with Deep Venous Thrombosis

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Background: The World Health Organization reported that thrombosis, particularly "venous thromboembolic disease" (VTD), is a major cause of death, and hence, is a public health problem. We recently reported that endothelial colony-forming cells (ECFCs) derived from endothelial cells (EC) (ECFC-ECs) from patients with VTD have a dysfunctional state that generates a reduction of its proliferative ability, which is also associated to senescence and reactive oxygen species (ROS).

Aims: We examine the status of cell cycle and expression of cyclin, cyclin dependent kinase (CDKs) and CDK inhibitors (CKIs) in ECFC-ECs patients with VTD.

Methods: ECFC-ECs obtained and frozen in our laboratory were analized in passage 3 to determinate by flow cytometer: status of cell cycle by KI67. We also used monoclonal antibodys to detect the expression of cyclin D3, cyclin A, cyclin B, CDK2, CDK4, $p16^{INK4}$, $p21^{CIP1}$ and $p27^{KIP1}$. The protocol was reviewed and approved by the Human Ethical Committee of the IMSS (FIS/IMSS/PROT/G09/771) and conforms to the guidelines of the 1975 Declaration of Helsinki. Informed written consent was obtained from all subjects before enrollment.

Results: Proliferation was reduced in ECFC-ECs from patients with VTD because more of 10% of cell population was arrested in S phase of cell cycle. The regulator proteins that are involved in this cell cycle status are Cyclin D3, CDK2, with a reduce expression, while $p21^{CIP1}$ and $p27^{KIP1}$ has are over expressed with respect to normal cells.

Conclusions: Our data shows that low proliferative potential in ECFC-ECs from VTD patients associated with cellular senescence is due to an anormality in the mechanisms related with the cell cycle, particulary, an increase of expression of Cip/Kip family members $p21^{CIP1}$, $p27^{KIP1}$ and arrest in S phase of cell cycle. Our results strongly suggest that patients with a history of VTD may be at risk of new thrombotic events by this cellular mechanisms.

PUBLICATION ONLY ATHEROTHROMBOSIS & STROKE

PO 01 | New Potential Marker for Analysis of the Organism State Under the Cardiovascular Disorders

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Background: The directly proportional relationship exists between level of the organism disorders and accumulation of the peptides up to 5 kDa in blood plasma. However, a clear answer to the question regarding properties of these peptides is absent nowadays.

Aims: To investigate new possible markers for analysis of the organism under the cardiovascular disorders (CVD).

Methods: Blood plasma was taken from 35 healthy donors and 6 patients with acute myocardial infarction, 11 patients with new onset unstable angina, 17 patients with progressive unstable angina,
7 patients with chronic stable angina, 6 patients with silent myocardial ischemia, 22 patients without proved coronary artery disease. Patients

were hospitalized in cardiology department of the Hospital #12, Kyiv, Ukraine. Peptides were obtained by the method of Nikolaichyk V. Concentration was counted in respect of calibration chart obtained after an analogical measurement of peptide 0,26 kDa. The purity of peptides fraction was controlled by 15% SDS-PAGE.

Results: The presence of peptides up to 5 kDa in all fractions of healthy donor as well as fractions of patients with all tested CVD was shown. The SDS-PAGE image was typical of all tested pathologies. The concentration of peptides separated from the blood plasma of patients with CVD was significantly higher in comparison with healthy donors. Thus, the most concentrated peptides pool (PP) fraction was obtained from the patients with the acute myocardial infarction, which was 14 times more concentrated than fraction of healthy people. The PP fraction in patients with progressive unstable angina was 6 times more concentrated. The PP fraction of patients with the new onset unstable angina, chronic stable angina as well as patients without proved coronary artery disease were 5 times higher than that in healthy donors.

Conclusions: The results showed that all tested CVD accompanied by increased concentrations of peptides up to 5 kDa. It could be used as a tool for monitoring of the organism state under the CVD.

PO 02 | Apolipoproteins and Serum Inflammatory Markers in Young Patients with Myocardial Infarction

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Background: CAD is gradually emerging as one of the major health challenges in Nepal and has been recognized among younger age group more frequently in recent years even in Nepal.

Aims: The present study aims to estimate the serum apolipoproteins and inflammatory biomarkers and thereby assess the risk factors prevalent in young patients of acute myocardial infarction attending the tertiary care centre of eastern Nepal.

Methods: This is a hospital-based cross- sectional study conducted from August 2015 to July 2016 in Thirty Eight patients aged 15-45 years presented with acute myocardial infarction. Eleven risk factors were studied namely: Gender, H/O Diabetes mellitus, H/O Dyslipidaemia, Family history, Smoking,Hypertension,Obesity,Alcohol, Altered Apo B/ApoA1 ratio,HsCRP leveland Serum Uric Acid Level. The study population were further divided into two groups: Group A with patients 35 years of age or less and group B with patients 36-45 years of age.

Results: The present study found that among the total study participants, 68% were Non-obese males. Majority of the population were hypertensive with the H/O Smoking and alcohol intake. Family history of acute myocardial infraction or sudden cardiac death was present in less proportion of the patients (32%). Serum Apo B was significantly raised compared to the Apo A in the patients, but their difference between the two groups was not statistically significant. Serum hsCRP and Uric acid was significantly higher in Group B compared to Group A patients.

Conclusions: The present study elaborates the use of Apo B/ Apo- A1 ratio in AMI in young adults in a tertiary care centre of eastern Nepal. The clustering of risk factors particularly three or more risk factors in an individual predispose to CAD at relatively younger age. This study depicts that Apo B/ Apo A-1 ratio is a better predictor of Myocardial Infarction in young adults compared to the other inflammatory biomarkers.

PO 03 | Particularities of Superficial Venous Thrombosis

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Background: Thromboembolic events (TEE) are very variable. Their clinical presentation, etiology and outcome depend on their localisation.

Aims: To determine clinical, etiologic and outcome characteristics of superficial venous thrombosis (SVT).

Methods: A retrospective and descriptive study of records of patients hospitalized for TEE during 20 years. We selected data of patients hospitalized for SVT. We determined clinical, etiologic and outcome characteristics of these patients.

Results: Among 1055 patients hospitalized for TEE, 139 (13.2%) patients presented SVT (84 men and 55 women). The average age of patients was 49.49 years [15-86].

SVT was associated to distal deep venous thrombosis in 10 cases, proximal deep venous thrombosis in 57 cases which 9 of them were complicated by pulmonary embolism. Thrombosis of superior cava vein was associated in 2 cases.

SVT were suspected in front of collateral venous circulation in 6 cases and venous cord in 37 cases. The other cases were accidentally discovered.

The more frequent factor risk of thrombosis found were : smoking (46%), varicose veins (32.4%), obesity (25.2%) and badrest (11.5%). On the other side, venous failure was shown in 6.5% of cases.

SVT were reported to Behçet disease in 20 cases (14.4%), hyperhomocysteinemia and neoplasms in 14 cases each one (10.1%), constitutional thrombophilia in 5 cases (3.6%) and antiphospholipid syndrom in 1 case (0.7%). SVT revealed underlying pathology in 24 patients (17.3%).

Only 7 patients were treated by heparin by cutaneous route. The other patients were treated by antivitamin K. Compression stockings were prescribed in all patients. But, only 29.5% wore them.

Post thrombotic syndrom was noted in 17 patients (12.2%). TEE recurred in 17 patients.

Conclusions: In our study, about half of SVT were lone, in the most of cases asymptomatic and idiopathic. No pulmonary embolism complicated SVT in absence of deep venous thrombosis. But, post thrombotic syndrome and reccurence were noted.

PO 04 | Venous Thrombosis in Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is auto-immun disease which affects several organs. Frequency of Venous thrombosis (VT) in this disease is estimated at 10%.

Aims: to determine characteristics of VT occuring during the SLE.

Methods: A retrospective and descriptive study of records of patients hospitatized for VT during 20 years. We selected data of patients where VT was attributed to SLE. We studied characteristics of these patients.

Results: Among 1055 patients hospitalized for VT, 14 (1.32%) patients had SLE (11 women and 3 men) with an average of age was 42.21 years [18-70]. SLE was associated to atiphospholipid syndrom in 4 cases.

VT was proximal in 8 cases associated to pulmonary embolism in 2 cases, distal in 2 cases and superficial in 1 case. Pulmonary embolism was lone in 3 cases.

As risk factors of thrombosis, smoking and varicose veins were noted in 3 patients for each one, bedrest and surgery in 1 patient for each one.

All patients were treated by antivitamin K during an average period of 31.42 months.

Post thrombotic syndrom and reccurence occured in one case each one.

Conclusions: In our series, SLE is a rare cause of VT principally proxiaml, occured in the absence of frequent risk factor. Complications were not frequent.

PO 05 | Clinical Profil of Digestive Thrombosis Patients

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Background: Digestive thrombosis is a rare disease. Its prognosis may be severe due to the local complications it causes : portal hypertension, Budd Chiari syndrome and digestive ischemia but also because of the underlying etiology.

Aims: to describe the clinical features of patients presenting digestive thrombosis.

Methods: Retrospective single center study having compiled patients' files comprehending the period between 2010 and 2016.

Results: The study group consisted of 12 patients. The male to female ratio was 0.5. The mean age at the time of diagnosis was 33 years.

The different locations were as follow : hepatic vein (six patients), portal vein (three patients), superior mesenteric vein (three patients) and inferior mesenteric vein (two patients).

Budd Chiari syndrome was decribed in four patients.

The clinical signs of discovery were abdominal pain in five patients, vomiting and ascites in one patient each. The other patients were asymptomatic.

All patients had an abdominal computed tomography scan which showed the digestive thrombosis.

The etiologies reported were: Behcet disease in three patients, Vaquez disease, activated proteine C resistance, cirrhosis and pancreatic pseudocyst in one patient each. No etiology was identified in four patients. All patients were treated with anticoagulant therapy. Three patients were also treated with predisone at the dose of 1 mg/kg per day and with immunosuppressive drugs because of their Behcet disease. The outcome was favorable in 8 cases. The average follow-up was 52 months.

Conclusions: The predominance of the Behcet disease as a main etiology of digestive thrombosis in our study can be explained by a recruitment bias. However, it needs more cases before drawing conclusions.

PO 06 | Prevalence of Resistance to Anti-platelet Therapy among Patients with Ischemic Stroke - Preliminary Results

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Background: Aspirin and Clopidogrel are currently the most prevalent antiplatelet agents used in the prevention of ischemic, non-cardioembolic strokes. However, variable response to antiplatelet therapy needs monitoring by platelet function studies.

Aims: To explore the prevalence of resistance to anti-platelet therapy with aspirin and/or clopidogrel among patients with ischemic, non-cardioembolic stroke.

Methods: Adult patients who developed ischemic stroke over the past two years and received treatment either with Aspirin or Clopidogrel or both agents were enrolled. Those with cardioembolic stroke or intracranial hemorrhage were excluded. Compliance with therapy was ensured by history and hospital records. Platelet functions were studied on blood samples from patients by platelet aggregometry and PFA-200 screen. Frequency of resistance to anti-platelet agents among patients with and without stroke recurrence over the period of anti-platelet therapy were compared.

Results: Of the 27 patients studied, 20 were on aspirin and 15 on clopidogrel. Of these, 13 were on dual antiplatelet therapy. Overall, 3 patients on aspirin and 6 patients on clopidogrel had resistance to anti-platelet effect. While the failure of aspirin effect was observed in 3 patients with monotherapy, 86% of the failures with clopidogrel occurred in patients on dual antiplatelet therapy. 15/27 patients had recurrent stroke: 6/15 among those with antiplatelet agent resistance, and 9/12 without resistance.

Conclusions: Resistance to the anti-platelet effect of clopidogrel (40%) appears to be more frequent than that due to aspirin (15%) in Omani population. No significant relation of failure of anti-platelet function with stroke recurence was observed in this small cohort(Chi square, p=0.33). Further larger studies are required to explore any possible relation of resistance to anti-platelet agents with recurrence of cardiovascular events.

PO 07 | Intracerebral Hemorrhage under Antithrombotic Medication in a Recent Year

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Background: Antithrombotic medication will be a risk of hemorrhagic complication. Meanwhile, direct oral anticoagulants (DOACs), which are recently introduced in the clinical use, are reported to show lower risk of intracerebral hemorrhage (ICH) compared with warfarin. However, the severity at onset and the outcome of ICH patients with DOACs are still controversial.

Aims: This study aimed to reveal the clinical features of recent ICH patients with antithrombotic medication including DOACs.

Methods: Between April 2014 and March 2015, ICH patients who admitted to our hospital were consecutively screened. Hematoma size was assessed by brain CT images on admission. Outcome was measured by modified Rankin Scale (mRS). Favorable outcome was defined as mRS< 3.

Results: Twenty-eight of 129 ICH patients (21.7%) were taken antithrombotic agents (7 warfarin, 4 rivaroxaban, 1 dabigatran, 8 aspirin, 3 thienopyridines, 1 cilostazol, 3 antiplatelet and anticoagulant and 1 dual antiplatelet agents). Mortality was 14.3% and 8.9% in patients with and without antithrombotic agents, respectively. Frequency of favorable outcome was 0%, 33.3%, 63.6% and 83.3% in patients with dual antiplatelet agents, single antiplatelet and anticoagulant, single anticoagulant and single antiplatelet, respectively. Hematoma size was not different between warfarin and DOACs (29.7ml and 31.3ml, respectively). Patients with DAOCs showed favorable outcome compared to patients with warfarin, although the difference of percentage was not significant (p=0.398: 66.7% and 43.3%, respectively).

Conclusions: Outcome of patients who were taking antithrombotic agents, especially to whom multiple antithrombotic agents were prescribed, is worse. The difference of outcome among different DOACs also needs to be investigated in the future.

PO 08 | Methylenetetrahydrofolate Reductasa (MTHFR) C 677 Mutation-risk Factor for Ischemic Stroke or Not - Case Report

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Background: Homozygosis for |MTHFR C667 T polymorphism can lead to high homocysteine levels and hyperhomocystinemia as an important risk factor for thrombotic events.

Aims: To determinate a role of MTHFR C 667 T mutation in a patient with an ischemic stroke documented by a magnetic resonance imaging.

Methods: We report a case of 42 years male patient with ischemic stroke. No traditional risk factors were found (non smoker, non hypertensive, non obesity, non diabetic, no vegetarian, no family history) to understand the reason for this thrombotic event. Because he was young and healthy person, we suspected that some genetic abnormalities should be the reason. Molecular study was very surprised for us - he was heterozygous for MTHFR C667 T mutation, but also heterozygous for MTHFR A 1298 C mutation, factor V (R2) mutation, LTA mutation and PAI 4G/5G polymorphism. Unfortunately, plasma homocysteine levels were not measured in the referent laboratory and we do not received a result who was very important for the future therapy.

Results: We reported a case of 42 years male patient with ischemic stroke.

Conclusions: In this case, we have a heterozygous for MTHFR C667 T mutation, which isolated, is not a risk factor for ischemic stroke, because those persons do not have high plasma levels of homocysteine. But without an influence of any environmental factor and family history, we suspected that the synergistic effects between all those heterozygous polymorphism were a strong risk factor for thrombotic stroke in our patient. We supported him with an anticoagulant therapy, higher doses of folic acid and B complex vitamins.

COAGULANT & ANTICOAGULANT MECHANISMS

PO 09 | Role Glycin-containing Olygopeptides in Platelet Aggregation

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Background: It was found that the final organism response to stress is the influence of catecholamine's in the blood system. A significant influence on the reaction may have both neuropeptides such as vasopressin and oxytocin and their products of proteolysis. So urgent is to study the influence of catecholamine's on platelet aggregation and the effect of these olygopeptides on the reaction conditions.

Aims: The aim of this work was to study the effect of C-terminal fragments of vasopressin and oxytocin on platelet aggregation under influence of epinephrine in the physiological norm.

Methods: Was used peptides representing the C-terminal fragments of vasopressin (Pro-Arg-Gly- NH2 - I), oxytocin (Pro-Leu-Gly-NH2- II) : To test with washed platelets, peptides are added in a concentration range of 10-1 - 10-10M or an equal volume of saline and the measured change in aggregation by the action of at a final concentration epinephrine 0.02 mmol / l. Aggregation was recorded on a aggregometer. The results were treated statistically.

Results: : In the first series of experiments examined the effect of the peptide I of washed platelet aggregation by the action of epinephrine. It was established that the peptide I induced a significant enhancement of aggregation in a concentration range of 10-5 - 10-10 M (p< 001). with maximum stimulation index 68%. The following series of experiments examined the effect peptide II on the platelet aggregation by the action of epinephrine. It is shown unlike peptide I this peptide virtually no effect on platelet aggregation induced by epinephrine. Only by adding the peptide at a concentration of 10-8M it caused inhibition of platelet aggregation.

Conclusions: Therefore, small regulatory peptides, which are products of proteolysis of neuropeptides, have the opposite effect on the platelets aggregation. Explore ways peptide proteolysis may lead to the development of new drugs for correction of various disorders in the body, hemostasis e.g.

PO 10 | Bleeding Disorders in Ashkenazi Jews

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Background: Bleeding Disorders in Ashkenazi Jews.

Aims: The aim of the exercise was to demonstrate bleeding disorders in Ashkenazi Jews.FactorXI (plasma thromboplastin antecedent).

Factor XI is frequently deficient in Ashkenazi Jews and this can result in a bleeding disorder This is often seen at circumcision but may result in menorrhagia in females.

Methods: The methods involved the demonstration of Bleeding disoders; predominantly Factor XI deficiency in Ashkenazi Jews. Nosebleeds or epistaxis may be severe. Factor XI concentrate is available but is often difficult to obtain. Fresh frozen plasma may have to be used. Ashkenazi Jews are of European descen tehile Sephardi Jews are usually of Spanish descent. But non-Jews may present with the disorder, mainly due to assimilation.

Results: The result showed a definite defect in the factor XI molecule with patients haaing a propensity to bleed, especially at laparotomies. There are two predominant mutations, type II and type IIII (using an older classification system). The type III mutation is an amino acid substitution (Phe283Leu) resulting in a missence mutation. This results in impaired dimerization and secretion of the FXI molecule. The seond is the type II mutation; this causes premature chain termination and results in very low levels of circulating FXI.

Conclusions: This finding is of great clinical significance as often patients do not know that they have a bleeding defect.

This is of great significance in Ashkenazi Jews. The type II mutation has been found in Iraqi Jewish and Israeli Arabic descent. Both mutations are thought to originate from a common founder, one occurring before and one after the divergence of the Jewish people!

PO 11 | Management of a Severe Congenital Protein C Deficiency Case

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Background: Congenital Protein C (PC) deficiency is a very rare condition associated with serious clinical manifestations.

Aims: Here we present a case with PC deficiency managed by FFP, anticoagulation and PC concentrate.

Methods: -

Results: A three day infant of consanguineous parents who were found to be asymptomatic carriers of heterozygous PC deficiency. presented to the emergency department with progressive skin necrosis on legs. Protein C activity was undetectable. She was initially treated with FFP at another center. At investigation she was found to have intracranial and retinal hemorrhage. Despite FFP and anticoagulation with low-molecular heparin her skin lesions progressed. Human derived PC concentrate improved skin lesions and intracranial bleeding and she was put on prophylaxis as well.

Conclusions: PC replacement is an established method for treating PC deficiency. It can also used for long term prophylaxis. Due to small number of cases, optimal management of this devastating condition is yet to be investigated.

PO 12 | Laboratory Detection of Thrombophilic Markers Frequency

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Background: Antithrombin III (ATIII), protein C (PC) and protein S (PS) deficiencies or inherited thrombophilic defects such activated protein C resistance (APCR), f.V Leiden (fVL) or prothrombin (II G20210A) mutation are potential markers for thrombophilia (increased tendency for venous or arterial thrombosis).

Aims: To represent association between low concentration of PC,PS, positive APCR activity and presence of mutation of f.VL or II G20210A mutation at patients with thrombosis.

Methods: 100 patients (21 to 70 years old) with a history of DVT, PE and AIM. Biological activity of AT III, PC, PS with Simens BERICHROM

ATIII ,BERICHROM PC, PROTEIN S Ac kits and resistance to activated PC (ProC® Ac R, Simens Bechring) were measured on automated coagulation analyser (BCS-XP). According to reference values ATIII, PC and PS activity below 60 % were regarded as deficiency. A ratio of activated PC below 2.1 indicated APCR. fVL and II G20210A polymorphism were detected with PCR specific polymorphism detection kits (both Applied Biosystems) on a ABI Prism 7000 equipment.

Results: 29 patients from 100 had APCR (1.64±0.25 v.s.2.24 ±0.51). 15 patients from all had decreased level of PC (43.67 %±18.53 v.s. 101.47%± 39.91) and 19 patients had decreased level of PS (45 %±15.18 vs. 89.6%± 39.68). All examined had normal ATIII (96.82%±16.96). 15 patients were heterozygotes and one was homozygote for f.VL. 9 patient were heterozygotes for II G20210A.1 patient with AIM was heterozygote for fVL and II, and 1 patient with Phlebothrombosis was homozygote for fVL and heterozygote for II. This patients were APCR(1.51±0.44). 14 patients with heterozygote fVL were at same time APCR (1.68±0.16).

Conclusions: Low concentration of ATIII, PC, PS and APCR are very useful laboratory markers for thrombophilia. Recognition of thrombophilic defects (fVL and II G20210A polymorphism) are complementary and changed the diagnostic approach for thrombophilia. They all together are potentional risk factors for predicting and developing of thrombosis.

DIAGNOSTICS AND OMICS

PO 13 | Red Blood Cells Distribution Width to Estimate Lupus Activity

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Background: Systemic lupus Erythematous is a multifactorial systemic autoimmune disease which can affect multiple organs. The general pathogenesis of this disease still needs a better understanding. Red cell distribution width is a measure of the red blood cells (RBCS) size variation.

Aims: was to study red blood cell distribution width (RDW) in systemic lupus Erythematous (SLE) patients as a lupus activity marker.

Methods: This prospective study was carried on Internal Medicine Department, Menoufia University Hospital from the period of March 2016 till august 2016. Our study included 58 Systemic lupus Erythematous patients with activity. Activity of patients was measured by Systemic Lupus Erythematous Disease Activity Index (SLEDAI).RDW was done as one of the parameters of complete blood count (CBC).Red cell distribution width was reported on the Sysmex XT. In this study we excluded any other causes of anemia and other connective tissue diseases.

Results: The study included 58 Systemic lupus erythematous patients, 20 systemic lupus patients with high activity and 38systemic lupus patients with very high activity. RDW was higher in systemic lupus patients with very high activity than in systemic lupus patients with high activity. There was a highly significant correlation between RDW and SLEDAI. There was a highly significant correlation between RDW and erythrocyte sedimentation rate (ESR).

Conclusions: There was statistically more increasing in RDW in lupus patients with very high activity than in those with high activity so RDW can be used as a lupus activity marker. RDW was associated with the inflammatory process of SLE.

PO 14 | Levels of von Willebrand Factor Antigen in Apheresis Donors and in Mild Bleeders with Near Normal Screening Coagulogram: A Study from a Tertiary Care Teaching Hospital, South India

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Background: Several studies have documented the influence of ABO blood group on plasma von Willebrand factor antigen (vWF:Ag) levels with significant low levels in O group, compared to other groups. Some forms of VWD can present with mild bleeding manifestations, with a normal screening coagulogram, with or without prolonged aPTT.

Aims: Among attendees of a tertiary care hospital in Southern India, to estimate vWf:Ag levels in

o apheresis donors and

o mild bleeders with near normal screening coagulogram

Methods: For apheresis donors (n=295), ABO blood group phenotype was done by standard tube method. The patients (n=39) enrolled had mild bleeding phenotype (ISTH BAT score of < 6 for females, < 4 for males and < 3 for children) whose routine coagulation

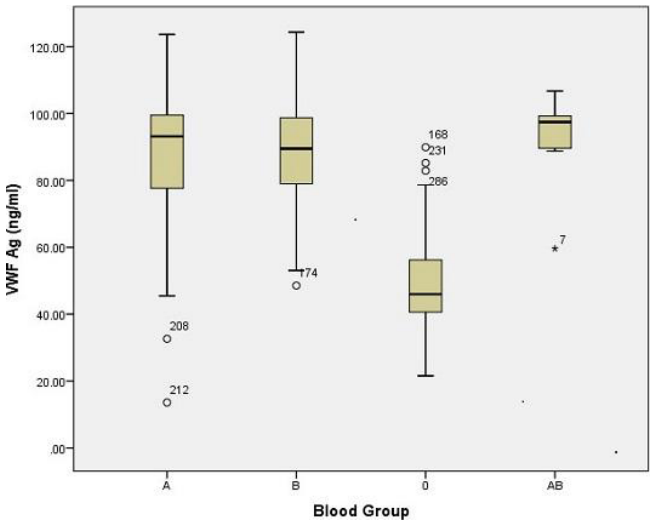

FIGURE 1 Comparision of vWF:Ag levels among various blood groups

screen (BT, PC, PT, aPTT) was either normal or showed mild aPTT prolongation. Estimation of vWF:Ag was done by ELISA method (Raybiotech, USA).

Results: All donors were males with mean age of 27.24 ± 7.25 years. The distribution of ABO blood groups was 46 (15.6 %), 135 (45.8%), 105 (35.6%) and 9 (3.1%) for A, B, O and AB respectively with mean vWF:Ag value being 87.53 ± 22.48 ng/ml, 88.84 ± 16.18 ng/ml, 48.72 ± 13.24 ng/ml and 92.04 ± 12.7ng/ml (Figure 1).

The mean vWF:Ag level was significantly higher for non-group O than group O individuals ($p< 0.05$).

Mean age of mild bleeders was 14.98 years, 74% were females, 82% had normal screening coagulogram. The average BAT score and vWF:Ag levels was 2, 3.7 and 3.2; 63.71± 20.93, 71.32± 21.59 and 68.74± 17.65 among males, females and pediatric patients respectively.

The overall mean vWF:Ag levels for donors and mild bleeders was 74.46± 25.2 ng/ml and 68.87 ± 18.93 ng/ml respectively.

Conclusions: This study emphasizes that vWF:Ag level is higher in non-group O than group O individuals.

The mild bleeders had vWF: Ag in normal range; however further work up is needed. Separate reference ranges of vWF:Ag for various ABO blood groups should be used for diagnosis of vWD.

PO 15 | Screening Test for Leiden Mutation Relevance

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Background: Protein C in active form (APC) is important inhibit regulator of coagulation cascade. Resistance to APC (RAPC) is mainly caused by FV Leiden mutation (FVL). In persons with FVL inactivation of factor Va is much slower and the time of thrombin formation due to factor Va is prolonged resulting in hypercoagulable state.

Aims: The aim of the study: to establish incidence of RAPC in patients suspected on thrombophilia; to compare results of genetic test regarded functional coagulation test.

Methods: We examined 335 samples from patients with clinical suspicion for thrombophilia. Samples of venous blood were collected in tubes with sodium citrate, centrifuged for 10 minutes at 1500xg to separate plasma for analysis. We applied screening RAPC determination test on automated coagulation analyzer. Principle of the test is activation of the endogenous protein C and clotting time measuring. In normal person activation prolongs result 2-3 times, but in person with Leiden prolongation is minimal. Result is calculated as a ratio (ACR) between clotting time with and without activator. Cut off value for ACR ratio was 2. Patients with smaller ACR ratio were suspected to be positive for Leiden mutation and were submitted to genetic analysis.

Results: Out of 335 samples which are examined for RAPC, 20 samples or 6% were positive. All of them were confirmed to be heterozygots for Leiden mutation by DNA analysis. We did not have false positive results confirming that test specificity is high.

Conclusions: Although we put the decision limit higher then recommended by manufacturer to avoid false negative results, percent of positive was significantly lower than in literature (20-25% in patients with indications for testing). It is close to percent for general European population (3-8% according to literature data). Since the test sensitivity (cut off 1.8) is 99.2%, reason for this may be uncritical test ordering. That remains the goal for the further investigation.

PO 16 | Simultaneously Label-free Measurement of Hemoglobin Concentration and Erythrocyte Shape by Scanning Flow Cytometry

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Background: Red blood cell (RBC) shape is not covered by modern hematology analyzers, but it has a significant correlation with pathologies. In our study we propose the simultaneously measurement of MCHC and erythrocyte shape with good accuracy combine with label-free measurement of the effective and total Band3 human erythrocyte protein.

Aims: Development of diagnostic system for screning blood cells shape to early detection of pathologies.

Methods: All experiments were carried out on the Scanning Flow Cytometer (SFC, fabricated by CytoNova Ltd., Novosibirsk, Russia, http://cyto.kinetics.nsc.ru). This instrument allows to measure light-scattering profiles (LSPs) of individual cells. Blood samples were taken by venopuncture and collected in a vacuum tube containing EDTA as anticoagulant. The sample was 1000-fold diluted in 0.9% saline and in isotonic solution of ammonium chloride.

Results: For each measured RBC we obtained the following morphological characteristics: hemoglobin concentration, sphericity index, surface area, volume, diameter, membrane spontaneous curvature, maximal and minimal thicknesses. The thickness-vs-diameter scatterplot can identify non-standard cells, such as top tail, associated with almost spherical RBCs, for example, reticulocytes. Our results confirm the possibility of precise, label-free, enhanced morphological analysis of individual intact RBCs. We determine for 15 donors the number of effective and total Band 3 protein and ration of membrane tension and elasticity for erythrocyte population. It specifies the current state of the donor anion exchange.

Conclusions: Lack of consumables and fast analysis time make the proposed method appropriate for routine clinical diagnostics, significantly increasing its descriptiveness and sensitivity to pathologies. The issued array of morphological RBC characteristics opens a new informative screening method in laboratory medicine.

FIBRINOLYSIS & PROTEOLYSIS

PO 17 | Interaction of Antithrombotic Preparation Pijavit with Proteinase Complex Longolytin and its Producer Imperfect Fungi Arthrobortys Longa

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Background: Imperfect fungi synthesize fibrinolytic substances and are the perspective sources of thrombolytic preparations. Longolytin - proteinase complex,- produced by imperfect fungi Arthrobotrys longa, dissolved experimental external thrombi in rabbits and inner veins thrombi in rats. Pijavit is pharmacological preparation from Hirudo medicinalis with powerfull anticoagulant and antithrombotic properties and can use instead of heparin.

Aims: The aim. To study the possibility to rise longolytin fibrinolytic (hence thrombolytic) activity by addition of new antithrombotic preparation from Hirudo medicinalis - pijavit.

Methods: In vitro pijavit 2-16 mg/ml was added to equal volume of longolytin 10-30 C.U. activity and fibrinolytic activity (F.A.) was determined on fibrin plates. In other experimental series pijavit was added to flask wih Arthrobotrys longa culture and cultural fluid F.A. was determined after 5 days of fungi culture growing and longolytin releasing.

Results: There were obtained 2 opposite results in vitro and in vital cells. In vitro longolytin F.A. was inhibited in dependence on pijavit dose and fully depressed in 16 mg/ml and more. There were depressed as plasminlike and plasminogen activator activity of longolytin general F.A. In contrast longolytin F.A. was increased by pijavit addition to Arthrobotrys longa culture. There were stimulated as F.A. on standard fibrin plates on 44% and on plates without plasminogen on 36%. Possibly this phenomenon was result of defense reaction of vital fungi's cells to appearing of great amount of proteinase's inhibitors, containing in pijavit. Pijavit created high anticoagulant activity, registered by thromboelastograpy in cultural fluid.

Conclusions: Combine using of 2 antithrombotic preparation not always results in cumulation of antithrombotic effect. It is necessary know properties of preparations Pijavit inhibited F.A. of longolytin in big doses, so its combine using must be very carefully.

HEMORRHAGIC DISORDERS, HEMOPHILIA

PO 18 | Immune Tolerance Induction in an Adult Haemophilia A Patient with a Long-standing Inhibitor

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Background: Inhibitor development occurs in up to 36% of severe haemophilia A (HA) patients. The clinically proven strategy to eradicate FVIII antibodies is immune tolerance induction (ITI).

Aims: Predictors for ITI success include e.g. the peak historical inhibitor titre, age and the period between initial inhibitor diagnosis and start of ITI.

Methods: We present the case of a 38-year-old patient (48 kg, 174 cm) with severe HA (< 1% FVIII) without ongoing regular FVIII therapy who had a high-titre inhibitor and required urgent orthopaedic surgery. More than 20 years ago, the patient had suffered an intracranial haemorrhage, and treatment with pd-FVIII resulted in inhibitor development (max. titre 160 Bethesda units (BU).

Results: At the time of admission on the orthopaedic department, the patient's FVIII inhibitor titre was 1 BU/ml. Turoctocog alfa (NovoEight®, Novo Nordisk) was used for FVIII replacement therapy during surgery, which was considered successful. On Day 7, an increased level of inhibitors to FVIII were detected, which peaked at 12.5 BU on Day 9. The classic Bonn protocol was selected as the ITI method. The patient was treated with 5000 IU turoctocog alfa twice daily, and 5000 IU FEIBA® once daily from Day 9 to 17, after which time FEIBA® administration was stopped. The patient's inhibitor levels declined and were no longer detectable by Day 17, although PK analyses on Day 20 showed a FVIII half-life of < 6 hours and recovery at < 66%. FVIII replacement therapy was continued and within 12 months all criteria for successful ITI were achieved. During the follow up of now more than30 months, no spontaneous bleeding occurred. Quality of life improved dramatically since the successful ITI und regular FVIII treatment.

Conclusions: Here we report a rapid success of ITI in an adult patient with HA having a long-standing inhibitor. Results suggest that surgery may not prevent ITI success and that HA patients with inhibitors should be offered ITI irrespective of the time since the first inhibitor detection.

PO 19 | Evaluation of the Quality and Safety of Care in Hemophilia Self-treatment

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Background: Self-treatment is an irreplaceable therapeutic strategy in the treatment of this chronic disease.

Aims: We evaluate the quality and safety of care for self treatment of hemophiliacs.

Methods: We started training patients to self-treatment in 2011. To date over 107 Hemophilia A and 6 hemophilia B , 26 hemophilia A,2 hemophilia B are self treatment. The main objectives of the training: Hemophilia, a diagnosis of hemorrhage, bleeding hazardous locations, hand washing, dose calculation and preparation of the product, practical training on peripheral venous approaches on artificial arm. Training in groups or individually.

Results: The medical team should monitor the use of clotting factor concentrates and evaluate the effectiveness of self-treatment

program. After training we noticed the rapid establishment of the substitution therapy at home at the first signs of bleeding and better control of pain in the majority of hemophiliacs. There is a slight decrease in factor consumption compared to hemophiliacs who are not self treatment. There is a reduction in truancy and in Dworkin with a significant improvement in quality of life with more independence and taking responsibility.

Conclusions: The quality and safety of care are conditioned by a commitment from the neat and care givers while ensuring the implementation of safety measures and the quality of self-treatment.

PO 20 | Participation of a Group of Healthcare Professionals in the Improvement of Hemophilia Treatment in Latin America

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Background: Red Lapi Association started 7 years ago with a group of healthcare professionals with experience in the treatment of hemophilic patients.

Aims: The objective of the Association is to work in the education of Specialists, General Practitioners, Bacteriologists, Nurses, as well as patients and their relatives. It has also supported the development of Hemophilia programs in Latin American countries and encouraged research and publications of their experiences. RED LAPI has counted with the participation of professionals from 13 countries.

Methods: Red Lapi has held 13 meetings. A section of each meeting is always dedicated to train members. We also work in the projects that are taking place and discuss about relevant issues with governmental bodies. A part of the meetings is dedicated to provide training to doctors and paramedics of the host country and also to local patients' associations in order to create awareness around the disease and help improve Hemophilia patients' care in Latin America.

Results: This work describes the following outcomes from Red Lapi:

The impact on the improvement of patients' care from 13 Workshops. These events were held in different cities with a total attendance of 463 people.

Activities to support the diagnosis and treatment of patients.

Publications: Diagnostic and therapeutic status of haemophilia in Latin America. The Journal of Haemophilia Practice, May 2014;1(2):30-34.

Handbook of Practical Hemophilia, 1st Edition Santiago de Chile, 2nd Edition Asunción Paraguay. Distributed in 13 Latin American countries. Adopted by the Ministry of Health of Paraguay as a manual for the National Hemophilia Program.

Collaborations with governmental bodies and advice provided to implement Hemophilia programs in Ecuador, Bolivia and Paraguay. Papers presented at international congresses.

Conclusions: In developing countries it´s very important to create international work groups to achieve positive results in the growth of national programs for the diagnosis and treatment of hemophilic patients.

PO 21 | Successful Non-operative Management of Intra-abdominal Hemorrhage in Patient with Hemophilia

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Background: Spontaneous intra-abdominal hemorrhages are rare bleeding episodes in hemophilia patients. That can cause many problems for severe hemophilia patients, the mortality rate increases significantly. Therefore it is very important to early diagnose and determine the treatment strategy urgently. Management of intra-abdominal hemorrhage range from conservative treatment to emergent embolization or surgery.

Aims: There is no clear guideline for managing intra-abdominal hemorrhage in patients with hemophilia. we describe two patients with intra-abdominal hemorrhage in whom bleeding was successfully controlled with non-operative management.

Methods: -

Results: One of the patients had gross hematuria and subsequent increasing abdominal pain. The other patient had significant periumbilical pain and mild fever. In our patients, we obtained an abdominal computerized tomography scan initially, in order to define the focus of bleeding, and rule out the presence of active bleeding. Intra-abdominal hematoma was observed in the both two patients, the size of the hematoma was approximately 11X4 cm, 7X5 cm, respectively. They treated successfully with coagulation factor and embolization and were discharged safely from the hospital.

Conclusions: We emphasize the role of active approach in the evaluation and non-operative management of an acute abdominal problem of this case without any surgical intervention.

PO 22 | Inhibitor Screening of Children with Hemophilia in the Diyarbakır - A Single Centre Study

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Background: Development of inhibitors in hemophilia patients is the most serious and life-threatening complication of hemophilia therapy.

Aims: The aim of this study was to determine the inhibitor prevalence in the children with hemophilia who were followed up in the Diyarbakır center at South-East Anatolia Region of Turkey.

Methods: 61 patients were included in the study (37 with hemophilia A, 8 with hemophilia B and 16 with von Willebrand disease). Ages ranged from 1 to 16 years (mean: 9.2 years). Out of 45 hemophilia A and B patients, 25 (55.5%) had severe hemophilia (factor level < 1%), 18 (40 %) had moderate hemophilia (factor level 1-5%) and two (4.5 %) had mild hemophilia (factor level >5-30%). The inhibitor positivity and titers were evaluated with Bethesda method.

Results: In this study, 7 patients were positive for inhibitors. Most of them 4 were low responders (< 5 Bethesda units), while 3 patients were high responders (>5 Bethesda units). Inhibitor was not found in any patient with hemophilia B and von Willebrand disease. Inhibitor prevalance was 18.9 % in all hemophilia A patients.

Conclusions: These results showed that the prevalence of inhibitor development was similar than the other centers of Turkey. It may be recommended that hemophilia patients should always be screened for inhibitors on regular intervals.

PO 23 | Liver Transplantation in Hemophilia Patients

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Background: Hemophilia is a sex-linked inherited bleeding disorder resulting in the deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B) and leading to lifelong bleeding complaints. The treatment consists of coagulation factor replacement. Prior to routine screening of blood products, many patients with hemophilia were infected with hepatitis C virus (HCV) and/or human immunodeficiency virus (HIV). As many of these patients are living longer now, they may develop end-stage liver disease and some will require liver transplantation.

Aims: To report our experience of liver transplantation in hemophilia patients.

Methods: A retrospective review of clinical notes and electronic case records.

Results: Between 2007 and 2016, 3 liver transplants were performed in 3 male patients with congenital hemophilia: 2 with hemophilia B and 1 with hemophilia A. The indication for transplantation was end-stage liver disease secondary to HCV infection. Two patients were co-infected with HIV and two had past alcoholic habits. A multidisciplinary team was involved in the peri-operative management and each patient had his own planning for the individualized optimization of clotting factor replacement strategy. No excess bleeding occurred in the peri or postoperative period. Overall survival patient rates at 1, 3 and 5 years, was 100% and factor VIII/IX levels became normal immediately after liver transplantation and remain within normal parameters.

Conclusions: In hemophilia patients with HCV associated end-stage liver disease, liver transplant has become an accepted treatment option. At our centre, the survival rates and peri-operative bleeding were similar to non-bleeding disorder patients, as reported by other Liver Transplantation Centres. Despite de data, concern remains about bleeding risks in the peri-procedure, however with close collaboration between the transplant team and the hemophilia treatment centre, it can be performed safely and successfully.

PO 24 | Chronic Osteolytic Lesions Secondary to Gigantic Pseudotumor of the Thigh in a Young Adult with Hemophilia B: Poor Prognosis and Outcome

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Background: Pseudotumor grows as a chronic, slowly expanding, encapsulated cystic mass as a result of recurrent hemorrhage in extraarticular musculoskeletal systems, Occasionally they cross the anatomic boundaries and could infiltratE and erodE the bones nearby. The bones usually implicated are femur, pelvis, tibia, and small bones of the hand.

Aims: To present an atypical case of hemophilic pseudotumor due to poor socioeconomic conditions and access to medical care that ended up in bad prognosis and health outcome

Methods: we present the case of a 30-year-old patient with hemophilia B with a history of irregular treatment with multiple hospitalizations during his childhood and a personal history of meningitis with epileptic syndrome, previous femur and ankle fracture, history of chronic anemic syndrome, and repetitive hematuria, consulted the emergency department with chronic ulcers of the lower limbs. Deformity of the limb, with a functional impossibility (wheelchair patient), a knee defect with a distal femur bone and proximal tibial fistula showing exposed. X-rays SHOWS osteolytic lesions of the entire femur and tibia with signs of chronic osteomyelitis secondary to a giant pseudotumor of the thigh that invaded both bones and compromised the skin as well. As a therapeutic intervention factor IX is administered, antibiotic, curettage, blood transfusion, psychological and nutritional support.

Results: This is the case of a patient with poor socioeconomic conditions who didn't consulted for medical care on time due to bad geographical access from where he lived. The patient presented rapid clinical deterioration and died after being admitted to our service.

Conclusions: Accurate knowledge of the extent and character of pseudotumors, gained through sonography, CT, and MRI, can be extremely useful in determination of proper management and follow-up

assessment . Bad prognosis and health outcome could be the result of poor access to medical care due to geographical or socioeconomical limitations in developing countries.

PO 25 | Pseudotumor ans Succeful Conservative Management in Hemophilia Patient with Inhibitors

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Background: Pseudotumors are a major complication in patients with hemophilia and can have a long life devastating effects, especially in the presence of inhibitors. The frequency and severity of hemophilic pseudotumours have decreased with the use of factor VIII replacement therapy. However, they may still occur in developing countries where facilities for diagnosis and treatment of hemophilia are not available. Only a few cases have been reported in the literature.
Aims: The purpose of our work is to showcase the experience in our Hemophilia Center in Barranquilla, Colombia and to highlight conservative management of hemophilia pseudotumor with long-term replacement therapy.
Methods: We report a 50 years old patient with severe haemophilia A plus low response inhibitor diagnosis with inguinal and pelvic pain, deformity in hip flexion, limp and palpable mass that was diagnosed with magnetic resonance as a pseudotumor of psoasiliac muscle with no involvement of the iliac crest. This patient underwent conservative medical treatment with rest, long term replacement of factor VIII therapy and physiotherapy with a remarkable clinical improvement; it was decided not to perform surgical treatment at the moment.
Results: Patient has developed satisfactorily and is still in periodical control observing the localized mass without increase in size or bone involvement of the iliac crest.
Conclusions: The behavior of the pseudotumour is different in children than in adults. In adults, they are usually observed at proximal bones, and do not usually respond to replacement treatment associated cost/benefit of interventional treatment versus replacement therapy, and the risk of recurrence should be taken into consideration when treating a hemophilic pseudotumor with the presence of inhibitor. **Conclusion:** The management of hemophilic pseudotumour aims at preserving function and includes conservative methods (immobilization, therapy), whenever this is feasible.

PO 27 | Unexpected Incidence of Inhibitor Development in a Hemophilic Population from the North of Spain

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Background: Inhibitor development is the major complication of therapy in developed world. It renders the treatment replacement ineffective and which increases both the costs of treatment and morbidity and mortality of the disease. Development of inhibitors is described in 36% and 5% of severe Hemophilia A and B patients respectively.
Aims: We describe our inhibitor population among our hemophiliac patients, as we notice we have a higher incidence and prevalence than it is described previously.
Methods: We have 54 patients diagnosed of hemophilia at the Hematology Department of the University Central Hospital of Asturias; 51 (95%) are HA (20 (37%) are severe); 3 (5%) are diagnosed of severe HB. We analyzed the variables correlated with development of inhibitor such as genetic mutation, age of diagnosis, number of exposures, type of treatment, symptoms, relapse, among others.
Results: In the period 1997- 2016, the overall incidence of inhibitor development in our severe hemophilia patient's population was 6 (26%); 4 (20%) patients having a severe HA and two (66%) patients having HB. The median age of the inhibitor detection was 11 months. The median of number of ED was 19 and one patient received continues infusion treatment of FVIII for intracranial hemorrhage in the diagnosis. The bleeding episodes included hemarthrosis, mucosal bleed, traumatic bleeding, hematuria and intracranial hemorrhage. A genetic study was performed in all of them: deletions and insertions were detected. Immunotolerance treatment was adopted in five patients (one was a transitory inhibitor); one patient with HB achieved immune tolerance, one patient with HA reached partial response, and two patients with HA and one patient with HB are refractory. In one HB patient, ITT had to be interrupted because a nephrotic syndrome development. In three refractory patients we used immunosuppressive treatment.
Conclusions: We find a higher incidence of inhibitor in our population than it is described. We have noticed a higher proportion of high risk mutations.

PO 28 | Hereditary Hypofibrinogenemia - Rare Familial Disease with Tendency to Increased Bleeding

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Background: Introduction: Hereditary hypofibrinogenemia is a rare familial disease with tendency to increased bleeding. Clinical manifestation vary proportionally to the depth of the deficit from solely post-surgery or post-traumatic bleeding to spontaneous haemorrhage of various intensity. Women tend to have spontaneous abortions and increased peripartum bleeding.
Aims: Case report: 21-year old woman was sent to our department in the 27th week of her first gravidity to find the cause of slightly prolonged INR. She had a history of more intensive periods and increased bleeding after the surgery of middle ear in childhood. The assessment

of fibrinogen level was not carried out at that time. We performed laboratory examination which confirmed slightly prolonged INR together with significantly prolonged thrombin time and decreased level of fibrinogen.

Methods: -

Results: The patient was carefully observed throughout gravidity. As she had no symptoms of bleeding and stable levels of fibrinogen 0,6-1,0 g/l, she was left without substitution. At the time of delivery she obtained 7 g of fibrinogen in fractions. We continued with the substitution for 6 subsequent days with 2 g dose per day, and next twice weekly 2g until the 21st day after delivery. Then we stopped the substitution (altogether the patient obtained 29 g). From the beginning of fibrinogen substitution until the 25th day after delivery we applied prophylactic dose of low-molecular weight heparin. The delivery of a healthy new born baby with normal weight was with no complications. For the whole peripartum and postpartum period the patient was free from increased bleeding or other complications. After the subsiding of gravidity hormonal changes we could trace the decrease of fibrinogen levels to 0,35 g/l.

Conclusions: We present a successful management of gravidity and peri and postpartum period in a patient with serious hypofibrinogenemia.

PO 29 | A Case with Factor X Deficiency and Malignant Melanoma

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Background: Factor X (FX) deficiency is an autosomal recessive congenital rare bleeding disorder that the bleeding tendency appears at any age, although the more severely affected (< 1% activity) presents early in life with umbilical-stump bleeding, CNS or GI bleeding. Common symptoms are at all severity levels which include epistaxis and menorrhagia. Therapy usually is administration of PCC and also a new pure FX concentrate.

Aims: Here we present a boy who was diagnosed with severe FX deficiency and malignant melanoma.

Methods: -

Results: Case: A boy whose brother has severe FX deficiency was diagnosed as severe FX deficiency after birth. He has been under PCC prophylaxis because of his frequent nose bleedings. He was admitted to our centre for his congenital giant nevus on his back. On physical examination, all systems were normal, the range of motion of his joints were full and there was a giant nevus with local hairy and nodular regions from his cervical region to lumbal region. He was consultated to Dermatology Department and a biopsy was performed some regions which were suspicious and was diagnosed as malignant melanoma. Then Positron Emisyon Tomografi (PET) was performed for the evaluation of metastasis. Bilateral axillary metastasis were found. His hemostasis control was done according to the protocol of our centre and he was operated. The majority of the nevus and bilateral axillary lenf nodes were exited. All materials which existed were malignant melanoma. The patient was evaluated as stage 3 malignant melanoma and Interferon was started. The patient's theraphy is still going on and none advers event had been seen.

Conclusions: Although malignant melanoma is the most frequent skin cancer in childhood, it is a rare cancer among the solid tumors. The congenital giant nevus is a risk factor for malignant melanoma. In this report, we presented a very rare case with FX deficiency and malignant melanoma that there is no similar case in the literature.

PO 30 | Inherited Thrombocytopathy - Impact on Patient's QOL

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Background: The QOL in case of women with inherited bleeding disorders is much lower due to objectively documented menorrhagia (reported to be 10% to 20%).

The gynaecological and obstetric management of these women, including appropriate anesthesia poses a lot of problems.

Aims: To highlight a difficult clinical problem

Methods: A case report and literature review.

Results: We present a case of 33 years old patient with rare bleeding disorder- thrombocytopathy due storage pool disorders. Defective platelet aggregation results from an intrinsic deficiency in numer of dense granules. Although applied treatment of menorrhagia had been individualized (III-B recommendation) there was no success.

Neither hormonal therapy (oral contraceptives [II-1 B], depot medroxyprogesterone acetate (DMPA) [II-3B], danazol [II-2B],) and non-hormonal therapy (antifibrinolytic drug tranexamic acid [II-1 B]) as well as desmopressin (II-1 B) have not increased QOL. As the patient was eager to plan pregnancy in near future, GnRH analogs [II-3B]) or local treatments (levonorgestrel-releasing IUS [II-1 B] had not be applied. Course of two pregnancies was unevenful. Before C-section performed due to orthopedic indications, transfusion of platelets was made. There was no major bleeding observed in the puerperium. In the offspring otherwise healthy, prolonged APTT with normal PLT count were noted.

Conclusions: QOL of patients with rare bleeding disorders is often low. A multidisciplinary approach is needed not only during pregnancy but in every stage of women's life.

PO 31 | Duodenal Dieulafoy´s Lesion in a Patient with Type 2 von Willebrand Disease: Case Report from Colombia

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Background: Recent studies have suggested that recurrent gastrointestinal bleeding (GIB) is a challenging complication in patients with von Willebrand disease (vWD).

Aims: .

Methods: We describe a 73 year-old Hispanic woman with Type 2 vWD who had recurrent GIB while on demand treatment with von Willebrand factor concentrate (Immunate®). The first episode was controlled with Immunate® *tis in die* 100 IU Kg^{-1}, but for the remaining 15 episodes, she required complementary treatments despite infusion of vWFC (blood transfusion, hypervolemic liquids, tranexamic acid, octreotide and proton bomb inhibitors infusions). We started thalidomide (50 mg *quaque die* -QD-), but GIB recurred and thalidomide was doubled; however, myelosuppression, dizziness and a superficial venous thrombosis in the left lower limb were detected; these findings disappeared spontaneously after suspension of thalidomide. We tried two more approaches:

1) Atorvastatin 10 mg PO *quaquenoctis*, but GIB recurred;

2) Prophylactic treatment with Immunate® 20 IU Kg^{-1} three times per week, then switched to Haemate-P® at the same dose of Immunate, but nonetheless, GIB recurred.

Finally in June 2016, and after two years of follow-up and multiple pharmacotherapy approaches, a new GIB occurred and Endoscopy revealed a Duodenal Dieulafoy´s lesion, managed with endoscopy injection of Adrenaline 1:10 000 + Argon plasma and hemoclips (#06). After this, our patient remained stable, without GIB manifestations during seven months, and prophylactic treatment was suspended after 6 weeks of a negative endoscopy.

Results:

Conclusions: Despite multiple treatment options and different invasive procedures, we think, as stated by Makris *et al*, that the diagnostic success of the procedure is higher in the actively-bleeding patient. We also think that identifying and controlling the lesion is an effective way to control recurrent GIB, even in the presence of other supportive care. In our case, prophylactic treatment proved to be ineffective in the prevention of recurrent GIB episodes.

PO 32 | Von Willebrand Disease Diagnosis in Algeria: A Monocentric Study

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder, due to quantitative and/or qualitative defects in von Willebrand Factor (VWF). It is characterized by a high clinical, biological and molecular heterogeneity. The VWD diagnosis is difficult and requires a panel of biological tests.

Aims: We report the clinical and biological characteristics of the VWD in 43 Algerian patients diagnosed in Lamine Debaghine hospital.

Methods: The VWD diagnosis is established upon three first level tests including the VWF Ristocetin Cofactor, the VWF Antigen concentration and Factor VIII activity, the bleeding score (BS) was also calculated. Second level tests are used for the subtyping including the RIPA, VWF-Collagen Binding, Plasmatic VWF multimers analysis and FVW-FVIII binding.

Results:

- Median age of diagnosis is 12 years old.
- Sex ratio M/F is 1:2.
- BS versus age: 9,7 for adults and 6,5 for children.
- Patients classification: 62 % type 1 of VWD, 08% type 2 (03 patients with type 2A and 01 patient with type 2M) and 30% type 3.

Conclusions: Type 3 of VWD seem to be more frequent in Algeria because of the high level of consanguinity. Molecular analysis should be performed for these patients to improve the understanding of VWD pathogenesis in our country.

MANAGEMENT OF THROMBOEMBOLISM

PO 33 | The Relevance of Personalized Therapy by New Oral Anticoagulants. Current Possibilities of Laboratory Monitoring and Control in Difficult Patients

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Background: Nowadays, routine laboratory monitoring in patients treated by the new oral anticoagulants (NOAC) is not accepted because of the proven effectiveness of fixed doses. NOAC is designed as an alternative to vitamin K antagonists (VKA). There are reports about the possible using of laboratory control of the NOAC in routine and integral tests for evaluation of hemostasis.

Aims: To justify the personalized selection of doses of NOAC under the control of thrombodynamic test and to demonstrate the need for monitoring of preventive doses to reduce the risk of re-thrombosis and bleeding.

Methods: The research included 49 patients with episodes of arterial or venous thrombosis and patients with a thrombotic readiness condition. The 25 patients received anticoagulants: low molecular weight heparins (8), VKA (4), NOAC (13). The examination included coagulation, D-dimer and Thrombodynamic test (TD). The control group included 18 patients with atrial fibrillation, which was planned appointment of the NOAC for primary prevention of stroke.

Results: Rivaroxaban was prescribed for 20 patients and Dabigatran - for 29 patients. Before the prescription based on TD hypercoagulation shifts were identified. In patients receiving the drug in 73.7% and 51.7% cases respectively was achieved normal coagulation. In other cases required dosage increased with the subsequent control of hemostasis. Only 10% the patients of the control group had pathological hypercoagulation due to results of D-dimer and 5% according to TD. There was normal coagulation in 100% after prevention by NOAC, 3 weeks later.

Conclusions: The prevention of thrombosis needs achieving the normal coagulation with low molecular weight heparins and then switching to NOAC under hemostasis control. Rivaroxaban demonstrated a higher frequency of achieving the normal coagulation. TD is useful for the initial evaluation of the hemostasis, for monitoring of anticoagulants, sensitive to the latent hypercoagulation and allows us to choose the optimal and safe dose of drugs.

PO 34 | Long-term Anticoagulation with Direct Oral Anticoagulants (DOACs) for the Prevention of Recurrent Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE)

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Background: To balance the long-term risks of recurrent venous thromboembolism (VTE) once anticoagulant therapy is stopped against the risks to continue the therapy.

Aims: To assess the benefit-risk trade off continued direct oral anticoagulant treatment in symptomatic VTE patients with at least 2 episodes of idiopathic VTE, treated for at least 6-12 months after the last episode, and in whom physicians had recognized the need to continue anticoagulation.

Methods: In the DOACs extension study in the patients that taked anticoagulants for 6-12 months a clinically important benefit was observed, but there are no data for a further continuation of therapy.

Our center has followed 34 patients on very long-term anticoagulation. Their medical history showed a median of 3 recurrent episodes of idiopathic VTE (range 2-5). Recurrent episodes were DVT in 26 patients, 2 episodes of PE in one patient, both PE and DVT in 7 patients. Patients have practiced DOACs, after the treatment of 6 months from the last thrombotic episode, for a period of 12-36 + months (median 19 months). One patient were treated with dabigatran, 10 with rivaroxaban and 23 with apixaban.

Results: There were no thromboembolic relapses, but a patient receiving rivaroxaban, with a history of two episodes of DVT and a mesenteric thrombosis presented an episode of cryptogenic stroke. There were three minor bleeding events, one with apixaban (bleeding from hemorrhoids) and two with rivaroxaban (1 epistaxis, and a patient who reported heavy periods, but not complicated by anemia). Neither major bleeding nor clinically relevant non-major bleeding was reported. There were no other adverse events related to DOACs.

Conclusions: In patients with symptomatic and recurrent VTE, in whom physicians have deemed necessary to continue the anticoagulation, a favorable benefit-risk profile of continued anticoagulation with DOACs was observed and would result in an important benefit in recurrent VTE.

PO 35 | Laboratory Tests for Management in Patient with Direct Oral Anticoagulants

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Background: A major advantage of these agents is the lack of a requirement for monitoring; however it´s recommended monitoring the drug for Rivaroxaban Apixaban and Endoxaban use anti-Xa chromogenic studies and for Dabigatran Hemoclot thrombin inhibitor and Ecarin clotting time (DTI test).

Aims: Detection of risk of develops bleeding with monitoring the drug in patient with direct oral anticoagulants.

Methods: We conducted a retrospective study with 227 patients who received direct oral anticoagulants (DOACs) between January 2015 and December 2016. One hundred eighteen patients (52%) receive Rivaroxaban, fifty patients (22%) receive Dabigatran and fifty nine patients receive Apixaban (26%). We analyzed the variables that's increases the bleeding risk such as age, weight, prothrombin time (PT) and activated partial thromboplastin time (aPTT), therapeutic range of the drug, and measurement of serum creatinine.

Results: We found 10% of toxicity with Dabigatran, a 7% with Rivaroxaban and a 3% with Apixaban. Thirty-five patients (15%) developed bleeding of which 11% patients had a minor bleeding and a 4% of patients had a mayor bleeding, we also found that 6% of patients with Dabigatran, 2.5% with Rixaroxaban and 1.5% with Apixaban developed thrombotic episodes. Twenty percent of patient didn't have therapeutic range of the drug. For each DOACs is shown in Table 1.

When we analyzed the patients who had hemorrhage we found that all patients with Dabigatran prolonged aPTT and the PT in 80%, for other DOACs is shown in Table 2.

Conclusions: In our series, in patients with dabigatran and who suffered bleeding, we found a significant prolongation of aTTP and PT, demonstrating the importance of laboratory tests prior to the administration of these agents and in emergency situations, for these reason should be include PT and aPTT, therapeutic level of the drug and creatinine measurement, within the emergency laboratory tests in patients that receive DOACs.

TABLE 1 Results of statistic study

Variable	Apixaban	Rivaroxaban	Dabigatrán
Percentaje	25.9%	51.9%	22%
Toxicity	3.3%	6.7%	10%
Thrombotic episodes	1.6%	2.5%	6%
Percenteje out of therapeutic range	8.4%	24.5%	22%
aPTT prolonged	8.4%	2.5%	80%
PT prolonged	16.9%	21%	4%

TABLE 2 Patients with bleeding

Variable	Apixaban	Rivaroxaban	Dabigatran
percentaje	34.2%	20.8%	7.8%
aPTT prolonged	8.3%	22.2%	100%
PT prolonged	25%	33.3%	80%
median therapeutic range	177	142	154
median serum creatinine	1.02	0.9	1.35
median age	74	81	71
median weight	66	72	65

PO 36 | Effective reversal of High Dose Dabigatran Intoxication by Idarucizumab in a Bleeding Polytrauma Patient

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Background: The monoclonal FAB fragment antibody against the oral direct thrombin inhibitor (DTI) dabigatran ("DABI") etexilate (Pradaxa™), idarucizumab (Praxbind™, "IDARU") has recently been licenced as a specific antidote for the reversal of the anticoagulant action of DABI in emergency situations.

Aims: Evaluation of efficacy of IDARU in a 63-year-old female, who had taken DABI for the prevention of stroke due to atrial fibrillation.

Methods: The patient had a severe trauma due to defenestration from 2nd floor presumably by suicidal attempt with complex fractures of pelvis, lower spine, distal right thigh, left ankle, and foot. Impaired renal function and enteroparesis induced by trauma and deep anaesthesia probably affected DABI resorption and pharmacokinetics.

Coagulation was monitored from citrate plasma by prothrombin time (PT), APTT, thrombin time (TT), clotting factors, e.g. factor II (FII). DABI levels were measured by a commercial thrombin inhibition test.

Results: Initial laboratory findings were trauma-/bleeding-related effects (table 1). During the next 4 days (d) clotting times of PT, APTT and TT gradually prolonged despite no anticoagulant had been applied. Further analysis revealed a profile typical for a high-dose DTI treatment (PT, APTT, TT prolongations, low FII activity). After confirmation of DABI intake, a DABI level > 10-fold higher than expected median peak level of 175 ng/ml according to SmPC 2 h after intake of 150 mg BID DABI was measured. As a decline of hemoglobin (Hb) indicated pelvic hemorrhage, a rapid reversal of DABI was done by 5 g IDARU i.v.. Further Hb decline was stopped, a marked decline of DABI and shortened PT, APTT, TT and increased FII level were observed (table 1). 5 h later a rebound of DABI occurred. 3 d later DABI was below detection limit.

Conclusions: IDARU is useful to reverse DABI anticoagulant effect. However, in case of very high DABI levels the standard dose does not completely neutralize the drug, and a rebound phenomenon has to be expected.

TABLE 1

	23.10.2016 7:33 p.m.	28.10.2016 2:20 a.m.	28.10.2016 8:43 p.m.	29.10.2016 4:07 a.m.	29.10.2016 9:26 a.m.	29.10.2016 8:00 p.m.	30.10.2016 4:11 a.m.	31.10.2016 3:59 a.m.	normal range
PT	10.8	33.8	79	13.6	22.3	17.2	16	9.7	7.6-9.8 sec
INR	1.2	3.4	>5.6	1.5	2.3	1.8	1.7	1.1	
APTT	28.3	89.2	111.6	67	68.9	55	52	34.5	25-35 sec
TT	-	>150	-	>150	>150	>150	>150	>150	<21 sec
II	71	11	2	45	23	34	38	95	83-145 %
VII	82	72	75	102	91	85	83	102	74-158 %
VIII	131.5	41	-	103.4	81.3	102.5	102.8	244.6	67-220 %
X	62	78	52	84	79	79	73	101	80-140 %
Dabigatran	-	-	1850	330 (after 5g Idarucizumab)	880	600	400	110	ng/ml

PO 37 | Does Intra-venous Unfractionated Heparin (UFH) Have a role In the Management of Acute Peripheral Vascular Ischaemia and Surgery In 2017?

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Background: Low molecular weight heparin (LMWH), at a weight adjusted dose without coagulation monitoring is commonly used in the treatment of venous thrombo-embolic events. Given its shorter half life and greater reversibility, unfractionated heparin (UFH) remains an accepted practice in peripheral arterial vascular interventions, however prescribing is demanding, monitoring is necessary with dosing revisions.
Aims: To determine the use and therapeutic efficacy of UFH in patients of a vascular surgical unit undergoing acute interventions, by analysis of prescribing, monitoring and outcomes.
Methods: A retrospective audit of an electronic prescribing system, from May 2015 to October 2016, identified 18 patients undergoing peripheral arterial vascular interventions and UFH therapy. Electronic records for peri-operative clinical details, prescribing and activated partial thromboplastin time ratio (APTT-r) testing were analysed, together with dedicated UFH dosing charts.
Results: Thirteen patients had had previous arterial events. Ten patients underwent surgical interventions, most commonly embolectomy, with eight managed conservatively for acute ischaemia. Heparin bolus therapy was documented for 17 cases, with infusions in 17, nine being continued for less than 24 hours, the longest being for six days. A therapeutic APTT-r was achieved at 12 and 24 hours in 6% and 22% of cases respectively and overall in only 50% during an infusion. There were three subsequent thrombotic events amongst the surgical cases, while two conservatively managed cases had progressive ischaemia. No excessive haemorrhage nor heparin induced thrombocytopaenia was recorded.
Conclusions: Most patients had had previous thrombotic events. UFH bolus therapy was common with infusions relatively limited in duration. Performance against a target APPT-r was poor. Whilst no excess haemorrhage was observed, the thrombotic/ischaemic outcomes suggest that the UFH schedule was of limited benefit and revision to stat bolus and, or LMWH should be assessed.

PO 38 | Oral Anticoagulants in Patients with Hereditary Protein Z Deficiency - A Case Report

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Background: Protein Z (PZ) deficiency can cause clinically relevant bleeding tendency. Nevertheless, also systemic anticoagulant therapy may become necessary in some patients with this rare bleeding disorder.
Aims: We present the clinical course and laboratory findings of a 85 years old female patient with PZ deficiency, in whom anticoagulation was indicated because of intermittent atrial fibrillation.
Methods: Case Report.
Results: The patient is well known in our hemostatic care unit since many years with a PZ deficiency (680-820 µg/l; ELISA, normal range 1.000-4.000) and suffers from spontaneous hematomas and easy ulorrhoea; also a pulmonary embolism occured after hysterectomy in her 40th year of life, and she also suffered from a deep vein thrombosis of the lower leg at the age of 79 years. A PZ deficiency could also be confirmed in her daughter suggesting a hereditary disorder. Because of intermittent atrial fibrillation oral anticoagulant (OAC) therapy now became necessary and was first started with phenprocoumon at a target INR of 2,0 - 2,5. Under this regime spontaneous bleeding of the thigh occured finally requiring clinical treatment. Therefore the anticoagulant was changed to apixaban 2x 2,5 mg daily. Now the patient suffered from dramatic spontaneous hematomas and bleeding of the mouth mucosa; measuring apixaban 5,5 hours after its intake revealed a peak concentration of 253 ng/ml (chromogenic essay; therapeutic range 69-221), while PZ concentration was 680 µg/l. Anticoagulation was now changed to low-intensity - phenprocoumon with a target INR of 1,7 - 2,0.
Conclusions: OACs should be used only with special care in patients with hereditary PZ deficiency, and routine laboratory monitoring of the therapy seems to be necessary even when novel OACs are used.

PO 39 | Prevalence of Laboratory Markers of Thrombophilia in Pregnant Women with Normal Obstetric Outcomes

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Background: The existence of thrombophilia amplifies the state of hypercoagulability inherent to pregnancy, which can lead to obstetric and perinatal complications.
Aims: We aimed to identify the prevalence of thrombophilia in pregnant women with normal obstetric outcomes.
Methods: Cross-sectional, prevalence study, with pregnant women in prenatal follow-up at HC-FMUSP. We included patients who denied previous diseases with single and spontaneous gestation and a morphologically normal fetus. We excluded women: with personal or familial history of 1st degree thrombophilia or venous thromboembolism or thrombophlebitis; who did not collect the requested tests; who developed severe forms of preeclampsia or placental insufficiency. We searched: factor V Leiden and prothrombin gene mutation; antithrombin, protein C and protein S activities; homocysteine serum levels,

lupus anticoagulant and anticardiolipin (IgG and IgM) antibodies. We collected the following information on delivery: gestational age, delivery route, Apgar scores and birth weight.

Results: 81 patients accepted to participate in the study, 62 collected blood samples for examination; one was excluded for diagnosis of thrombophlebitis in this gestation. Data are available on 50 deliveries; four cases were excluded (two cases of fetal malformation, one case of altered Doppler velocimetry and one case of superimposed preeclampsia). The prevalence of factor V Leiden (heterozygosis) was 1.9% and mutation of the prothrombin gene (heterozygosis) was 1.8%. 20.0% presented antithrombin < 79%; 58.5% presented protein S < 55%. The concepts were born with gestational age of 39.7 ± 1.0 weeks and weight of 3242 ± 407 grams; 15.2% was small for gestational age (according to Fenton curve), 2.2% presented Apgar score < 7 in the 5th minute of life.

Conclusions: Laboratory markers for thrombophilia may undergo changes during pregnancy. Women with altered antithrombin or protein S functional dosage are being called for new blood collection at least two months postpartum.

PO 40 | Variation of Clotting Factor Levels of Pregnant Women Attending Antenatal Clinics in Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo State, Nigeria

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Background: An estimated 50,000 Nigerian women die each year from complications of pregnancy and childbirth, accounting for 10% of global estimates of pregnancy maternal death.

Aims: This cross sectional study was set out to evaluate the effects of pregnancy on some coagulation factors; FV, FVII, FVIII, FIX, plasma Fibrinogen Concentration, platelet count and PF-3 availability.

Methods: A total of 578 subjects comprising 396 pregnant women and 182 non pregnant women were recruited for the study using standard manual methods.

Results: Factor VIII (% activity) (217.24±1.95) and platelet count (X10^9/µl 339.98±4.69) of the pregnant women were significantly higher than the Factor VIII (% activity) (199.13±1.02) and platelet count (X10^9/µl) 208.54±7.09 of the non-pregnant women (p< 0.05) respectively. Thrombin was significantly reduced in pregnant group over the non-pregnant group (p< 0.05). Plasma fibrinogen concentration (% activity) (86.28±0.43) of the non-pregnant women was found to be significantly higher than the pregnant women (78.53±0.59) (p< 0.05). The PF-3(sec) and platelet count (×10^3/µl) of the 2nd trimester versus 3rd trimester were found to be statistically lower than those of the 3rd trimester (p< 0.05). The Plasma Fibrinogen concentration (% activity) of Gravida 1 (77.07±1.18) was significantly lower when compared with Gravida 2 (79.26±1.05), Gravida 3 (81.51±0.98) and Gravida 4 (78.40±1.05) (p< 0.05) respectively.

Conclusions: We observed that coagulation factors were significantly increased when measured together in pregnant women. Further investigation reveals factors VIII and fibrinogen to be significantly higher, while thrombin was significantly decrease in pregnant women respectively. Moreso, haemostatic attention should be given to pregnant women throughout the course and events of pregnancy to prevent or reduce the risk of haemorrhagic disorders and possible disseminated intravascular coagulation (DIC).

PO 41 | Catastrophic Antiphospholipid Syndrome in Obstetrics Practice (Clinical case)

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Background: catastrophic antiphospholipid syndrome (CAPS) is an uncommon, often fatal, variant of the antiphospholipid syndrome that results in a widespread coagulopathy and high titres os antiphospholipid antibodies and affects predominantly small vessels supplying organs with the development of multiorgan failure.

Aims: To consider CAPS development and to observe the pregnancy after CAPS.

Methods: since 2001 to 2016 we discovered 17 patients with CAPS development and observed the pregnancy after CAPS.

Results: Patient Z.24 years old, was admitted to hospital at term of 27-28 weeks. Her first pregnancy was finished with spontaneous abortion. In one hour after hospitalization, eclampsia was developed and patient was delivered with cesarean section. The premature girl (weight 980 g, height 24 cm) was born. Blood loss composed 700 mL. After delivery the infusion therapy was started, including fresh frozen plasma, anti-aggregational agents, hypotensive and uterotonic drugs, corticosteroids and antibiotics. Due to polyorganic failure, plasmapheresis and anticoagulant therapy LWMH were conducted. During hemostasis research LA and APA IgG/IgM (148 GPL, MPL) were revealed.The state of patient was severe for several days, but the level of APA became lower - 87 GPL, MPL, LA was positive. On the 20th day after delivery, the woman was discharged home in satisfactory state and was recommended to continue LMWH therapy for 3 months more.

Conclusions: The diagnosis may be missed as it is well known that the antiphospholipid antibodies may be negative at the time of the thrombotic events, only to reappear later. Besides this, serological samples might be uninformative because of previous cytostatics and steroid therapy. The development of obstetric complications in patients with APS makes it necessary to suppose presence of the catastrophic variant of this syndrome. All patients with a history of previous thrombotic events or pregnancy losses, should be tested for the presence of antiphospholipid antibodies.

PO 42 | Personalized Antithrombotic Prophylaxis in Patients with Multigenie and Combined Forms of Thrombophilia and APS

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Background: patients with multigenic and homozygous forms of thrombophilia - this is the group of women with repeated nonspecific thromboses, unusual localization thrombosis, arterial and venous repeated thromboses repeated placenta-mediated complications. In the history of such patients - the syndrome of fetal loss, premature detachment of the normally located placenta, thrombosis during the admission of hormonal contraceptives, hormone replacement therapy. The standard approach to it is not acceptable. They require a personalized approach to the choice of therapy, the selection of the dose medications, assessment of the effectiveness and the individual definition of the duration of the treatment and prevention of complications.

Aims: to observe the pregnancy of women with multigenie and combined forms of thrombophilia and APS, to evaluate the efficiency of treatment and personalized approach to each case.

Methods: from 2001 to 2016 years we observed the pregnancy of 59 women up to the age of 37 years with multigenic and combined forms of thrombphilia.

Results: all patients with thrombosis history genetic trombophilia received LWMH. Women with APS - LWMH and antiaggregants, intravenous immunoglobulin. The presence of CAPS demanded the plasmapheresis and FFP. Itcases of ADAMTS-13 deficieny - LWMH and antiaggregants. Assessment of the effectiveness of the treatment was carried out on the basis of anti-Xa activity (theraputic level =0.5-0.8). 57 children were born from 54 women. There have been no cases of hemorrhagic and thrombotic complications.

Conclusions: Only the personalized approach to the diagnostics of thrombophilia and choice of the treatment allows you to prevent the development of obstetric complications.

PO 43 | Thrombophilia and IVF

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Background: Women with thrombophilia have been shown to be at increased risk, not only of pregnancy associated thromboembolism and vascular complications of pregnancy, including pre-eclampsia and fetal loss, but also of IVF (In Vitro Fertilisation).

Aims: Whether the thrombophilia can be a cause for IVF failures.

Methods: 25 women with a single or multiple IVF failure were followed. 18 pregnant women, whose first IVF had failed (1 group), and 7 women had repeated IVF failure (3 or more-2 group), all with obstetric history of complications in pregnancy, and with individual or family history of thrombosis. Testing for thrombophilia was conducted before the pregnancy, i.e. in the period of the planning of the pregnancy.

Results: The prevalence of thrombophilia was assessed in all 25 women Genetic thrombophilia was found in all 25 women, multi genetic thrombophilia in 18 (11 in first and 7 in second group). Mutation of methylenetetrahydrofolate reductase (MTHFR-C677T) was found in 10 women in the first group, and in all of the women in the second, f.V Leiden-5, prothrombin 20210A-4 combined with deficiencies in protein S and C-1 and antithrombin III-3 and antiphospholipid antibodies (b2-glycoprotein I) thrombophilic factors were found in 9 women. Hyperhomocysteinemia was found in 8 women with MTHFR mutation (4 in each group). All women with successful pregnancy after IVF (only one woman doesn't become pregnant) failure received therapy before and during the pregnancy. One woman had a miscarriage (loss of cardiac function in the fetus). 26 healthy newborns were born from 23 women with thrombophilia and IVF failures.

Conclusions: The data stated above suggests that thrombophilia plays a role in the genesis of IVF failure, with a higher prevalence in cases of multi genetic and combined thrombophilia. Women with IVF failures should be screened for thrombophilia. Intervention with antithrombotics might improve pregnancy outcome in this women. Further research is certainly needed to shed light on this very important matter.

PO 44 | Risk Factors and Outcome of Thromboembolism in Pregnancy and Postpartum Period: A Two Years Retrospective Analysis in a Tertiary Care Hospital of Pakistan

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Background: Venous thromboembolism complicates 0.5 to 3.0 per 1,000 pregnancies and is the leading cause of maternal mortality in the most developed countries.

Aims: To evaluate the risk factors and clinical outcome of thrombosis in pregnancy and postpartum period.

Methods: A retrospective two years analysis was done from January 2015 till December 2016 at Aga Khan University Hospital. Ethical exemption was sought from institutional ethical committee. Informed consent was obtained. Files coded with thrombosis and pregnancy was reviewed for their associated risk factors and outcome. Frequencies were generated for quantitative variables.

Results: A total of 11 pregnant and postpartum females were diagnosed with thrombosis. The median age was 30 years(22-42 years). Out of 11, 8 had postpartum thrombosis. The commonest site was deep venous thrombosis(DVT) of lower limbs (55%), followed by cerebral venous sinus thrombosis (18%), inferior vena cava thrombosis, pulmonary embolism and upper limb DVT(9%) each. C-section and prolong bed rest was found to be risk factors for post-partum thrombosis. No identifiable risk factor was found for ante-partum

thrombosis. Out of 11 females, 8 (72%) were screened for heritable thrombophilia, of which two were found to have low protein S levels. Single maternal death occurred in postpartum due to pulmonary embolism following inferior vena cava thrombosis. Single induced abortion was carried out for warfarin induced embryopathy in a patient with antepartum upper limb thrombosis.

Conclusions: Thrombosis was most commonly seen in postpartum period while C-section and immobilization was found to be commonest associated risk factors. Thrombophilia screening was performed during active thrombotic episode; hence making the diagnosis of heritable thrombolphilia questionable in two females with low protein S. Maternal mortality was found to be 9%. A prospective study needs to be done to determine risk factors of thrombosis in pregnancy and postpartum period with their long term outcome and recurrence.

PO 45 | The Clinical Significance of Determining Homocysteine Levels and Functions of the Natural Anticoagulants in Women Taking Combined Oral Contraceptives and Planning Pregnancy

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Background: Hormonal contraception can activate hemostatic system as a side effect, deplete natural anticoagulants and promote the development of thrombotic and pre-thrombotic state.

Aims: Investigation of the basic indicators of hemostatic system in women who have been taken hormonal contraceptives during a long time (3 to 5 years).

Methods: Since 2010 have been examined 105 patients who received hormonal contraceptives (oral contraceptive, containing 30 mcg of ethinylestradiol with 2 mg of dienogest). The study included women who had no family history, and no their own thrombotic history.

Results:

1. The level of homocysteine was above normal in 63% of patients
3. Resistance to activated protein C was detected in 45% of patients.
4. Antithrombin III at the lower limit of the normal value in 68 % of patiens
5. Platelet aggregation was increased in 78% of patients.

Conclusions: Considering the undoubted influence of long-term use of hormonal contraceptives on hemostatic system with obvious signs of pro-thrombotic state, pregnancy is not recommended immediately after stopping use hormonal contraceptives. It is recommended to use B-vitamins, anticoagulants, antiplatelet agents, folic acid.

PO 46 | Case Reports of Successful Management of Pregnancy Complications and VTE in Women with Inherited Thrombophilia

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Background: Inherited thrombophilia (Factor V Leiden, Prothrombin G20210A and MTHFR C677T gene mutations) has been suggested as a possible cause of recurrent pregnancy loss (RPL) and venous thromboembolism (VTE). Inherited thrombophilia is a genetic disorder of blood coagulation resulting in a hypercoagulable state, which can result pregnancy complications. Inherited thrombophilia is a main risk factor of thrombosis during pregnancy and postpartum.

Aims: According to this fact, that management of pregnancy complications is different, the aim of analysis of case reports was to show our experience in successful management of pregnancy complications and VTE in women with inherited thrombophilia.

Methods: Under observation were 20-26 years old 2 pregnant women who had recurrent pregnancy loss and venous thromboembolism. In Patients PCR analyses were used for detection of inherited thrombophilia and duplex ultrasound for thromboembolism and pregnancy complications. In both cases, prophylaxis of thrombosis during the pregnancies was done using subcutaneous injection of enoxaparin 40 mg per day.

Results: Patient L.M. 26-year-old with a previous medical history of venous thromboembolism (DVT of the left leg) and pregnancy loss. Patient's genotype: homozygous mutation of factor V Leiden.

Patient T. A. 20 -year-old with a previous medical history of venous thromboembolism (DVT), one pregnancy loss and one episode of premature birth with infant *death*. Positive *family history* for VTE (Patient's mother and grandmother suffered with VTE). Patient's genotype: triple heterozygous form of FVL/PTH/MTHFR mutations.

In both cases pregnancy and delivery were uncomplicated for both the mother and the fetus, and a normal vaginal delivery took place.

Conclusions: Prophylaxis of thromboembolism and pregnancy complication during the pregnancy in patients with homozygous carrier of Factor V Leiden and compound thrombophilia can be safe and effective using prophylaxis dose of LMWH.

PO 47 | Control of the Anticoagulants and Antiplatelets during the Pregnancy in Women with Complicated Obstetric Medical History with Routines and Global Tests for Assessment of Hemostasis

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Background: There is algorithm of initial examination and rules of treatment by low molecular weight heparins (LMWH) in women with complicated obstetric medical history.

Aims: To prove the personalized choice of dose of LMWH and acetylsalicylic acid (ASA) under control by tests for assessment of hemostasis; to show the need for individual control for improving the pregnancy.

Methods: The 78 women with complicated obstetric medical history were included in research and divided into 4 groups: infertility, early abortions, late abortions, premature birth. All patients were examined to exclude congenital and acquired thrombophilia, as well as evaluation of hemostasis by routine and global tests: thrombodynamics (TD) and thromboelastometry (NATEM). The dose selection was under the control of the TD, D-dimer and XIIa-dependent fibrinolysis. The algorithm have been developed and implemented control of the D-dimer and XIIa-dependent fibrinolysis every 2 weeks, the TD every 4-6 weeks from the date of a positive pregnancy test and up to 34 weeks, the NATEM is at 36 weeks.

Results: The patients who have not received LMWH before including to the study were found significantly more pronounced chronometric changes of hypercoagulation according to the TD, and the largest percentage of spontaneous clot formation (33%). Due to our algorithm, the dose of Enoxaparin was increased up to 0.4 ml per day in 65% of patients; to 0.6 ml in 13%; to 0.8 ml in 5.2%; to 1.0 ml in 3.4% of patients. The dose of ASA of 50 mg per day was unchanged in 77% of patients; it was increased to 100 mg in 15% and up to 150 mg in 8% of patients. In all patients the pregnancy ended well.

Conclusions: TD is useful for the evaluation of the hemostasis, for monitoring of anticoagulants, and it is hidden hypercoagulation-sensitive. The D-dimer index is useful for evaluation of pro-thrombotic readiness before prescribing the LMWH; however, it is less useful in monitoring the effectiveness during the prescribed therapy by LMWH.

PATHOGENESIS OF THROMBOEMBOLISM

PO 49 | β2GPI Titer and its Clinical Correlation with Laboratory Antiphospholipid Antibodies Syndrome Presented by Fetal Loss

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Background: Despite international efforts to standardize laboratory testing for antiphospholipid antibodies (APA), significant variation in the performance of different assays remains a critical problem.

Aims: To study the clinical utility of Beta2 glycoprotein1 (β_2GPI) and anticardiolipin (ACL) antibodies, in conjunction with the activated partial thromboplastin time-lupus anticoagulant (APTT-LA) & dilute Russell viper venom time (dRVVT) for diagnosis of APA in patients with repeated pregnancy loss.

Methods: The study was conducted on 40 Egyptian females attending Ain Shams University Hospitals with history of ≥3 consecutive early miscarriages, or one fetal loss more than 10 weeks. An informed consent is obtained from all patients. Laboratory testing was done in a two-step procedure for the coagulation-based assays; screening by APTT-LA & dRVVT screen; Positive patients were further tested by mixing study and dRVVT confirm respectively. All patients were tested for ACL IgG &β2GPI IgG. Data were analyzed statistically using SPSS version 24.

Results: Fifteen patients had prolonged APTT-LA time, only 2 were confirmed positive after applying Rosner index and these 2 patients showed positivity with ACL, while only one was positive for β2GPI. Twenty-five patients were positive for dRVVT screen, only 17 were confirmed positive by dRVVT correction ratio. Thirteen patients were positive for both APTT-LA & dRVVT screen ratio. Perfect agreement was found between Rosner Index & ACL, fair agreement was found between Rosner Index &β2GPI, slight agreement was found between dRVVT screen and APTT-LA, fair agreement was found between ACL &β2GPI (Kappa index=1.0, 0.4, 0.3, 0.4 respectively). Only Rosner index was significantly correlated with number of abortions (P=0.01).

Conclusions: dRVVT correction ratio showed high sensitivity and specificity 100% for each. APTT-LA showed low sensitivity 57.89%, mixing study improved the specificity for diagnosis of APS.

PO 50 | Antiphospholipid Syndrome in Pregnancy: A Case Report

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Background: Antiphospholipid syndrome (APS) is an autoimmune disorder, associated with pregnancy complications such as preeclampsia, thrombotic episodes, autoimmune thrombocytopenia, fetal growth restriction, and recurring fetal loss.

Aims: APS is one of the few treatable causes of pregnancy loss with chances of more than 70% successful pregnancy rate if adequately treated.

Methods: A 29- year old pregnant patient 8 weeks´ gestation(gw) with previous unexpected fetal death in the third trimester (35 gw) was referred to our Institute for detailed coagulation screening including Tr, PT, APTT, TT, DD, AFA, LA, AT III, PC and PS.The results were within normal range, maintaining so during the whole pregnancy. LA was twice positive. No systemic autoimmune disease was found. Low-dose aspirin and a prophylactic dose of low molecular weight heparin (LMWH) were introduced (enoxaparin a 40mg/day, s.c.). Later on, she was screened for APC-R (negative results obtained) while heterozygous mutation of MTHFR C677T gene was found. Normal homocystein levels were measured. From 27gw, weekly

cardiotocography (CTG) monitoring was performed with occasional tachycardia till the 32 gw detected. Aspirin was discontinued at the 30 gw and the prophylactic dose of LMWH was increased to 60mg /day. From 34 gw, daily CTG monitoring and ultrasonography was performed. Placenta was rapidly aging and more frequent tachycardia was registered. On 35w6d, a caesarian section was carried out . The thromboprophylaxis was continued six weeks after delivery with no side effects recorded.

Results: Rest regimen was recommended and carefull monitoring by both the gynecologist and coagulation disorder specialist with in time initiation of anticoagulation. A preterm c-section was performed which resulted in successful pregnancy outcome.

Conclusions: Attentive clinical care with regular check-ups followed by double drug regimen of combining LMWH with low-dose aspirin has proved to be a successful therapeutic management in reducing the obstetric complications.

PO 51 | One Hundred Days of Repetitive Thrombosis - Diagnostic and Therapeutic Challenge

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Background: Factor V Leiden thrombophilia is characterized by a poor anticoagulant response to activated protein C(APC) and an increased risk for venous thromboembolism (VTE). Antiphospholipid antibody syndrome is an autoimmune disease characterized by the presence of thromboembolic complications. The authors therefore present the case of a 50-year-old woman with multiple thrombotic events and the presence of antiphospholipid antibodies in combination with heritable thrombophilia, which, to the best of the authors' knowledge, is the first time to be reported in Republic of Moldova.

Aims: Through this case report, the authors aim to describe the evolution of this complex pathology, probably because of its challenging diagnosis and the limited treatment.

Methods: we described the evolution of case.

Results: We present a rare case of women which during 100 days of hospitalization has developed eight thrombotic events in a row (stroke, DVP, VTE, MI, peripheral radial thrombosis, sparing artery Thrombosis), both in the venous system as well as in the artery, followed by disabling serious patient (deep paresis on the left, left leg amputation, blindness on the right and hemianopsia on the left, etc.) Thrombophilia screening tests showed patient to be a heterozygous carrier of MTHFR gene mutation (both C and A) and heterozygous FVL mutation, in presents of positive anti-beta-2-glycoprotein 1 antibodies. Because of thrombosis extension, inherited prothrombotic condition and the relatively young age of the patient, we decided to continue life-long oral anticoagulant therapy.

Conclusions: The authors conclude that clinicians need to have a high index of suspicion of trombophillia and/or APS in patients who present with a thrombotic episode - clinicians should investigate for the presence of antiphospholipid antibodies and genetic tests, as early diagnosis may influence the course of the disease. Finally, patient education on the importance of drug compliance, periodic monitoring, and prevention of thrombosis is indispensable.

PO 52 | Pulmoner Thromboembolism: A Rare Cause of Respiratory Distress in Infants with Neuroblastoma

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Background: Pulmoner thromboembolism (PTE) is rarely seen in children. In patients with malignancy who developed respiratory distress, PTE must be also considered in differential diagnosis.

Aims: Here, we presented a rare case with neuroblastoma who had PTE due to chemotherapy without L- asparaginase.

Methods: A 12-month old boy was admitted to the hospital with an intraabdominal mass. Abdominal ultrasounography showed a mass lesion surrounding the aorta, containing intense calcification. He had a metastatic lesion at the corpus of L1 vertebra in the whole body magnetic resonance imaging (MRI). The patient diagnosed the stage IV of neuroblastoma with a biopsy and chemotherapy consisted of cisplatin, etoposide, cyclophosphamide was started. Ten days after sixth course,he developed respiratory distress. The patient had respiratory rate of 60/min, intercostals, and suprasternal withdrawals, no cough. Lung sounds and cardiac examination were normal. O2 saturation was 76 % in room air. Blood gas analysis revealed PH:7.47, PCO2:26.3 PO2:32.6, HCO3:19.1 and O2 saturation 68.7% respectively. Radiography of chest and cardiac echocardiyography were normal. High resolution computerized tomography of thorax revealed that pulmonary parenchyma was normal and there was a thromboembolism in the pulmonary artery of the laterobasal segment of the right lower lob. The patient was diagnosed as PTE and enoxaparine was started at the dose of 1 mg/kg twice every day. Respiratory distress was improved at 13 th day of anticoagulant therapy. The appearance of tromboembolism disappeared in Thoracal CT at 15th days of anticoagulant therapy. Later, chemotherapy, according to the protocol of TPOG-2009, was continued without problems.

Results: -

Conclusions: In childhood, PTE is especially common after congenital heart diseases, nephrotic syndrome, long-lasting permanent catheterization and use of L- asparaginase. The development of pulmonary thromboembolism due to chemotherapy is a rare but important reason to be kept in mind.

PO 53 | A Challenging Deep Venous Thrombosis in a Child

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Background: Popliteal vein thrombosis is an unusual presentation of deep venous thrombosis (DVT) in children.

Aims: We report a 10-year-old girl who presented with a 2-month history of pain and swelling of the popliteal fossa of the lower leg.

Methods: She was referred as popliteal vein thrombosis by the Division of Vascular Surgery Unit of the State Hospital, and the Department of Orthopedics of our university hospital to our unit. She was on low-molecular-weight heparin (LMWH) therapy. After the detailed clinical, laboratory, and radiological evaluation; the diagnosis was confirmed. All test results were in normal range except the mildly increased levels of homocysteine and lipoprotein(a) at the thrombosis work-up. Folic acid was added to LMWH. After, these detailed evaluations, the patient was followed by clinically and radiologically (doppler ultrasonography) in every two weeks intervals.

Results: However, two months later, USG revealed a new developed saccular aneurysm of the popliteal vein in addition to the popliteal vein thrombosis. The diagnosis was confirmed by the computed tomography angiography, also. The patient was referred for the surgical excision to the three very experienced Vascular Surgeons of different hospitals. But, they suggested the follow-up of the patient without surgery, LMWH therapy was continued, and the patient was followed-up closely, again. However, the second new aneurysm was detected in her follow-up. Wide surgical excision was done at that time, and the histopathological diagnoses were popliteal venous aneurysm and suspected angiomatoid fibrous histiocytoma (in a small area). However, molecular studies could not be done for AFH. Given the intermediate malignant potential of this tumor, the patient was evaluated again for the distance metastasis. There is no metastasis or recurrence following about four years after surgery, and she has no complaints.

Conclusions: In conclusion, the evaluation and follow-up should always be done carefully and closely in children with unprovoked DVT.

PO 54 | Use of Direct Oral Anticoagulants in Multiple Myeloma Patient with Atrial Fibrilation

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Background: The role of the direct oral anticoagulants (DOACs) in oncological patients who are receiving treatment remains unexplored for the time; irregular monitoring and drug interactions entail bleeding and thrombosis risk. There are some information in thrombosis disease and the results reported are encouraging; but there is no data in atrial fibrillation.

Aims: The main goal of this study is analyze the safety on the use of direct oral anticoagulants in patients with multiple myeloma who were receiving treatment.

Methods: A retrospective study was conducted of patients with multiple myeloma who were treated with chemotherapy and vitamin K antagonists (VKA) because of atrial fibrillation. These patients had had irregular monitoring; drug interactions, previous bleeding, and we decided to change to DOACs. The main variables investigated were minor and major bleedings, thromboembolic events, multiple myeloma chemotherapy. Patients with severe interaction between drugs were excluded. Demographics and laboratory data were collected. Statistical analyzes were performed using SPSS 21.0 (SPSS Inc.,Chicago, IL).

Results: Four patients were analyzed, all of them were men. The median age was 79.5 (73-81). Mean of CHADVASC2 and HAS-BLED was 3 and 1.25 (1-2) points respectively. Only one patient had an exitus not related with drug. Chemotherapy received was Melphalan- Dexamethasone (50%) and Bortezomib-Melphalan-Prednisone (50%). DOACs received were Apixaban 5 mg (25%), Rivaroxabán 20 mg (25 %) and Rivaroxaban 15 mg (50%). There were no thrombotic events that would have required removal drug. There were 2 patients with minor bleeding (50 %) and 1 patient with major bleeding (25%) this one had risk factor for bleeding (colon polyposis). Two of these patients discontinued the drug (50 %) for bleeding.

Conclusions: In spite of having a short serie of patients we suggest that DOACs should be safed in patients with atrial fibrilation and multiple myeloma. Further studies are needed to confirm this hypothesis.

PO 55 | Clinical Analysis of 399 Cases of Malignant Tumor Complicated with Venous Thrombosis in the Uygur and Han nationality in Xinjiang

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Background: To investigate the characteristics of Uygur and Han patients with VTE in XinJiang,in order to improve their diagnosis and treatment and improve their prognosis.

Aims: To investigate the characteristics of Uygur and Han patients with VTE in XinJiang,in order to improve their diagnosis and treatment and improve their prognosis.

Methods: The clinical data of malignant tumor complicated with VTE were retrospectively

analyzed between 2010 to 2015 in Tumor Hospital of Xinjiang. To explore the morbidity and clinical characteristics of Uygur and Han patients with malignant tumor complicated with VTE.

Results: The mean age of onset of VTE in Xinjiang Uygur was higher than that in Han .The level of D-dimer in patients with malignant tumor was higher than that in normal group .The number of cases of gynecological system tumors, gastrointestinal tumors, lung cancer and hepatobiliary tumors were higher in Han than in Uygur patients (P< 0.01).The number of cases of pancreatic cancer and lymphoma were higher than those of Uygur, the difference was statistically significant (P < 0.01).The number of esophageal cancer in Uygur was higher than that in Han, but the difference was not significant. Tumor associated VTE more occurred in the lower limbs, the incidence of the left lower limb was 43.9%, and 25.1% of the right lower limb,and 12.8% of the bilateral lower limbs. Lower incidence of upper limb, only 1.8%. The incidence of pulmonary embolism was between the upper limbs and lower limbs, it´s 16.5%. The incidence of left lower limb, right lower limb, pulmonary embolism (P < 0.01) and bilateral lower limbs (P < 0.05) were higher in Han than in Uygur. The difference was statistically significant. There was 55.6% of PE patients showed sudden chest pain, 23% of patients showed chest tightness and shortness of breath, 11.1% of patients showed hemoptysis,and the other showed cough, sputum.

Conclusions: There are differences in the age, tumor location and thrombus location between Uygur and Han with VTE in Xinjiang Uygur and Han.

PO 56 | Circulating Neutrophil-extracellular Traps (NETs) Are Elevated in Patients with Endometriosis

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Background: Neutrophil-extracellular traps (NETs) are related to the pathogenesis of inflammatory and autoimmune conditions. Endometriosis is considered a chronic inflammatory condition.

Aims: To evaluate plasma circulating NETs levels in a cohort of patients with endometriosis.

Methods: In this case-control study plasma circulating NETs levels were assessed in patients with surgically confirmed endometriosis (group E, n=82) and in patients undergoing benign adnexal surgery without surgical findings of endometriosis (group C, n=35). The median age, Body Mass Index and tobacco use were similar in both groups. Venous blood samples were obtained at the time of surgery before preanesthesic medication intake. Plasma circulating NETs levels were measured as histone-DNA complexes (i.e. nucleosomes) by a quantitative sandwich-enzyme-linked immunosorbent assay (ELISA). Results were expressed as a ratio between mU of patient/ mU of standard sample. Statistical significance was defined as a p-value < 0.05.

Results: Plasma circulating NETs levels were significantly higher in the group E compared with the group C ([median (25th; 75th percentiles)]: group E: 0.734 (0.484; 1.363); group C: 0.541 (0.411; 0.653); p =0.005)). The sub-analysis of endometriosis patients with deep infiltrating endometriosis (group DIE) or without DIE (group non-DIE) demonstrated that plasma NETs levels were higher in the DIE group (p = 0.02).

Conclusions: Patients with endometriosis have significantly higher NETs levels than controls, which seem to be mainly due to increased levels in the subgroup of DIE patients. Circulating NETs may be involved in or related with the pathophysiology of this gynecologic condition.

PO 57 | Thrombophila Associated with an Unusual Presentation of Liver Fibrosis/Cirrhosis and Splanchnic Vein Thrombosis

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Background: Some patients who had no common causes of liver fibrosis/cirrhosis, yet developed liver fibrosis/cirrhosis and splanchnic vein thrombosis.

Aims: To show that hypercoagulation may be another rare cause of liver fibrosis.

Methods: All laboratory tests and image studies were performed routinely. Magnetic resonance elastography (MRE) of the liver was used to evaluate the extent of liver fibrosis which were correlated with the Metavir score (F0-F4). Informed consent was obtained from patients.

Results: Patient I, a male was born in 1956. He has protein C (PC) deficiency with a functional level of 13% and heterozygous p.Gly324Ser substitution at exon 9 of the PC gene. He suffered from left proximal deep vein thrombosis (DVT) in 1998, superior mesenteric vein thrombosis (SMVT) and receiving resection of small intestine in 2003, portal vein thrombosis (PVT) with presence of collateral circulation and liver cirrhosis in 2004. Patient II, a male was born in 1950. He has familial protein S (PS) deficiency with a functional activity of 12% and heterozygous c.531-534 + 4 del at exon 6 of PS gene. SMVT occurred in 2011 and intestinal resection was done then, PVT with cavernous transformation and liver fibrosis were detected in 2012, MRE of the liver showed F2 and F3 score in patient I and II, respectively. FVIII:C/PC ratio, a good biomarker of hypercoagulation in thrombophilia, revealed a higher value, i.e. 13.25 and 2.50 in patient I and II, respectively, as compared to normal control (Mean±SD 0.85±0.28, ranges 0.35~1.56). All had no HBV (hepatitis B virus) and HCV-infections, alcoholic consumption, antiphospholipid antibodies, homocysteinemia, abnormal level of anti-mitochondrial antibody and anti- smooth muscle antibody.

Conclusions: These 2 patients had no common causes of live fibrosis/cirrhosis, yet they developed liver fibrosis and splanchnic vein thrombosis, hypercoagulation associated with thrombophilia my cause extrahepatic splanchnic vein thrombosis and intrahepatic microthrombosis leading to fibrosis.

PO 58 | Antithrombin Activity in Newly Diagnosed Diabetis Mellitus Patient seen at the University of Benin Teaching Hospital, Benin City, Nigeria

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Background: Thromboembolic complications contribute significantly to morbidity and mortality in diabetic patients. The role of antithrombin in the pathogenesis of vascular complications in diabetics has not been investigated in our environment.

Aims: This study aims to evaluate the role of antithrombin activity (AT) in the pathogenesis of vascular complications in newly diagnosed diabetics.

Methods: This is a cross sectional study conducted at the University of Benin Teaching Hospital, Benin City. Sixty two newly diagnosed diabetics were recruited consecutively from the consultant outpatient unit between April and October 2015 and 54 non diabetic controls from the general populace. Venous blood was collected each subject into citrate and EDTA containers for determination of AT activity and haematological parameters respectively. AT activity was measured with Technoclone chromogenic AT kit (Technozyme Lot no:0551B00.02) and full blood count was analyzed using Hematology Analyzer, ERMA INC (Tokyo model FCE 2.0).

All participants gave informed consent. The study was approved by the Research and Ethics Committee of the hospital.

Result was analyzed with SPSS version 16. Student T test was used to compare difference in mean, Chi-square and Fisher´s test were used to test association of AT deficiency with vascular complications. Pearson correlation coefficient was used to correlate AT activity with BMI and haematological parameters.

Results: The mean AT activity in newly diagnosed diabetics was significantly lower than in controls (83.3 ± 30.0 vs 92.8 ± 20.0%; p = 0.050). The incidence of AT deficiency in newly diagnosed diabetics was 32.3%. AT deficiency was associated with overweight/obesity and thrombocytopenia. Microvascular complication was associated with AT deficiency.

There was a significant negative correlation between BMI and AT activity (r = --0.276; p = 0.030).

Conclusions: Low AT activity is associated with microvascular complications in newly diagnosed diabetic subjects.

TABLE 2 Correlation between AT activity, Age, BMI and Haematological Parameters in Newly Diagnosed Diabetics

		Antithrombin Activity (%)	
	units	r	p value
Age	yrs	0.076	0.556
BMI	Kg/m2	-0.276	0.030
HbA1c	%	0.112	0.565
Hgb	g/dL	-0.029	0.824
WBC	(x 109 cells/L)	0.090	0.488
Platelet	(x 109 cells/L)	0.220	0.086

TABLE 1 Association between AT deficiency with Clinical and Laboratory Parameters in Newly Diagnosed Diabetics

Variables	Sub category	Deficient AT Activity n = 20	Normal AT Activity n = 42	Statistical test	p value
Age (years)	<40 ≥40	3 (15.0) 17(85.0)	5 (11.9) 37(88.1)	Fishers	0.705
Gender	Male Female	7 (35.0) 13(65.0)	11(26.2) 31(73.8)	$\chi 2 = 0.510$	0.475
BMI (Kg/m2)	<25 ≥25	2 (10.0) 18(90.0)	18(42.9) 24(57.1)	Fisher´s	0.010
Hgb (g/dL)	<10 ≥10	3 (15.0) 17(85.0)	3 (7.1) 39(92.9)	Fisher´s	0.377
WBC count (x 109 cells/L)	<4 4 - 11 ≥11	3 (15.0) 16(80.0) 1 (5.0)	2 (4.8) 36(85.7) 4 (9.5)	Fisher´s	0.340
Platelet count (x 109 cells/L)	<150 ≥150	8 (40.0) 12(60.0)	6 (14.3) 36(85.7)	$\chi 2 = 5.124$	0.024
Vascular complications	Yes No	11(55.0) 9 (45.0)	32(76.2) 10(23.8)	$\chi 2 = 2.862$	0.091
Type of vasculopathy	Micro Macro Both	6 (54.5)) 2 (18.2) 3 (27.3)	3 (9.3) 20(62.5) 9 (28.2)	Fishers	0.001
Specific Complications	Stroke IHD Diabetic foot Nephropathy Claudication Retinopathy Neuropathy	0 (0.0) 0 (0.0) 2 (10.0) 0 (0.0) 4 (20.0) 6 (30.0) 3 (15.0)	3 (7.1) 2 (4.8) 3 (7.1) 3 (7.1) 8 (19.0) 26 (61.9) 11 (26.2)	Fisher´s	0.545 1.000 0.654 0545 1.000 0.019 0.509

PO 59 | Portal, Mesenteric and Splenic Vein Thrombosis in a Patient with Homozygous Factor V Leiden Mutation (A1691G) Receiving Oral Contraception

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Background: Women on oral contraceptives (OC) are at increased risk of venous thromboembolism (VTE). In factor V Leiden (FVL) carriers receiving OC the risk is even higher although most of FVL carriers remain asymptomatic despite long term OC use.

Aims: Report on clinical manifestations of thrombosis in homozygous carrier of factor V Leiden receiving oral contraception.

Methods: Factor Leiden mutation (G1691A) and mutation G20210A of prothrombin gene were detected using RFLP/PCR method. (EURx, Polska)

Results: Patient with no family history of venous thromboembolism, on oral contraceptives (third generation progesterone), during appendectomy at 43 was diagnosed with thrombosis of the portal, splenic and mesenteric vein. Low molecular weight heparin (LMWH) was administered followed by VKA (vitamin K antagonists). Due to unique thrombosis location and young age diagnostics for thrombophilia and myeloproliferative disorders was carried out. No bone marrow proliferative disorder was determined but factor V Leiden (homozygote) was detected. Family testing revealed that asymptomatic mother (65y) and brother (44y) are homozygotes of factor V Leiden.

Conclusions: The ethiology of splanchnic vein thrombosis is multifactorial therefore the diagnostic process of the entity should comprise searching for genetic and acquired risk factors of VTE.

PO 60 | Deep Vein Thrombosis in a Family with Leiden Mutation (FVL, G1691A), Antithrombin Deficiency (AT) and ARhD (+) Blood Type

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Background: In people with inherited thrombophilia the risk of deep vein thrombosis (DVT) is related to the ABO blood type. Blood type AB individuals with thrombophilic mutations are at significantly higher risk of DVT. The mechanism by which the non-O blood types contributes to thrombosis risk in FVL carriers is probably related to factor VIII levels [Morelli et al. 2005].

Aims: To establish the risk factors for familial thrombosis.

Methods: Factor V Leiden (FVL) mutation (G1691A) was identified by PCR/RFLP with restriction enzyme Mnl1 (Eurx, Poland) and the AT activity was assessed using SXa-based assay (Innovance, Antithombin, Siemens).

Results: Two cousins of sibling parents were admitted to IHTM at the same time. The younger one (son of the brother), a 31 year old white-collar worker (obesity and hypertension) was diagnosed with VTE due to prolonged immobilization. The elder one (son of the sister) a 40 year old computer programmer (no obesity, hypercholesterolemia or addictions) reported trauma-related swelling and redness of the left lower limb and was diagnosed with DVT (Doppler ultrasound). Incidents of sudden death were reported in families of both siblings; maternal grandparents of the elder cousin as well as the paternal grandparents and father of the younger one and sudden death of the paternal grandfather of the elder. Our studies revealed:· homozygous mutation in FVL and congenital AT deficiency (58%) in the elder cousin. · heterozygous FVL mutation and AT in his mother (49.46%), · heterozygous FVL mutation in his father and his two children· AT deficiency (65.92%) in his younger son. No congenital thrombophilia was determined in the brother of the elder cousin. Heterozygous FVL mutation and AT deficiency (50.13%) were determined in the younger cousin. Both were ARhD (+) blood type.

Conclusions: The thrombotic risk is significantly higher in families with AT, FVL mutation and ARhD (+) blood type. All members of both families with FVL mutation and AT were of ARhD (+) blood type.

PO 61 | Prothrombotic Mutations, Protein C Deficiency and Hyperhomocysteinemia in a Patient Presenting Recurrent Venous Thrombosis

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Background: Patients with recurrent VTE may present complex hemostatic defects. In approximately 10% of Factor V Leiden carriers Prothrombin G20210A mutation is also detected. In Polish Factor V Leiden carriers a frequent concomitance of heterozygous protein C deficiency or prothrombin G20210A polymorphism has been confirmed (10% and 30% respectively).

Aims: Analysis of the clinical course of recurrent VTE in a patient with polygenic defect and hyperhomocysteinemia.

Methods: case report

Results: A 33 year old man -a sedentary lifestyle, 2 risk factors for VTE (exam-induced stress, long trip) presented swelling, redness and pain in the left lower limb. Doppler ultrasound revealed DVT in saphenous and popliteal veins. Anticoagulation therapy with Enoxaparin 1 mg/kg bw/7 days and warfarin-3 months was effective. The second VTE event occurred 5 years later following injury of the same limb. Doppler ultrasound confirmed proximal vein thrombosis. Six-month effective anticoagulation therapy followed. Eight months later the patient reported pain in the limb after intense exercise. Acute VTE was detected in the external iliac vein. The study confirmed heterozygous Factor V Leiden (G1691A) mutation and heterozygous prothrombin G20210A mutation, congenital protein C deficiency -0,32 IU/dl (0,70-1,40 IU/dl) and hyperhomocysteinemia - 28.5 mmol / L (n: 5.0-12.0 mmol -/- l). Family history: the grandfather had a heart attack and died of stroke later at the age of 59. The father died of heart attack, the mother had a heart attack and died of stroke. The patients' brother had DVT in both lower limbs, suffered extensive stroke and died at the age of 29.

Conclusions: Recurrent VTE was caused by several risk factors: genetic defects (Factor V Leiden and prothrombin G20210A mutations), protein C deficiency, elevated serum homocysteine, trauma, stress and sedentary lifestyle.

Co-occurrence of venous and arterial thrombosis in the family suggests the presence of complex genetic/environmental defects in other family members as well.

PO 62 | Rise and Fall of Thrombophilia Law in Argentina

B. Grand, A. Rossi

On behalf of The Women Health Issues in Thrombosis and Hemostasis Group (WHITH-CAHT) and the Argentine Thrombosis and Hemostasis Cooperative Group (CAHT), Buenos Aires, Argentina

Background: In Argentina the relationship between thrombophilia and pregnancy complications increased its popularity.Celebrities from entertainment programs made public their obstetric complications associated to thrombophilia and claimed for early detection and treatment of this "disease that attacks pregnant women". Even a law petition was presented to the Congress.

Aims: To describe how the Thrombophilia Law was voted and vetoed in Argentina.

Methods: 2015 Law projects titled "Psychophysics protection of women with thrombophilia" were presented to the Congress.

Objectives: Early detection of thrombophilia in women in fertile and prefertile age; inclusion of tests and treatments as obligatory in public/private health programs.

CAHT and WHITH-CAHT activities 2015

(1) Scientific Meeting with representatives of the city legislature and Health Ministry;2016

(2) A document signed by 35 experts in Thrombosis and Hemostasis that explained the reasons why the law was unnecessary and had serious scientific and ethical questioning;

(3) Scientific Meeting with representatives from the National Ministry of Health;

(4) Petition of a meeting with the Commission of health of the Congress.

Results: The(1)took part , but a few days after Deputies voted in favor of the law. The (2) was read in (3). In spite of our medical opinion, the Chamber of Senators voted in favor. The medical voice was ignored; the meeting with the commission of health was not done. We decided to inform through the media(newspapers, interview on the radio/TV) and social media.The president vetoed the law and argued in the Official Gazette: the tests are expressly not recommended to be routinely carried out adding that it has been criticized/questioned by medical societies Argentine Thrombosis and Hemostasis Group and Hematology in coincidence with Ministry of Health.

Conclusions: We have 2 voices regarding thrombophilia and pregnancy complications; new worldwide active medical education interventions and strategies of communication for women in reproductive age are needed.

PO 63 | Hypofibrinogenemia and Thrombosis

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Background: Congenital hypofibrinogenemia is a blood clotting disorder characterized by a quantitative deficit that results in hemorrhage of varying magnitude. It is a rare disease with the presence of a notion of consanguinity, transmitted by the autosomal recessive mode.

Aims: Show the importance of the hemostasis assessment.

Methods: We report an observation of hypofibrinogenemie.

Results: Sir M 45 - year - old , stemming from non a consanguine marriage , married and father of 4 children followed in our department of hematology for congenital hypofibrinogenemia, admitted on 12/11/2016 for edema of the lower limb Right with necrosis of the 5th toe homolateral.

hEmogramme: WBC: 18700 ele / mm3 HB: 12: 5 g / dl, Platelets: 328000ele / mm3, TP < 14% Fg: 0.7 g / absence of PDF and the dosage of the factors of coagulations are normal IHepatic Impairment: correct, Renal assessment: correct, The dosage of the proteins c, s and antithrombin = normal,

Echo-Doppler of the lower right limb Morphological and hemodynamic study of the arteries and veins of the lower right limb.

Lymphedema of the lower right limb The main causes of thrombosis have been eliminated

The necrosis of the toes which led first to the amputation of the right 4th and 5th toes but the necrosis was not controlled twenty days later, the necrosis had spread to the 1st and 3rd toes that were necrectomy But in vain necrosis was still extended to the second toe which led to the amputation of the forefoot two days after the Doppler echo of the right lower limb showed total acute thrombosis of the right pedal artery Which alas led to the amputation of the middle third of the right leg On 25/1/2016 a good cicatrisation.

Conclusions: In this case, Hypofibrinogenemia is associated with thrombosis whereas the tendency to thrombosis is seen in dysfibrinogenemia so it is imperative in the biological assessment of thromboses to perform a TP haemostasis, TCA and fibrinogen balance which will Diagnosis of previously unrecognized fibrinogen deficiency.

PO 64 | Role of Thrombophilia Testing in Retinal Vein Occlusion: Single Center Experience from Oman

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Background:

- Retinal vein occlusion (RVO) is a serious retinal vascular disorder.
- he role of thrombophilia disorder and RVO still not clear.

Aims:

- To demonstrate any relationship between studies parameters and RVO.
- To clarify any significant relationship among RVO and thrombophilia.
- To describe RVO in Omani Population.

Methods:

- A total of 11 patients referred by the ophthalmologist with RVO (central RVO, n=7; branch RVO, n=4) prospectively enrolled in this single institution study after an informed consent.
- Several routine biochemical parameters of Complete blood counts, renal liver function, serum triglycerides (mmol/L), Lipoprotien 1a (g/L) total cholesterol (mmol/L) and homocysteine (mmol/L).
- Thrombophilia screening included factor V Leiden, prothrombin geneG20210A and JAK2V612F mutation analysis; autoantibody assays like ANA, ANCA, LA, ACA, anti-beta2 glycoprotein activity and coagulation proteins estimations including protein S, protein C, antithrombin III and factor VIII levels were also performed.

Results:

- The study enrolled a total of 11 patient's, Their ages ranged between 25 to 75 years (mean ± SD; 48.8 ± 13.6) with a mild male preponderance (54.5%).
- Majority of patients developed CRVO(64%), with the remaining developing BRVO (36%).
- 27.3% and 45.5% had history of diabetes and essential hypertension, respectively.
- None of the thrombophilic factors were abnormal.except one patient each, showed positivity for anti-B2 glycoprotein antibody (11.1%), prothrombin gene G20210A heterozygosity (14.3%) and elevated serum homocysteine level (23.3 mmol/L.
- Normal Biochemical studies(S. lipoprotein 1a levels, S.triglycerides, S.total cholesterol, S.homocysteine).

Conclusions:

- 91% of patients were below the age of 60 years.
- CRVO (64%) was apparently more prevalent that BRVO (36%).
- 27.3% and 45.5% had history of diabetes and essential hypertension, respectively.
- Thrombophilia testing in RVO might not be cost effective.

PLATELETS – CLINICAL

PO 65 | Acute Severe Thrombocytopenia Induced by Abciximab: A Case Report

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Background: Glycoprotein (GP) IIb/IIIa inhibitors are frequently used during percutaneous coronary interventions (PCI), reducing ischemic complications, improving outcomes. Severe acute thrombocytopenia is a serious adverse event of these drugs. In this setting, the balance between risk of sgnificant bleeding and thrombosis requires a careful approach.

Aims: Report a case of a patient with an acute myocardial infarction (AMI) who developed severe acute thrombocytopenia induced by abciximab.

Methods: A 69-years-old male was admitted to emergency room with a 2-hour history of chest pain radiating to the left arm. He had a previous history of AMI in 2006 treated with thrombolysis followed by coronary artery bypass grafts. The electrocardiogram showed ST elevation, cardiac biomarkers were positive, full blood counts and coagulation tests were normal. Aspirin 250 mg, ticagrelor 180 mg and morphine 2 mg iv were given. PCI was performed with incomplete thrombus aspiration. Abciximab was then given (0,25 mg/Kg iv bolus followed by a 0,125 µg/Kg/min infusion for 12 hours). Five hours after beginning abciximab, severe thrombocytopenia was detected (platelets < $10x10^9$/L), with normal hemoglobin and no signs of bleeding. Abciximab was immediately stopped. One platelet pool and methylprednisolone (500 mg pulse iv) were given. Four hours later, platelet count was $38x10^9$/L. Periodic monitoring of blood counts was performed (day 3: platelets $107x10^9$/L). Patient had hemoptoic expectoration for 4 days. On day 5 aspirin 100 mg od was started (platelets $157x10^9$/L) and clopidogrel 75 mg od was started on day 8.

Results: Platelet count completely recovered in 5 days. He did not have any major bleeding or ischemic event, his clinical situation improved and he was discharged on day 10.

Conclusions: Thrombocytopenia is a rare complication of GPIIb/IIIa inhibitors. Due to bleeding risk, it is important to monitor platelet count before and during treatmen. Management of this patient was successful.

PO 66 | Prolactin Level in Patients' Sera with Hepatitis C Virus Related Thrombocytopenia

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Background: PRL is involved in the activation of many immunological responses. It enhances the progression of the immune process in autoimmune diseases. Autoimmunity is a common finding in chronic hepatitis C. There was a significant association between hyperprolactinemia (HPRL) and infection with HCV genotype 3 and genotype 4.
Aims: The aim of this work was to evaluate serum levels of prolactin (PRL) in a group of patients with HCV-related thrombocytopenia compared with patients with hepatitis C virus (HCV) without thrombocytopenia and controls.
Methods: This study was carried at Internal Medicine Department, Menoufia University Hospital, from the period of Mars 2015 till December 2015. Subjects were calssified into Group (I): fourty one chronic hepatitis C patients with thrombocytopenia. Group (II): thirty five chronic hepatitis C patients without thrombocytopenia. Group (III): twenty five control healthy individuals with matched age and sex.
Results: We found hyperprolactinemia in patients with HCV-related thrombocytopenia and it was significantly higher than in patients with chronic hepatitis C patients without thrombocytopenia than controls. Also, there was association between hyperprolactinemia and low platelet count. Low platelet count was positively correlated with the degree of liver fibrosis.
Conclusions: This study shows that hyperprolactinemia is present in a subset of patients with HCV-related thrombocytopenia, so anti-prolactin may be useful in treatment of this category of patients.

PO 67 | Study of Serum Prolactin in Primary Immune Thrombocytopenic Patients

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Background: ITP is a disorder characterized by immune-mediated accelerated platelet destruction and suppressed platelet production. Hyperprolactinemia (HPRL) has been described in many autoimmune diseases such as systemic lupus erythematosus.
Aims: The aims of this work were to study serum prolactin (PRL) levels in patients with primary immune thrombocytopenia (ITP) and to investigate its possible correlation with disease activity and manifestations.
Methods: The study was carried out on 40 cases of primary ITP patients (group I) and 50 healthy controls (group II). PRL was measured directly in the serum samples by VIDAS PRL kits using the ELFA technique for all patients and controls.
Results: Moderate HPRL (serum PRL 30-200 ng/ml) was present in eight (20%) of primary ITP patients, but was not present in any of the 50 controls. Among 22 patients with platelet count below 30 000/µl, eight (36.4%) patients had HPRL and 14 (63.6%) patients had normal PRL levels. HPRL was associated with lower platelet counts.
Conclusions: This study shows that HPRL is present in 20% of patients with primary ITP. Also, patients with HPRL have a lower platelet count than patients with normal PRL levels.

PO 68 | Immun Trombocytopenic Purpura in a Patient with History of Non-Hodgkin Lymphoma and Common Variable Immune Deficiency

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Background: Development of immune thrombocytopenic purpura (ITP) after complete remission of Non-Hodgkin lymphoma in a child is a rare entity.
Aims: Herein, we present a 9 ½ years old boy presented with thrombocytopenia who had a past medical history of Non-Hodgkin lymphoma in remission for 4 years and common variable immunodeficiency diagnosis (CVID).
Methods: While he was on intravenous immunoglobulins (IVIG) treatment on a monthly basis for the CVID, he developed petechia and hemogram showed platelet count of $7x10^9$/L. A diagnosis of ITP was established after the initial investigations including viral serology and bone marrow aspiration examination to rule out relapse of Non-Hodgkin lymphoma with bone marrow involvement. Bone marrow examination revealed increased megakaryocytes of 8-10 in number in x10 magnification. IVIG treatment at dose of 1 gr/kg was administered with a good response in platelet count $272x10^3$µ/L.
Results: Autoimmunity is a well known entity in lymphomas and CVID.
Conclusions: Although, CVID has been reported to be associated with immune cytopenias and lymphomas, the previous lymphoma diagnosis in our patient prompted a diagnostic challenge. Therefore, ITP may develop as a representation of this phenomenon.